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Emerging Trends and New Vistas in Applied Sciences - 2022

First International Conference

On

" Emerging Trends and New Vistas in Applied Sciences - 2022"

12TH FEBRUARY 2022

Compiled and Edited by

P.Arivazhagi | P.Sivakumar | A.Mangaiarkkarasi | V.Karunakaran
G.Gayathry | C.Harisudan | K.Sivagamy | P.Savitha
R.Meenatchi | P.Silambarasan | M.Radha | S.Ratnasamy

SOCIETY FOR NATURE AND APPLIED SCIENCES

In Colloboration with

SRI VENKATESWARAA MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTRE
PUDUCHERRY, INDIA

First International Conference
ON
"Emerging Trends and New Vistas in Applied Sciences"
(ETNVAS 2022)

12th February, 2022

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Society for Nature and Applied Sciences (SNAS)
(Regn. No: SRG/TRY/112/2021)
In collaboration with
Sri Venkateshwaraa Medical College Hospital and Research Centre
(SVMCH & RC), Puducherry, India

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in collaboration with
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FOREWORD

The role of applied sciences in overall growth and development of Indian economy is quite significant and laudable as it is clearly visible by the growth, agricultural production, infrastructure development, greater access to affordable health services and overall Gross Domestic Product of India. Keeping in view the huge potential and role played by all the applied sciences in improving the lifestyle and standard in the country at present and near future it is essential that quality research output need to be disseminated.

To commemorate the foundation of Society for Nature and Applied Sciences, Trichy the "FIRST INTERNATIONAL CONFERENCE ON EMERGING TRENDS AND NEW VISTAS IN APPLIED SCIENCES (ETNVAS 2022)" organized in collaboration with *Sri Venkateswaraa Medical College Hospital and Research Centre, Puducherry.* Indeed, it is interesting to have all in one platform from applied sciences *viz.*, agriculture, medicine, veterinary, fishery, forestry, Engineering, life science, post harvest, biotechnology and pharmaceuticals representing students, scientists, renowned experts, farmers and industry representatives to deliberate and discuss on the emerging trends and new vistas in applied sciences for converting the output into outcome which finally plays major role in the growth and development by affordable technologies, cost effective protocols, methods and products for the end user.

I am delighted to be part of this initiative and hope that the scientists, academicians from life sciences, faculty members, research scholars & students to join in ENTWAS 2022 will be able to enhance your knowledge in novel research areas & technologies in various disciplines of sciences *viz.*, Agriculture and allied sciences, medical sciences, engineering sciences & life sciences. I appreciate the efforts taken by SNAS & SVMCH&RC in organizing the event in hybrid mode through virtual and physical means to encourage the participants from national and international level considering the pandemic situation. To honor the best performers in various capacities awards and prizes are also included. I wish the participants make full use of the interactions and enhance their capabilities.

(SMITHA.R, I.A.S.)

SRI VENKATESHWARAA MEDICAL COLLEGE HOSPITAL & RESEARCH CENTRE

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FOREWORD

I am very much delighted to know about that, our Institution (SVMCH&RC) has been planned to conduct an International Conference in collaboration with Society for Nature and Applied Sciences (SNAS) on 12th February 2022.

It is known that any conference is considered as a meeting place of likeminded people to discuss about common concern with advancement in the respective field. We are providing a platform for the faculty and PG students to do their Oral/ Poster/e-Poster presentation. The National and International resource persons from the reputed Institution by giving Orations are sharing their knowledge to the beneficiaries.

By conducting the conference, one can improve their presentation and communication skills, to know about latest research in their field, to participate in the discussion in order to refine their ideas, to get to know about other people and to get new friends and to visit new places.

I appreciate all the delegates who have registered with the expectation to get newer ideas from this conference. My special appreciation for the faculty members and PG students who are doing their presentation in this scientific forum. I congratulate the Organizing and Co-organizing Secretaries and Organizing Committee Members for taking keen efforts to make this International Conference a grand success.

Wishing you the best !!!

Dr. S. RATNASAMY, B.Sc., M.S.,
DEAN

Society for Nature and Applied Sciences
(REGN. NO: SRG/TRY/112/2021)

Dr. R. SAVITHA, Ph.D
Women Scientist (DST)
President

Tamil Nadu, India

Dt:09.02.2022

PREFACE

The role of applied sciences in overall growth and development of the Indian economy is quite significant and laudable because the transformation took place clearly visible by the growth, agricultural production, infrastructure development, greater access to affordable health services and overall Gross Domestic Product of India. Keeping in view the huge potential and role played by all the applied sciences in improving the lifestyle and standard of the country men at present and near future is entrusted in the high quality research outputs which need to be disseminated.

Modern India had a strong focus on science and technology, realizing that it is a key element for economic growth. India is among the topmost countries in the world in the field of scientific research. It is evident that the government of India has allocated Rs 14,217 crore in the 2022-2023 toward Science and Technology. It is clearly visible that investment is the guiding force for our nation. In the present era of the country's development the all around path breaking finding in the applied sciences research paves a key role in positioning India in the world arena and inclusive growth development. The outputs obtained in the form of technologies, new findings, protocols, designs, and formulations in all facets of applied sciences play a major role in terms of outcome in growth and development.

To commemorate the foundation of Society for Nature and Applied Sciences, Trichy the "FIRST INTERNATIONAL CONFERENCE ON EMERGING TRENDS AND NEW VISTAS IN APPLIED SCIENCES (ETNVAS 2022)" organized in collaboration *Sri Venkateswaraa Medical College Hospital and Research Centre, Puducherry.* Indeed, it is interesting to have all in one platform from applied sciences *viz*., agriculture, medicine, veterinary, fishery, forestry, Engineering, life science, post harvest, biotechnology and pharmaceuticals representing students, scientists, renowned experts, farmers and industry representatives to deliberate and discuss on the emerging trends and new vistas in applied sciences for converting the output into outcome which finally plays major role in the growth and development by affordable technologies, cost effective protocols, methods and products for the end user.

Society is very sure that the recommendations emerging out of this conference shall go a long way in developing practically feasible strategies and action plans for sustainable promotion of technologies, protocols, methods and products from applied sciences in the country as well as the globe. We also take this opportunity to convey our sincere gratitude to our organizing committee members who have put their valuable efforts in bringing out the research papers and abstracts in the form of a reference book on this occasion.

P. Savitha

PRESIDENT

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FOREWORD

It is very nice to see people doing great things in the world every day. It has been my pleasure and indeed great privilege for me that the First International conference on Emerging Trends and New Vistas in Applied Sciences is conducted by SVMCH & RC in collaboration with Society For Nature and Applied Sciences.

The goal of this conference is presenting the most recent abstracts of original research paper, case reports & case series with various topics like Medical sciences, Life sciences, Agriculture sciences and Engineering sciences done by many young researchers from across the world. The transition towards the interdisciplinary collaboration emanates new scientific problem and research progresses that are not confined to a single disciplinary context. This conspire effort of presentation and publishing represents the hopes and strength of the organizing team. The courage also go after their dreams and make this positive impact.

I congratulate the organizing team for preparing and successfully conducting this conference and publication.

Dr.A.Mangaiarkkarasi. M.D.,
Vice Principal (Academics)

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THEME I AGRICULTURE AND ALLIED SCIENCES

INTEGRATED AGRICULTURE FOR RURAL BIO-ENTREPRENEURSHIP AND NUTRITIONAL SECURITY IN INDIA

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Agriculture at present has the trend of using an industrialized production model, where crops and animals are produced in systems that are increasingly specialized, simplified and concentrated, to address growing food, feed and fiber demands. Within this model, external inputs (e.g. irrigation, synthetic fertilizers, chemical pest control, feeds, growth hormones) have been utilized to achieve production goals, often at the expense of environmental quality and key ecosystem services. Continuation of this model of agricultural production has resulted in loss of ecosystem services, increased ecosystem simplification and species extinction. Responses to future challenges in agriculture should include the development of novel systems that are highly productive, minimize damage to the environment, and effectively utilize renewable resources. Achieving these multiple goals will be a momentous task, as it will require development and implementation of more complex, diverse and management-intensive production systems than currently employed. Furthermore, future agricultural production systems will need to be adaptable to respond to unforeseen environmental challenges. Integrated agricultural systems have been purported to possess attributes to address these challenges.

Integrated agriculture is a production system with multiple enterprises managed in a dynamic manner that interact in space or time and these interactions result in a synergistic resource transfer among enterprises. Integrated agriculture involves farming systems with environmental, economic, social, and intergenerational sustainability. In an integrated, sustainable agriculture system, the goal is not necessarily producing immediate outputs, but rather maintaining a system of healthful production, over time. The major components of a sustainable system include economy, environment, and community. The resources that must be managed in such a system takes all of these related components into account.

Integrated Agriculture: Background and Rationale

The background

The remarkable growth of Indian agriculture over the last five and half decades, i.e. after the advent of Green Revolution (i.e. cereal production) technologies, has ushered in an era of self-reliance in food grain production, improved rural prosperity and has brought in an element of resilience into agriculture (Evenson and Gollin 2003). Food-grain production, which was 50.8 million tonne (mt) in 1950-51, was raised to 196.8 mt by 1997. The impact of the Green Revolution was so impressive that India became a role model for many developing countries. Concerted efforts made by researchers, farmers and policy makers transformed India from begging-bowl to bread-basket status within a short period of a decade or so. Obviously, this proved a matter of national pride and great satisfaction to the scientists and farmers of the country.

In the backdrop of past glory, unabated growth of the Indian population and its large scale (60%) dependence on agriculture, continue to pose a serious challenge for planners and agricultural scientists alike (Falcon *et al*. 2005). Assuming present trends, the Indian population is estimated at 1.3 billion by 2020, sharing resources with a very large livestock population. On the basis of present consumption patterns, estimated total requirement for food grain will be around 300 and 350 mt by 2020 and 2030, respectively, as against present production of approximately 270 mt. For these two years, the demand for edible oil, milk, vegetables and fruits is expected to rise to 7.9 and 9.5, 93.1 and 119.5, 93.6 and 110.7, and 53.7 and 70.5 mt. Similarly, a 30–50% increase in demand is anticipated for marine and livestock products from present levels of 5.4 and 3.6 mt, respectively.

In other words, to keep pace with the food requirements of such a large population, there is an urgent need to accelerate all aspects of agricultural food production with due consideration to restoration and conservation of natural resources, which can only be achieved through sustainable resource management and the adoption of farmer-participatory holistic approaches (termed the farming systems approach). The farming systems approach is considered a resource management strategy to achieve economic and sustained productivity that meets the diverse requirements of the farm household whilst preserving the resource base and maintaining a high level of environmental quality (Lal and Miller 1990).

The structure of Indian agriculture is undergoing transformation. A notable feature of changing farm structure is the dominance of smallholders whose number has increased over time and will continue to do so in future (Table 1). The traditional monoculture and disciplinary approach is unable to meet the growing and changing food and nutrition demand and improve the livelihood of these smallholders on a sustainable basis (Mahapatra and Behera 2011). Therefore, an integrated approach to farming is critical to sustain agricultural production, maintain farm incomes, safeguard the environment and respond to consumer concern about food quality issues (Yadav and Prasad, 1998). However, the potential contribution of integrated agriculture to the development of a more sustainable production system has largely been ignored. Moreover, decline in per capita availability of land from 0.5 ha in 1950-51 to 0.15 ha in 2009 and a projected further drop to less than 0.1 ha by 2020 point to the urgent need of developing strategies and agricultural technologies that enable adequate employment and income generation, with small and marginal farmers at the fore front. The problems

encountered by these 2 groups of farmers are different than those having large holdings. These farms need multi-enterprise farming activities that are complementary and technically compromising in the interest of the productivity of the whole farming system. The crop and cropping system based perspective of research needs to make way for farming systems (holistic)-based research particularly with regard to small farmers (Jha 2003). Integration of land-based enterprises, such as aquaculture, poultry, duckery, apiary, field- and horticultural-crops within the biophysical and socio-economic environment of the farmers is important to make farming more profitable and dependable. Adoption of an individual farm enterprise in isolation cannot sustain the farm family, but the integrated approach holds the promise of addressing the issues of sustainable economic growth of Indian farming communities. Integrated agriculture systems benefit from synergisms among different enterprises, diversity of produce, and environmental soundness.

Integrated Agriculture: Rationale

Some of the reasons for shifting from a specialized production system to an integrated production system are: (i) specialized farms operating on marginal profit, (ii) economic vulnerability with specialized production, (iii) high cost of fuel and nutrients, (iv) pests (i.e., weeds, insects, nematodes, and pathogens) becoming more damaging with monocultures, (v) yield decline due to long-term management-induced constraints on soil chemical and physical characteristics and biological diversity, (vi) spatially and temporally improved nutrient cycling on a field and landscape level with integration of enterprises, and (vii) conservation of soil and water resources with greater adoption of system-based holistic management approaches. Few other important aspects of integrated agriculture are given below:

Integrated agriculture is an important pathway to bio-diversity

There has been an ever increasing dominance of economically-driven highly intensive farming systems over ecologically-oriented traditional agricultural systems all over the globe. Such a shift is rapidly reducing the diversity of cropping systems and diminishing the quality of available habitats for various organisms associated with agricultural landscapes, and hence adversely affecting the existing biodiversity. The Indian scenario provides an excellent example. Market-oriented intensive agricultural production systems are replacing ecologically-oriented extensive traditional farming systems, and hence leading to rapid changes in the agricultural landscape. It is widely accepted that the major practices of the intensive systems that adversely affect farm level biodiversity are application of synthetic fertilizers and pesticides, cultivation of but a few high yielding varieties, continued mechanization of agriculture and the removal of semi-natural habitats in farm areas argued that conservation and sustainable use of biodiversity contributes significantly to sustainable development and mitigation, and adaptation to climate change.

IA promotes a rich culture of biodiversity through maintaining a multi-enterprise systems of flora and fauna. Behera *et al*. (2007) reported 21 species of cropped plants in an integrated farming systems under eastern Indian conditions, comprising root crops, leafy vegetables and greens, flowers, fruits seeds and nuts, agro-forestry plants, trees, and medicinal plants besides the usual business of growing field crops such as rice, wheat, and green gram for grain purpose and mustard and toria for oilseeds. Such a mosaic of plant and crop species contributes for a better quality of life for the farmers by providing various items of food, fodder and fuel for the farm family (Behera *et al*. 2018). Rearing of cows, goats, etc. along with fish in farm ponds helps balance the diet of the farm family. Such multi-enterprise farming plays a vital role in making the farming system sustainable through different cropping, biodiversity and ecosystem services. Intensification of farming has a clear impact on biodiversity (Amjath-Babu and Kaechele 2015). The process of intensification includes the combined effects of ploughing frequency, fertilizer and pesticides applications, and specialization of production.

The traditional farming systems of India are relatively stable and in equilibrium. The species complexes in traditional systems exemplify the co-existence of plants, humans, draught animals, friendly birds, beneficial insects, pollinators, earth-worms, soil micro-organisms and bio-control agents. Agricultural biodiversity and associated traditional knowledge are essential to the climate change resilience of these landscapes but their roles are largely overlooked by researchers and policy makers (Mijatovic *et al*. 2013). Modern farming systems, which evolved in response to the growing needs of society to ensure food and nutritional security, have progressively replaced traditional farming. More intensification of crops and cropping systems in modern farming has led to a decline in the genetic pool and an erosion of biodiversity, and the links among the components and enterprises are broken causing unsustainability (Dent 1990). It is important that diversity is assured while attaining high production levels and profitability.

Integrated agriculture is an important pathway to nutritional security

Biodiversity is believed to contribute to human nutrition through many pathways, including increasing dietary diversity and quality, enhancing income, enhancing resilience, and providing resilience to climate change. In integrated agriculture systems a variety of produces are produced which helps in family nutrition at the farm and regional level, thus contributing nutrional security. Citing one example, in the integrated agriculture production systems at Delhi a variety of products such as cereals – wheat, maize; pulses – pigeonpea, pea, greengram; oilseeds – mustard, groundnut; Fibre –cotton; Leafy vegetables – raddish, mustard, spinach; vegetables – brinjal, raddish, baby corn, tomato, drumstick; legume vegetables – country bean; spices –ginger, coriander, onion; Fodder –berseem, corn (stover), baby corn (stover), sweet corn (stover); fruits – guava, kinnow, lemon, banana; milk, egg, meat, fish and biogas etc. were produced which helped in the better nutrition of farm family and livelihood of the farmers (Behera *et al.,* 2018).

Integrated agriculture contributes to food safety and ecosystem services

Over 60% of ecosystems of the world over are declining for which ecosystem system services are declining gradually. Integrated agriculture which is based on agro-biodiversity principles has the potentiality to reverse the trend. Agro-biodiversity can be used as a resilient crop production system due to its usefulness in self-sufficiency, erosion control, carbon sequestration, soil fertility build up, drought resistance, functional biodiversity and organic production systems. Agrobiodiversity and its link to ecosystem properties have cultural, intellectual, aesthetic and spiritual values that are important to society. Such a diversity not only serves as strong pillar to sustainability, risk minimisation and climate resilience, but also provides ecosystem service for the farm family to maintain a healthy life by meeting around 70-80% of the farm family requirement for various products including modern form of renewable energy (biogas). The produces are of better quality due to no or minimal use of chemicals (Behera *et al*., 2018). Agricultural intensification through intensive use of fertilizers and mechanization reduces the biodiversity and this made the necessity for integration agriculture which depends less on these modern inputs. Specialization of farms as pure cropping or pure intensive livestock production systems is considered a mainstream path for modernization and development, which creates a negative impact on biodiversity and ecosystem services. For example, in Argentina, the cultivated area of crops increased 45% between 1990 and 2006, while use of fertilizers increased 400% (Gavier-Pizzaro *et al*., 2012). Consequences were loss of habitat heterogeneity, particularly loss of avian diversity and associated ecosystems services that benefit crop production.

Integrated agriculture contributes to system stability and it is the best framework for developing sustainable agricultural system

Integrated agriculture systems provide opportunities to capture ecological interactions among different land use systems to make agricultural ecosystems more efficient at cycling nutrients, preserving natural resources and the environment, improving soil quality, and enhancing biodiversity. Moreover, diversifying agricultural production could utilize labor more efficiently at farm and/or regional scales (Hoagland *et al*., 2010). Monoculture and continuous cropping or rice-wheat and rice-rice systems has resulted in various disadvantages, e.g. degradation of natural resources, build-up of diseases and pests, and decline in factor of productivity (Ayyappan and Arunachalam 2014, Singh 2015). All these have endangered the basic fabric of sustainability in some of the most productive zones of India. Crop-animal systems in Asian agriculture display a wide diversity in cropping patterns, livestock species and use of the resource base. There is evidence of positive and economic benefits from crop-animal inter-actions that promote sustainable agriculture and environmental protection (Devendra 2002). Under the stress of intensive agriculture, environmental degradation has been reported in many economically developed countries from excessive use of high energy inputs, such as fertilizers and pesticides. Use and recycling of locally available inputs and integrating them with the minimum needed quantities of external inputs would enhance the sustainability of the farming process. Use of locally available inputs besides being environmentally friendly can keep production costs within the affordable reach of the peasant farmers. Indigenous technological knowledge has a substantial stake in this process. Integrated agriculture are useful owing to increased diversification, intensification, improved natural resource efficiency and increased productivity, as well as increased sustainability.

Integrated agriculture is renewable agriculture

Everywhere in the world, intensification of agricultural production has been driven by a large use of non-renewable resources that often impair environmental sustainability, as well as by a huge simplification of agricultural systems at all levels of organization, i.e. field, farm, landscape and region. Particularly in industrialized countries, agriculture has become highly specialized in response to political and economic constraints, leading to a large decline in number of farms, despite a large increase in physical and labor productivity. Intensification and specialization of agricultural systems in industrialized countries has come with increasingly negative impacts on the environment (Tilman *et al*., 2002), which is now considered unacceptable by society. Consequences of specialization and increasing labor productivity through simplification of crop management and greater external inputs are water contamination, sinking groundwater levels, rising atmospheric greenhouse gas concentrations, soil erosion and dysfunction, and loss of biodiversity. On the other hand, integrated agriculture explores the natural mechanisms of the farming systems and depends less on external inputs. Integrated agriculture has the inbuilt mechanism of generating bio-resources by recycling the by-product or wastes of one enterprise as input for other enterprise. In an integrated agriculture system at Indian Agriculture Research Institute, New Delhi the requirement of external inputs was drastically reduced through fish-duck, poultry-fish, apiary-crop, dairy-biogas production-crop integration (Behera *et al*., 2018). Renewable energy by production of biogas, solar energy and nutrients in the form of compost/manure are produced in the farm itself for which ecology of the farm is improved. Besides, in a pond-based integrated production systems pond serves as a nutrient and water harvesting structure.

Integrated agriculture: An antidote for intensification?

Reduction of crop diversity within landscape mosaics and within crop rotations due to the disappearance of forage crops and grassland areas reduces the potential attainment of ecosystem services traditionally served by diversified crop–livestock systems, such as improving soil structure, water infiltration, nutrient cycling, soil organic C sequestration, and soil biological diversity; and controlling weed communities, insects, and disease populations. This lack of biogeochemical and ecological controls due to the loss of diversity within cropping

systems has been partly compensated by the use of synthetic fertilizers and pesticides, yet which can unfortunately generate unacceptable loads of pollutants to air, water, soil, and neighbouring native biotic communities. Moreover, biodiversity at a landscape level for a large range of taxa (plants, insects, small animals and birds) within intensive cereal cropping systems is highly dependent on the spatial continuity and diversity of the landscape mosaic. In particular, permanent vegetation within undisturbed fields (i.e. forage and grasslands) plays an important role in landscape biodiversity by controlling meta-population dynamics .

Integrated Agriculture: Principles

"The wise live without injuring nature is as the bee drinks honey without harming the flower" - Lord Buddha. Integrated agriculture is based on the principle of holistic and system approaches to agriculture which comes from the major philosophy of the country that is Vasudhaiva Kutumbakam. Whole world is one family, and all human being and organism live on the earth are member of this family. Its agriculture version also believes in diversity and co-existence linking with ecology and nature. Practising integrated agriculture, we give justice by providing opportunity to others to co-exist with a natural harmony. This has been well recognised and valued by ICAR and Ministry of Agriculture in their demonstration of the same by showcasing the achievements in tableau of 2018 and 2019. In 2018, ICAR showcased its thrust and achievement in the form of Integrated Farming Systems for the development of vast small and marginal farmers of our country. This was more appropriately designed in the context of NEH region. NEH region is rich in its culture and traditions and bio-diversity. In this model, the rich culture was depicted at the background which is important that the existing farming systems of the region must not be tempered. It was demonstrated as a potential tool to double the farm income. During 2019, Kisan Gandhi was presented by ICAR on republic day tableau. The tableau got first prize. It depicted the Gandhi's vision on agriculture. i.e. Integrated agriculture (IA). Gandhi ji believed on non-violent agriculture, which can be interpreted as organic agriculture- not using chemical fertilizer, not destroying the ecosystem and safe environment for the animal so that humanity can get safe agricultural produce for consumption. Other principles on which IA is based are: maximum replacement of off-farm inputs by recycling and efficient utilization of farm wastes and by-products; exploring the natural mechanisms of a farming systems in the way of complementarities of co-existence of various organisms, enterprises, water harvesting and natural resource conservation.

Benefits of Integrated Agriculture (Ia) Systems

The advantages of IA include pooling and sharing of resources/inputs, efficient use of family labour, conservation, preservation and utilization of farm biomass including non-conventional feed and fodder resources, effective use of manure/animal waste, regulation of soil fertility and health, income and employment generation for many people and increase economic resources. It improves space utilization and provides diversified products. IA is a strategy to ensure sustainable use of the natural resources for the benefit of present and future generations. The followings are the important benefits from implementation IA in a region/country.

1. **Productivity:** IA provides an opportunity to increase economic yield per unit area per unit time by virtue of intensification of crop and allied enterprises.
2. **Profitability:** Improves profitability by reducing production costs through recycling wastes and by-products of one enterprise as inputs to other enterprises.
3. **Sustainability:** IA helps for optimal and effective utilization of wastes and by-products of linked components. It gives emphasis for achieving agro-ecological equilibrium through reduced build-up of pests and diseases .
4. **Balanced food:** Components of a varied nature are linked to produce different varieties of products and produce, which serve to provide a balanced diet for the farm family.
5. **Environmental safety:** In an IA, waste materials are effectively recycled by linking appropriate enterprises and components, thus minimizing environment pollution. It is recognized that single enterprise based farming endangers ecological security. For example, burning rice residues is common practice in intensively rice-wheat cropped areas of India (e.g. the Punjab, Haryana, Western Uttar Pradesh), resulting in vast nutrient loss and increasing the concentration of GHG in the atmosphere. Such situations could be avoided by agricultural diversification with the introduction of more enterprises (e.g. animal husbandry) on the farm. Rice straw can be used as animal feed and turned into manure for sustaining soil health. Also, as an IA systems take into account effective resource use and nutrient recycling and makes the farming less dependent on external inputs, it helps minimize environmental pollution occurring due to heavy use of external inputs.
6. **Resource recycling:** Effective recycling of waste materials and by-products (crop residues and livestock wastes) is practiced in an IA systems. Therefore, there is less reliance on outside inputs (e.g. fertilizers, agrochemicals, feeds, energy). This leads to a more stable production system.
7. **Year-round income :** IA provides a flow of money for the farmers throughout the year by way of the sale of a variety of farm produce (e.g. milk, egg, mushroom, vegetables, fruits, food grains).
8. **Risk minimization:** IA provides a stable and sustainable production system through diversified crops and enterprises, which helps in risk minimization and resilience to climate change. Single commodity based agriculture is always endangered by natural hazards such as floods, drought, and disease epidemics. During 1999-2000 in India, many cotton growers in Andhra Pradesh, Maharashtra and

Karnataka committed suicide as their crops were heavily damaged by pests. Adoption of IA systems would help farmers escape such situations and reduce the risk involved in crop failure.

Rural Bio- Entrepreneurship

An entrepreneur is one who creates his own business i.e. a person who organise, operates ad assumes the risk of a business venture. Entrepreneur is an economic agent who plays a vital role in economic development of a country. Rural Bio-entrepreneurship plays a key role for the nation due to its potential for employment and doubling the farmers income. Bio-entrepreneurship plays an important role for inclusive growth by large scale involvement faming communities. Entrepreneurship development is a practice or means to improve entrepreneurial skill among people. In other words it is the inclusion, advancement and grooming of entrepreneurial skills in to a person needed to establish and successfully run an enterprise. Rural bio-entrepreneurship is developing entrepreneurship by taking agricultural activities/enterprises/rural bioresources as a business venture. It may deal with entire value chain like production, processing or distribution. There is ample scope and opportunity for developing entrepreneurship by focussing on bio-resources in the rural areas. The activities – vermincomposting, aquaculture, mushroom cultivation, floriculture (cut flowers), post harvest technology and value addition, production agri- inputs - bio-pesticides, improved hybrid seeds, bio fertilizers, fish seed production, dairy farm with fodder cultivation, broiler goat rearing, bee keeping, production of bio control agent, poultry feed production, coir making, bamboo furniture making and green house technology, custom hiring centre of farm machinery etc. offer opportunities for developing bio-entrepreneurship in rural areas. In recent years, custom hire services and agro clinics also provide very good opportunity for the rural youth for developing entrepreneurship. Many farmers and rural youth have adopted integrated farming fystems (IFS) as business venture and it has proved to be very sustainable and accepted agro-business model.

Entrepreneurship and skill development go together. Govt. of India has launched an important programme “Skill India” in a massive scale which is needful for bio-entrepreneurship development in our country. ICAR in its education programme is giving a lot of focus on entrepreneurship development through student READY (Rural Entrepreneurship Awareness Development Yojana) programme. There is need to develop the skill of the farmers and other stake holders with the objective to empower them for developing entrepreneurship. There is need of developing skills for technical, managerial and entrepreneurial skill of the farmers and rural youth. Similarly, marketing is another integral part of the entrepreneurship. The farmers should be linked for the market. Market demand is important driver for bio-entrepreneurship.

There are self employment avenues can be taken up in rural areas in the agricultural field by innovative farming activities like crop production, seed production; fruits and vegetables, cut flowers, nursery of horticultural crops and livestock breeding farms. Similarly input marketing fertilizer/pesticides/seed production trading, agricultural implement and machinery fabrication and custom hiring services etc. provide very good opportunity for self employment of the agricultural graduates and rural youths. For rural bio entrepreneurship women entrepreneur can play an important role. There are many schemes operating in our country for encouraging and motivating women for entrepreneurship development. Such schemes are - Prime Minister Rojgar Yojana (PMRY), Training Rural Youth for self employment, Integrated Rural Development Programme, Indira Mahila Yojana, Mahila Vikas Nidhi, National banks for Agricultural and Rural Development schemes etc. These schemes are very complementary for Bio-entrepreneurship development in India. At present these has been an institutional shift from extension to entrepreneurship building. Agriculture is increasingly changing to agro-business in rural India. Extension has a large role to understand markets and analyse market trends as agriculture is increasingly turning out to be a business proposition.

Integrated Agriculture Systems: The Road Map

The followings are the important areas for promoting IAS : (i)The systems and interdisciplinary approach; (ii)Targeting research and development in rainfed areas; (iii) Markets , marketing and upgrading value chain; (iv) New extension strategy for 21st century Agriculture; (v)The developmental farm model (DFM); (vi)Energy Self-sufficient Integrated Farming Systems (E-IFS); (vii)Farm Development card (FDC); (viii) Empower farmers and entrepreneurs; and scientist ; (ix) Synergy through collaboration and linkages; and (x) Prescriptive Agriculture.

Conclusion

Today’s agriculture and food systems are deeply rooted from the era of cheap energy and fertilizers, an assumption of static climate, and the ability of entities to ‘externalize’ environmental and social costs. With society currently facing the end of cheap energy and a growing awareness of climate change linked to rising concentrations of greenhouse gases, additional pressures are likely to emerge expanding human population and increasing competition for scarce water supplies. To meet multiple demands, e.g. supporting livelihood, conserving biodiversity, off-setting emissions, adapting to climate change integrated agriculture is very promising. An integrated agriculture system represents multiple crops (cereals, legumes, tree crops, vegetables, etc.) and multiple enterprises (livestock production, fish farming, bee keeping, etc.) on a single farm. Promotion of IA system is important for establishing sustainable agriculture. It provides scope for exploring synergistic interactions of the components of systems, and to enhance resource-use efficiency and recycling of farm by-products. The innovative approaches, DFM, E-IFS, FDC, prescriptive agriculture, synergy through collaboration and linkages and farmers empowerment are the keys to promote integrated agriculture for rural bio-entrepreneurship and nutritional security in Indian.

Table 1. Number and Area of Operational Holdings by Size Group, 2010-11

Category of holdings	Number of holdings			Area			Average size of holdings		
	2000-01*	2005-06*	2010-11*	2000-01*	2005-06*	2010-11*	2000-01*	2005-06*	2010-11*
Marginal (less than 1 hectare)	75408 (62.9)	83694 (64.8)	92826 (67.1)	29814 (18.7)	32026 (20.2)	35908 (22.5)	0.40	0.38	0.39
Small (1-2 hectares)	22695 (18.9)	23930 (18.5)	24779 (17.9)	32139 (20.2)	33101 (20.9)	35244 (22.1)	1.42	1.38	1.42
Semi-medium (2-4 hectares)	14021 (11.7)	14127 (10.9)	13896 (10.0)	38193 (24.0)	37898 (23.9)	37705 (23.6)	2.72	2.68	2.71
Medium (4-10 hectares)	6577 (5.5)	6375 (4.9)	5875 (4.2)	38217 (24.0)	36583 (23.1)	33828 (21.2)	5.81	5.74	5.76
Large (10 hectares and above)	1230 (1.0)	1096 (0.8)	973 (0.7)	21072 (13.2)	18715 (11.8)	16907 (10.6)	17.12	17.08	17.38
All holdings	119931 (100.0)	129222 (100.0)	138348 (100.0)	159436 (100.0)	158323 (100.0)	159592 (100.0)	1.33	1.23	1.15

No. of holdings: ('000), Area operated: ('000 hectares), Average size: (hectares). The figures in parenthesis represent % of total. Source: ASG, 2015.

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ANTIMICROBIAL RESISTANCE - A GLOBAL THREAT

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Antimicrobial drugs were used for treating microbial infections. Antimicrobial resistance had developed as a major threat worldwide, endangering the efficacy of antibiotics and causing a serious risk to global public health. Antimicrobial resistance (AMR) is the abilityof microorganisms to resist the effects of an antimicrobial drug to which it was previouslysensitive (Anderson and Lewis, 1965). Injudicious use of antimicrobial agents by health professionals and the livestockindustry, enhanced global migration and wildlife spread are considered as the major reasons for the emergence of the prevalence of drug-resistant bacterial infections. In addition, the spontaneous evolution, mutation of bacteria, and transfer of plasmids amongthe bacterial species are significant contributors to antimicrobial resistance (http://www.fao.org).

Antimicrobial resistance (AMR) is the ability of microorganisms - bacteria, viruses, fungiand parasites – to resist the effects of an antimicrobial drug to which it was previously sensitive (Bilal Aslam *et al*., 2018). The significant global increase of AMR could be associated with increased use of antibiotics in human and animal clinics and animal production, selection pressure, poor sanitation, wildlife spread, and poor sewage disposal system (www.worldbank.org). In food animals, antibioticsare commonly used in cattle, chicken, and pigs and it is estimated that by 2030 such use will increase up to 67% in the most populated countries of the world (http://ecdc.europa.eu). Numerous organizations, like the Centers for Disease Control and Prevention (CDC), Infectious Diseases Society of America, World Economic Forum, and the World Health Organization(WHO) have declared antibiotic resistance to be a "Global public health concern (Friedman *et al*., 2015). The CDC assessed antibiotic-resistant bacterial infections according to seven factors: clinical impact, economic impact, incidence, 10-year projection of incidence, transmissibility, availability of effective antibiotics, and barriers to prevention (Golkar *et al*., 2014).

Global surveillance programs that monitor resistance in specific bacterial pathogens, such as Mycobacterium tuberculosis and Neisseria gonorrhea, have been developed. In addition, a number of regional surveillance programs have been monitoring AMR in selected geographical areas, such as the Central Asian and Eastern European Surveillanceof Antimicrobial Resistance (CAESAR), the European Antimicrobial Resistance Surveillance Network (EARS-Net) (Holmes *et al*., 2016) and the Latin American Antimicrobial Resistance Surveillance Network (ReLAVRA) (Humphrey *et al*., 2005).

Antimicrobial resistance development

In addition to the mutation in various genes on the chromosomes of the microorganism, exchange of genetic material among microbes plays a vital role in the spread of antibiotic resistance (Holmes *et al*, 2016). Plasmid transmission among bacterial species is the most important phenomenon which may transfer genes of antibiotic resistance to the susceptible bacterialorganism. Antimicrobials may additionally exert a selective pressure to the emergence of resistance (Munita and Moore,2016; Stokes and Gillings,2011). Another important facet known as "One Health" also plays a significant role in the transmission dynamics of antibiotic resistance(Holmes *et al*, 2016). Hence antibiotic resistance is a global health challenge, involving the transfer of bacteria and genes between humans, animals and the environment. Although multiple barriers restrict the flow of both bacteriaand genes, pathogens recurrently acquire new resistance factors from other species, thereby
reducing our ability to prevent and treat bacterial infections (Joakim Larson and Carl-Fredrik Flach, 2021). Injudicious use of antimicrobial growth promoters in farm animals is associated with the transmission of resistance to humans via animal products. Resistant bacteria and mobile genetic elements, plasmids may make their way from animals to humans through various means (Anderson and Lewis, 1965; Humphrey *et al*., 2005). At the community level, food contamination is the most important mode of transmission especially for resistant pathogens of the family Enterobacteriaceae. The influence of the environment on antibiotic resistance is also a concern. In the agriculture sector, microbicides usage might also contribute to antimicrobial resistance development. Nosocomial infections with multi-drug resistant bacteria, such as Acinetobacter baumannii, Enterobacter spp., Klebsiella spp. and Salmonella enterica serovars have beenrecognized in hospitalized dogs, particularly in intensive care units, for many years (Irizarry *et al*., 2016).

Among gram-positive pathogens, a global pandemic of resistant Staphylococcus aureus and Enterococcus species currently poses the biggest threat (CDCP, 2013). Vancomycin-resistantenterococci (VRE) and a growing number of additional pathogens are developing resistance to many common antibiotics (Golkar *et al*., 2014). The global spread of drug resistance among common respiratory pathogens, including Streptococcus pneumoniae and Mycobacteriumtuberculosis, is epidemic. Gram-negative pathogens are particularly worrisome because they are becoming resistant to nearly all the antibiotic drug options available, creating situations reminiscent of the pre-antibiotic era(Rossolini *et al*., 2014).

Hence, AMR is a major concern for the rapid global spread of resistant bacteria that cause common infections and that resist treatment with existing antimicrobial medicines (Francesca Prestinaci *et al*., 2015).

Impact of AMR

Human health impact: In case of resistant infections, patient require longer hospitalization stays as well as more intensive care units (ICUs) and isolation beds areneeded in order to prevent the spread of the infection (Friedman *et al*., 2016). Moreover, the treatment of resistant infections becomes challenging resulting in high fatality rate. Also, the AMRposes a significant health problem for patients with cancer and organ transplantation. Livestock health impact: The increase in resistance to antimicrobials will make treatments on animals ineffective and cause the infections to become more severe (www.fao.org). Economic impact: AMR increases the treatment costs; it is estimated that AMR couldcost from $300 billion to more than $1 trillion annually by 2050 worldwide (www.worldbank.org).

Conclusion

Antimicrobials are very important for the treatment of microbial infections. Hence, tominimize the AMR development, it became essential that the antimicrobial use should beappropriate. Following the correct drug with recommended dose and duration is mandatory. Proper wastage disposal (hospital sewers, community sewers, wastewater treatment plants) would be quite helpful in the prevention of development of AMR. Medicinal plants could be used as an alternative for antimicrobial drugs for producing a better antimicrobial action. With the rise of multiple resistant bacterial strains being isolated across the world, research into natural plants with antibacterial properties have become imperative as an alternative method for the treatment of bacterial infections. Plant derived phytochemical compounds can elicit their antibacterial mechanisms by damaging the bacterial cell membrane, suppressing virulence factors in bacteria, inhibiting bacterial nucleic acid production and preventing bacterial biofilm formation.

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MAINSTREAMING GRAIN ZINC AND IRON CONCENTRATIONS IN CIMMYT WHEAT GERMPLASM

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Abstract
Genetic biofortification through breeding offers sustainable solution to the micronutrient malnutrition problems in the target countries. Great progress has been made in the past decade in transferring alleles for high-zinc (Zn) and iron (Fe) from diverse genetic resources into elite wheat breeding lines. However, the major challenge is to maintain simultaneous and high rates of genetic gains for grain yield and grain Zn to meet the food and nutritional security through the continuous delivery of biofortified varieties that are competitive to replace non-biofortified varieties successfully. Although a few intermediate effect QTL regions are identified for grain Zn, both yield and Zn content are quantitatively inherited. Increased breeding efforts and new approaches are therefore required to combine them in high frequency in CIMMYT's elite germplasm, ensuring that Zn levels are steadily increased to the required levels across the CIMMYT breeding pipelines. The addition of Zn as a core-trait will be achieved through significant acceleration in the breeding cycle, expanding population sizes, extensive Zn phenotyping, yield testing, phenotyping for biotic and abiotic stresses, molecular-assisted selection and genomic selection. While continuing to increase agronomic performance, high Zn alleles has been added as a core-trait. Zn content will be increased in breeding lines annually along with the frequency of elite lines with high Zn that have potential to be released by partners. A genomics assisted "rapid cycle recurrent selection" scheme achieved through rapid generation advancement approaches are expected to enable CIMMYT wheat breeding program to mainstream grain Zn in all the breeding pipelines in about 10 years.

Introduction

Micronutrient deficiency or "hidden hunger" affects more than two billion people globally and is particularly prevalent in the poorest rural communities in developing countries, where people do not have access to and/or can't afford a more nutritious diversified diet. These communities rely on staple foods such as wheat, rice and maize that are sustaining but deficient in vitamin A, iron, iodine and zinc. The deficiencies contribute significantly to the global disease burden, reduce productivity and prevent children from reaching their full potential by impairing physical and cognitive development and increasing their susceptibility to infectious and immunity system-related diseases. Zinc plays a crucial role in controlling gene expression, and its deficiency affects the entire body, including the immune system, metabolism, and development. Lack of dietary zinc is a major public health problem in developing countries, increasing the risk of mortality, stunted growth, diarrhea, and respiratory illnesses in infants and young children, pre-term delivery in pregnant women, and other adverse health outcomes (Black et al., 2013). An estimated 17% of the world's population is at risk of inadequate zinc intake (WHO, 2014).

Several approaches have been developed to alleviate micronutrient deficiencies in human nutrition, in particular diversification of home food production, supplements and fortification. The latter two methods are widely used, although they tend to reach the urban population more easily than the rural population, which is much more affected by malnutrition. A complementary approach is biofortification, or the improvement of the nutritional quality of staple foods, which is cost-effective, has high potential (Bouis, 2002; Bouis et al., 2014), and directly targets rural populations. For over a billion people in South Asia (India, Pakistan, Bangladesh and Nepal) and Ethiopia, our main target region/countries, wheat is an important source of Zn; therefore, the strategy of mainstreaming biofortification in the CIMMYT spring bread wheat breeding program would have a significant impact on a huge number of people who consume varieties derived from CIMMYT breeding research.

Biofortified wheat as a delivery vehicle for Zn

Biofortified wheat has several potential advantages as a delivery vehicle for wheat in South Asian and Ethiopian diets. Much of the wheat produced in both regions is milled in the communities in which it is grown and consumed as whole-wheat flatbreads. The use of whole wheat meal in food products means that more Zn, which is concentrated in the outer aleurone layer, is retained during milling (although phytate, which interferes with Zn absorption, is also higher in the aleurone) (Cakmak et al., 2010). The tendency to consume wheat directly on the farms and in the communities where it is grown means that industrially fortified wheat from large, commercial mills is unlikely to reach poor rural communities. Wheat is one of the main agricultural crops in India and Pakistan, with over 100 and 26 million tons of annual production, respectively(Poole et al., 2021). In these countries, wheat is a major dietary staple and typically consumed as whole meal flour, which is used to prepare a variety of traditional flat breads like *chapatti* and *roti,* often cooked on a hot pan or in the traditional clay oven "tandoor" throughout rural and urban areas(Kumar et al., 2019). Our target population living in rural areas consumes mainly whole meal or high extraction rate flours which, compared to "white" flours produced with a lower extraction rate, contain more Zn-rich grain components. Wheat flour currently contributes more than 40% of Pakistan's daily caloric intake, with per capita wheat consumption averaging around 124 kg per

year, one of the highest in the world(Prospects and Situation, 2021). In India, flour demand is growing steadily but the Indian milling industry is still very fragmented, and almost two-thirds of the flour is produced in small stone mills or "chakki". For these reasons, rural populations will benefit more from biofortified wheat than from industrial fortification with Zn, Fe or other food supplements. CIMMYT-derived wheat varieties, which are rapidly and widely adopted in both India and Pakistan, are an excellent delivery vehicle for high Zn wheat, and the self-pollinated, true-breeding nature of the species means that the trait is likely to be stable for several generations of on-farm seed production.

Progress in the last decade

So far, significant progress has been made in the targeted development and deployment of biofortified wheat with enhanced Zn concentrations (and grain Fe as an added secondary trait, as Fe and Zn are highly correlated), reaching more than a million households with biofortified wheat varieties in South Asia (Velu et al., 2019).Identification of high Zn parents and biofortification breeding began in 2004 at CIMMYT. It has involved transferring high Zn genes from selected landraces, spelt wheat and emmer wheat-derived synthetic wheat, among other genetic resources found to carry higher levels of grain Zn. New sources of high Zn had to be identified, because the variation and absolute content in hexaploid elite wheat lines was too low. The target to increase grain Zn was 8 ppm considering the baseline of 25 ppm in popular varieties grown in South Asia (Velu et al., 2012). Biofortified wheat varieties bred at CIMMYT that produced up to 5% higher yields compared to commercial checks were released in target countries in India, Pakistan, Nepal and Bangladesh which are focus countries for zinc enriched wheat. In 2014, the target for breeding Zn was raised to 12 ppm over currently grown commercial checks, based on a revised estimated recommendation from nutritionists and the identification of low frequency lines, such as 'Zinc Shakti, that possess higher Zn levels.

A decade of research efforts at CIMMYT have shown that there is significant genetic variability for Zn content in wheat germplasm developed through crosses with wild relatives and landraces, indicating that Zn content is amenable to rapid breeding progress. Although a number of QTL with moderate effects on grain Zn have been found in different germplasm sources, the genetic control of the trait appears to be best treated as polygenic. Recent work by CIMMYT has shown that it is a moderately heritable trait (with roughly the same heritability as yield) and that gains of approximately 0.5 ppm in grain Zn content per annum should be possible. Under the previous round of support from HarvestPlus, CIMMYT devoted about 20% of its total breeding effort to its high-Zn wheat pipeline. Selection in Zn-homogenized fields in Mexico is predictive of high grain Zn in South Asia, allowing CIMMYT to identify a first wave of lines with substantially increased Zn content while delivering roughly 90-95% of the agronomic performance of non-biofortified elite lines. However, to remain competitive, the performance of Zn-enhanced lines/varieties must be equal or superior to that of current non-biofortified elite lines/varieties, to ensure that smallholders who produce for both home consumption and the market adopt them. Since both yield and Zn content are invisible polygenic traits, increased breeding efforts and new approaches are required to combine them at high frequency in CIMMYT's elite germplasm, ensuring that Zn levels are steadily increased to the required levels across the CIMMYT breeding pipeline; in other words, "mainstreamed."

Novel tools to accelerate genetic gains

The main focus of the Zn mainstreaming efforts at CIMMYT aimed to close the yield gap of about 6-7% between biofortified and non-biofortified lines and to achieve simultaneous genetic gain for grain yield and high Zn and other necessary traits, thereby fostering adoption of high Zn varieties. When breeding for the additional trait "Zn content", a reduction in gain for yield can only be minimized by making the breeding program more efficient. The simultaneous improvement for grain Zn (and Fe as secondary added trait) and grain yield potential along with other agronomic traits, resistance to diseases, stress tolerance and end-use quality. Since grain Zn, yield potential and several other traits are polygenically inherited, it is likely that only modest simultaneous incremental genetic gains will be achieved in each breeding cycle. We are further optimizing breeding stages, increasing the size of segregating populations, developing advanced lines faster by reducing the cycle time, and piloting a more rapid breeding cycle assisted by genomics. For this reason, A concerted effort in terms of necessary changes within the breeding program implemented to strengthen the culture of continuous improvement in wheat breeding by testing, optimizing and adopting new approaches for increasing breeding efficiency and genetic gain, to significantly increase rates of genetic gain for grain Zn and grain yield simultaneously. As part of the optimization and piloting of breeding for Zn mainstreaming, we applied the "breeder's equation" of:

i) Shorten the breeding cycles that thus increase genetic gain per year. The proposed rapid generation advancement approaches to accelerate the breeding process are the "rapid bulk generation advancement method" (RBGA) aiming for 3-year cycle and the second is the "rapid-cycle, recurrent-selection" breeding scheme (RCRS) aiming at two years breeding scheme (Fig 1). The RBGA and RCRS breeding methods, coupled with genomic selection approaches, would accelerate Zn mainstreaming by reducing the cycle length from the current 4 years to 3 and 2 years, respectively, and resulting advanced lines evaluated in 3 to 4 selection environments of Ciudad Obregon, Mexico for agronomic and nutritional quality traits for selection of parents with more precision.

ii) Selecting parents for recycling more accurately by using breeding values based on phenotypic data and genotypic data from different selection environments for grain yield, grain Zn and Fe, and other

agronomic traits combined with pedigree and genotypic data (GBLUP). Training populations and prediction models specific for biofortification breeding is being generated at CIMMYT. Prediction models developed using novel statistical genetic models (GBLUP) incorporating all the available genomic and phenomic information, being validated and utilized in the RCRS breeding pipeline for selection of potential parents and progenies with high breeding values for Zn and grain yield, to accelerate higher genetic gains for grain Zn and grain yield simultaneously. Several QTL mapping and gene discovery studies at CIMMYT and by other partners identified promising QTL regions and KASP markers are being developed. Promising markers will be used as fixed effects, in addition to thousands of markers used for the genomic prediction pipeline, to select potential parents and progenies.

iii) Optimized selection index estimates for grain yield and grain Zn for parental selection and designing better crosses to achieve higher rates of genetic gain. In addition, selection of potential parents for new crosses using genomic estimated breeding values and use of a selection index approach by specifying appropriate economic weights of traits such as Zn versus yield. Secondly, which traits should be included in the index such as "must-have" traits, and what are the genetic correlation and variance components to be included in the selection index matrix. Interestingly, a strong positive genetic correlation (approximately 0.7) between Fe and Zn content across the CIMMYT wheat germplasm shows that, for every genetic standard deviation (GSD) increase in Zn content achieved through breeding, Fe content will be increased by approximately 0.5 GSD. Because screening for Fe is much more difficult than screening for Zn (Fe screening is complicated by contaminant Fe from, e.g., soil or threshing equipment) and high-throughput methods such as XRF do not yet have the sensitivity to accurately measure index elements for Al and Ti contamination, it is likely that a focus on Zn improvement will prove to be the most efficient way of achieving simultaneous gains in Fe content. Additionally, since the bioavailability of Fe is smaller than Zn bioavailability, Fe must be increased to significantly higher levels to reach target nutritional levels and achieve measurable impacts on human health.

iv) Robust phenotyping of main breeding pipeline bread wheat (BW) materials for Zn and Fe from three environments for Stage 2 lines showed large variation for grain Fe and Zn with identification of superior lines with high Zn and grain yield and other agronomic traits (Fig 2).

Fig. 1. The proposed Rapid Bulk Generation Advance breeding scheme

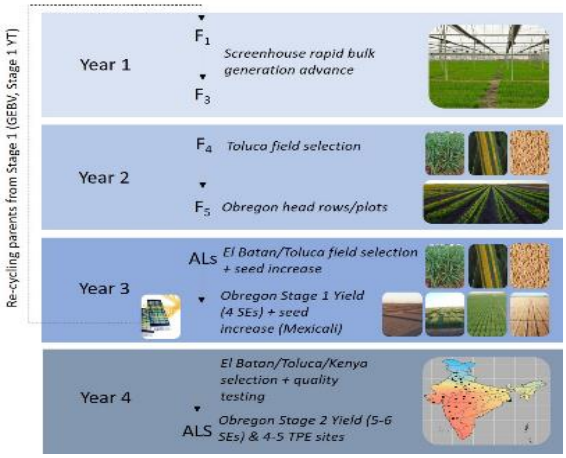
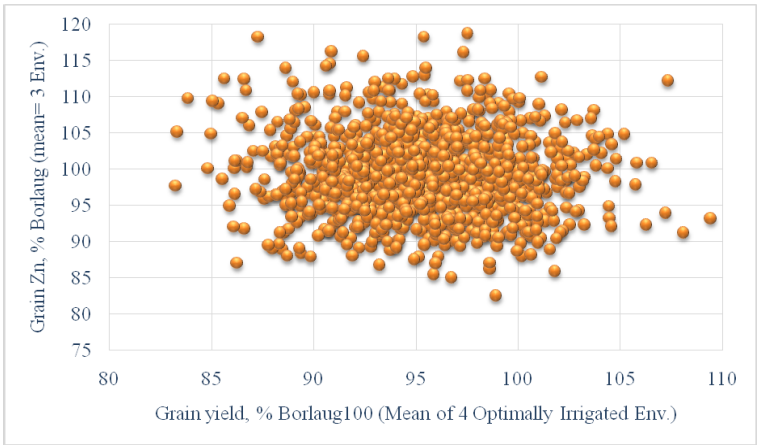


Fig. 2. Grain yield and Grain Zn distributions for 1,120 entries in Stage 2 Yi



Conclusion

Utilizing all these model tools and methods, the Zn mainstreaming objectives will be realized within 10 years of time where 75% of all elite spring wheat lines developed by CIMMYT are enriched for Zn; that is, they contain an additional 12 ppm (40% higher) more grain Zn content than currently grown cultivars, with the baseline Zn value of 25 ppm." This is being achieved by increasing annually about 20% of crosses in all the bread wheat breeding pipelines involved at least one high Zn elite parent, whereas for the Zn pipeline aimed at increasing grain Zn levels while maintaining competitive yield levels as a population improvement approach, Eventually, all the elite high Zn lines sent to target countries (South Asia and Ethiopia) for potential variety releases will meet the full Zn target.

Acknowledgment

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SUSTAINING THE CROP PRODUCTIVITY WITH WATER AND NUTRIENT MANAGEMENT STRATEGIES UNDER CHANGING CLIMATE SCENARIO

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The agricultural scenario of the Kerala state is unique, characterized by diversity of crops and multiplicity of cropping situations. However, the low crop productivity associated with high production costs is a great concern in Kerala's agriculture. Additional stresses come from climate disruption, variability and change, resulting in impacts on water availability and temperature regimes over time and space. This paper describes about the Best Management Practices (BMPs) available. To assess climate change related impacts on crop suitability and agro-ecologically attainable yield, the study applied the Agro Ecological Zonation (AEZ) methodology, which is jointly developed by Food and Agriculture Organization (FAO) and the International Institute for Applied Systems Analysis (IIASA). Range of scenarios for crop productivity for years 2041–2070 (2050s) and 2071–2100 (2080s) using the climatic condition based on four RCPs(2.6, 4.5, 6.0, and 8.5) adopted by the Intergovernmental Panel on Climate Change (IPCC) were generated using AEZ simulation modeling. Major crops grown in Kerala are coconut, arecanut, rubber, black pepper, coffee, tea, cardamom, paddy, tapioca, cashew andvegetables.The AEZ analysis for these crops showed that for ensemble mean of RCM outputs, under rain-fed conditions, yields of banana, arecanut, rubber, coffee, and black pepper show declines between 2.89 and 86.18 %. However, coconut and rice, under rain-fed conditions, show very minor increases of 3.17 and 0.99 % respectively. Under irrigated conditions, yields of coconut, arecanut, coffee, and black pepper show a decline between 3.83 and 86.18 %. However, if we look at all the models and different RCPs,results show that with few exceptions in most of the cases yields tend to decline with climate change. In addition, the assessments for soil nutrient flow were carried out to evaluate the soil fertility status and nutrient budgeting of major crops/cropping systems at different spatial scales (plot, farm, and district level) using NUTMON-Toolbox. Results exhibited a trend of depletion of N and K from soil reserve, whereas, P was positive, indicating the need for carefully redefining N and K management strategies. These results suggest that there is an urgent need to return to water and nutrient efficient crops and assure best possible use of scarce water and precious soil nutrients resources. Drip fertigation was chosen as one of the BMPs and it was demonstrated across Kerala. Results from the project showed that the application of nutrients through drip fertigation improved the crop yield of all the demonstration plots and the increase in yield over control ranged from 13 to 317%. Drip fertigation resulted in improvement of water and nutrient use efficiency and maintenance of soil health. Benefit-Cost ratio (BC) of drip fertigation worked out under the project ranges from 2.05 to 3.50 for selected crops. Besides, strategies needs to be adopted for improving the water and nutrient use efficiency were also discussed. Investments in climate smart agriculture, micro-irrigation practices especially drip fertigation, improved water conservation practices, development and management of natural resources through watershed and afforestation activities, conservation of crop biodiversity, etc. needs to be given focus to improve the production from farms.

Continuous intensive agriculture without adequate attention on rational soil management poses serious threat to the sustainability of agro-ecosystems. One of the consequences of irrational soil management under intensive cropping is the decline in soil fertility. This nutrient mining does not get the same public attention as droughts, pest infestation *etc.*, since it is a gradual process and not associated with catastrophes and mass starvation and therefore largely invisible (Surendran and Murugappan, 2006). Also pertinent is that many soil test methods do not readily reveal nutrient mining because the available fraction extracted is buffered well by supply from other nutrient pools, as is often seen with K availability in some high CEC soils. The change in soil nutrient stocks over time has to be measured in order to quantify the extent of nutrient mining and also to provide an early caveat on adverse trends in nutrient inflows and outflows from the farm (Smaling et al., 1993; Stoorvogel et al., 1993). Crop yields harvested with the present level of fertilizer recommendations and the yields harvested in many fertilizer response experiments with higher levels than the present level have unequivocally revealed that the fertilizer optima calculated in present day research are erroneous and in many cases sub-optimal.

This paper envisages a wholesome approach in different phases covering all the above aspects in order to ensure real optimum character to these fertilizer optima and sustainability to agro-production systems that are vital to break the crop yield barrier both in Kerala and Tamil Nadu. Even though the study was carried in both the states , here we are discussing about the state of Kerala only. Kerala experiences a humid tropical climate, characterized by the presence of heavy rainfall, high relative humidity, alternate wet and dry periods, abundant sunshine and high ambient temperature. It is a leading producer of commercial agricultural commodities in the country. Major crops grown are coconut, arecanut, oil palm, rubber, coffee, tea, cardamom, paddy, tapioca, cashew etc. The agricultural scenario of Kerala state is unique, characterized by diversity of crops and multiplicity of cropping situations. However, productivity is very low when compared to other Indian states or international averages. Low fertile lateritic soils, nature of topography with undulating terrain, coupled with high intensity rainfall leads to top fertile soil loss through severe erosion and nutrient loss through leaching, which

might have been one of the contributing factors for low productivity. There are also other possible reasons for this low productivity. However, at this juncture, the low yield of crops associated with high cost of production is a great concern in Kerala's agriculture. Natural resource management issues are inherently complex since they involve the ecological cycles, hydrological cycles, climate, animals, plants, geography etc. All these are dynamic and interrelated. Additional stresses come from climate variability and climate change, resulting in impacts on water availability in time and space, potentially increasing rainfall variability, resulting in more powerful monsoons and more frequent floods and droughts, and changing the water requirements, climatic suitability and yield of crops as well as the incidence of pests and diseases. Productivity of almost all the crops except rubber is lower in Kerala compared to other Indian states and a sustainable solution needs to be evolved. In this background, DST-TIFAC sponsored a project to CWRDM under India – IIASA program on "Evaluation of soil nutrient budgets at field, farm and regional level in humid tropics of Kerala and development of a model for management of soil health" to assess the soil fertility status and nutrient balance / budgeting of major crops /cropping systems at different spatial scales (plot, farm and district level) in Kerala and come out with a Decision Support System (DSS) for managing soil fertility and sustaining the crop productivity. This was done in a holistic manner through soil fertility assessment in terms of nutrient stocks / flows as an individual crop / cropping system / mixed crop and farm as a whole through field experiments and also at a district / /regional level to know about the status of their soils and the strategies needed to sustain the fertility, besides exploring the possibilities for increasing the crop productivity in a environmentally sustainable way.

Field experiments results showed that 125 % of recommended dose of NPK fertilizers recorded the highest yield of 7685.9 kg $ha^{-1}$and this treatment significantly improved the yield, when compared to the other treatments. The results indicated that the soil loss ranged between 0.0 and 14.44 t ha^{-1} for different rainfall events of low to high. Results on mean quantity of soil loss in relation to the rainfall revealed that the quantity of soil loss was high under high rainfall events and was low in the case of low rainfall events. The correlation was significant with r value of 0.90 and R^2 value of 0.81. Data collected from the field experiment with respect to all inflows and outflows are being compared using *transfer functions or* in-built regression equations of NUTMON-model by linking nutrient input with rainfall for calibration and validation (Vlaming et al., 2001). The observed and predicted values of soil loss using NUTMON model is matching in most of the cases. But in a few cases, the model prediction is not similar to the observed values and hence, under such cases, the other environmental variables such as rainfall intensity, preceding soil moisture and crop canopy cover are being analyzed to eliminate the error percentage. Another experiment was also conducted to assess the runoff, soil loss and subsequent nutrient losses during the cropping period from the runoff plots, similar to the previous experiment. Besides, nutrient lost through leaching also has been assessed using soil water samplers. In addition, as management strategies, two treatments were included as 1. Contour trenches of 50*50*50 cm across the slope and 2. Growing pineapple as strip crop across the slope. The observed and predicted values of soil loss using NUTMON model is matching in most of the cases. Based on the two experimental data, the model was validated.

For regional level, Kozhikode district has been chosen and 20 farms have been selected covering all the agro ecological units. The result revealed that majority of the farms were low or medium in N and K. Soil available N is under low level in all the selected 20 farms. Soil available K was medium in 12 farms and low in 8 farms. In the case of P, 15 farms recorded high P values, whereas, only 5 farms recorded low P content. Nutrient budgeting was also done for individual farms and the flow diagram will help us to identify where the nutrients are flowing out from the farm. The Soil Health Cards have been distributed to these farmers with fertilizer recommendation for several crops. Collected data from all these farms were fed into the data processing module of NUTMON and the nutrient balances were computed using the NUTMON for regional level nutrient budgeting. The results showed that in majority of the farms, N and K were in negative balance whereas P was positive confirming that the existing nutrient management practices are not a sustainable one in the long run. Similar is the case for Kozhikode district as a whole. The per-hectare N and K balances was found to be –9.5 for N and -17.2 for K in kg ha^{-1} yr^{-1+}, whereas P registered a positive balance (+ 7.4 kg $ha^{-1}yr^{-1}$) in Kozhikode district. In a nutshell, NUTMON-Toolbox at different spatial scales (*viz*., micro (plot), meso (farm) and macro (district/ regional levels) exhibited a trend of depletion of N and K from soil reserve, whereas P was positive, indicating the need for carefully redefining N and K management strategies.

A decision support system (DSS) was developed as an output by combining all the data bases generated from the project, apart from using all available secondary data sources. The DSS viz., CWRDM- Integrated Crop Nutrient Management Software (CWRDM- ICNMS) will help in generating nutrient recommendation for most of the crops in Kerala by considering all the inflows and outflows from the farm. Decision Support Systems (DSS) CWRDM -ICNMS will serve as a tool to identify the depletion of nutrients and will help to suggest the management options using a systematic approach. A documentation of climate change and the increasing climatic variability with future projections for the crops of Kozhikode have also been compiled in this study. In addition, increase in temperature, uncertainty in rainfall and increase in carbon fertilization has been studied in detail and about their impacts on soil and water conditions and a focus has been given on its influence on crop productivity under humid tropical conditions in the current study. To overcome the negative impacts of climate change, apart from the developed DSS of nutrient management, several other adaptation strategies needs to be focused on to improve or at least sustain the productivity (Surendran *et al.,* 2016). Brief descriptions of the adaptation options, which potentially can reduce the vulnerability of agriculture to the effects of climate change,

are also discussed. The output obtained from these works are a cost effective, eco-friendly conservation and management technology for higher input use efficiency, agricultural productivity & profitability without deteriorating natural resources for the entire farming community in Kerala and Tamil Nadu.

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CURRENT STATUS OF AGRI AI APP FOR ONFARM DETECTION PEST AND DISEASE

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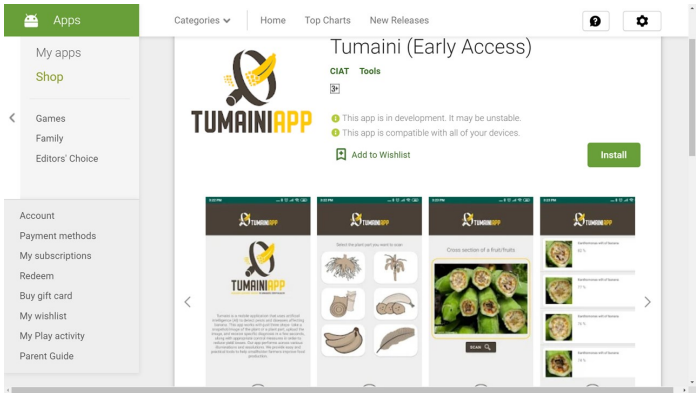
Worldwide, numerous diseases and pests affect theproduction of Agriculture crop plants. Novel and rapid methods for the timely detection of pests and diseases can/will guide surveillance strategies and have control measures applied in a more targeted and timely manner. As Artificial Intelligence (AI) has been successfully applied in various fields, it has freshly moved in the domain of just-in-time crop disease detection. In this paper, we are reporting our experience on testing the Tumaini AI-powered app to detect major Musa pest and diseases in banana landscapes in Tamil Nadu (TN), Southern India and also to sharing our experience on testing accuracy and application of AI technology in Agriculture.

Banana crops are prone to damage by several types of pests and diseases. Once the pest or disease afflicting a crop is identified, swift and targeted action can reduce the extent of outbreaks and potentially save entire harvests.

CGIAR researchers at the Alliance of Bioversity International and CIAT have developed a digital tool to help farmers better protect their banana crops. The tool is a mobile application that merges expertise on banana genetic resources with artificial intelligence to quickly identify common afflictions that threaten bananas, allowing farmers and extension workers to act quickly and save their crops.

The smartphone app, called Tumaini – which means "hope" in Swahili – helps banana farmers scan plants for signs of five major diseases and one common pest. Farmers use the app to upload a photo of an affected crop, which is then scanned for symptoms of pests and diseases using image-recognition technology, drawing on a dataset of more than 50,000 images. Tumaini records the data, including geographic location, and feeds it into the database. The app then provides a diagnosis and recommends steps to address the affliction.



The Tumaini app has so far demonstrated a 90% success rate in detecting pests and diseases

Other existing crop disease detection methods focus primarily on leaf symptoms and can only accurately function when pictures contain detached leaves on a plain background. The novelty of Tumaini is that it can detect symptoms on any part of the crop – including the fruit, bunch or plant – and can read low-quality images, even those containing background noise, like other plants or leaves, to maximize accuracy.

Tested in Colombia, the Democratic Republic of the Congo, India, Benin, China, and Uganda, the Tumaini app has so far demonstrated a 90% success rate in detecting pests and diseases. The work is a step toward creating a satellite-powered, globally connected network to control disease and pest outbreaks, say the researchers who developed the technology.

Research on the app was published in 2019 in the journal *Plant Methods* and became one of the year's most successful research papers for Bioversity International and CIAT. It also generated significant media attention and sparked inquiries from industry stakeholders regarding possibilities to expand the use of the app. To date, some 3,000 farmers are using the app in the field, with numbers climbing after a second version released in 2020 allowed for offline use.

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GENETIC VARIABILITY STUDIES IN FLORAL ORGANS RELATED TRAITS IN MEDICINAL LAND RACES AND SEGREGANTS IN RICE (*ORYZA SATIVA* L.)

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Abstract

Morphological characteristics of different floret traits is considered as greater impotance in rice to increase of self pollination for reliable seed production. Normally the florets of rice are adichogamous, most of the florets are self-pollinated at the time of floret opening. Synchrony between floret opening and anther dehiscence may contribute to the high rate of self-pollination. However, the synchronization is not always perfect and some rice florets hybridize naturally. The rate of natural hybridization varies among varieties of rice, suggesting that there is a difference in the reliability of self-pollination among varieties. Subsequent cytological features in the development of anther, pollen grains and ovary of rice have much in common with anther and pollen development biology of other members of gramineae. The experimental material consisted of four traditionally and nutrient rich medicinal land races and two improved high yielding varieties along with selected F_2 segregants were used in randomized block design during kharif 2012-2013. Among them medicinal land races Kavuni recorded hightest significant mean value for anther breadth, stigma length, stigma breadth, style length, style breadth and pollen fertility. Higher values of PCV and GCV were recorded for stigma breadth followed by ovary breadth. All the characters exhibited high heritability. High heritability and genetic advance were observed for stigma breadth, ovary breadth, style length, anther breadth, ovary length, anther length and style breadth revealed the role of additive gene action. This paper reports a correlation between morphological characteristics of floral organs and the reliability of self-pollination in rice. Understanding these processes will not only contribute greatly to the basic knowledge of crop development biology, but also to the development of new nutritional varieties for rice breeding in the future.

Key words: Genetic variability, PCV, GCV, Heritability, Genetic advance, Gene action, Self pollination, Anther dehiscence, Anther, stigma, *Oryza sativa*, Rice, Floral traits.

Introduction

Rice (Oryza sativa) is the world's major staple food and manipulation of pollen fertility is particularly important for the demands to increase rice grain yield. Besides the food security, under nutrition and malnutrition is the raising problem in the developing countries. As rice being a staple food, even a small increase in nutritive content in rice would have a significant impact on human health. Conventional biofortification (breeding staple food crops with high nutritive content) of cultivated varieties using nutritive rich landraces of rice can serve the purpose. Medicinal landraces like Navara is highly nutritive and are rich in minerals like potassium, sodium, calcium, micronutrients *viz.*, iron and zinc. It also contains higher proteins, carbohydrates and vitamins like thiamine, riboflavin and niacin etc. Though there is no scientific data on the medicinal properties, they are being used in ayurveda in treating diseases like arthritis, cervical spondylitis, muscle wasting, skin diseases and neurological problems (Deepa *et al*., 2008). Many high yielding modern rice varieties, some landraces are still popular in farmer's fields due to their adaptability to different agro climatic conditions, unique characteristics and special uses. Biofortification of cultivated high yielding varieties with these landraces with conventional method decrease the hunger of malnutrition. Microsporogenesis and male gametogenesis are essential for the alternating life cycle of flowering plants between diploid sporophyte and haploid gametophyte generations. Towards a better understanding of the mechanisms controlling rice male reproductive development, we describe here the cytological studies. The rice inflorescence architecture is quite different from those major cereal crops, in addition rice florets have an asymmetric structure with five types of floral organs with characteric numbers, one lemma and one palea in the first whorl, two lodicules in the second whorl, six stamens in the third whorl and one pistil in the innermost whorl. Rice is basically an autogamous plant propagating through seeds produced by self pollination. Fertilization occurs in a spikelets which has six anthers with more than 1,000 pollen grains in each and an ovule with a branched stigma. Immediately after the spikelet open at flowering, pollen is dispersed and germinates on the surface of the stigma. Each rice flower contains both male and female parts, which allow each flower to pollinate itself without the need for other flowers, or other rice plants. Breeding for high-yielding rice is one of the pragmatic solution in addressing a food shortage problem that is caused by a marked increase in the global population coupled with decreasing trend in available land and limited water for agriculture (Shanti *et al*., 2010). Nowdays traditional land races rich in nutrition introgressed with high yielding cultivated rice varieties of hybrid rice plays a role in successful utilization. The study revealed a wide variation for several phenotypic floral traits contributing to the seed production efficiency of nutrionally rich rice, such as pollen fertility and morphological traits of floret such as length and breadth of anther, stigma, style and ovary. Among them anther length is especially emphasized as a major component in increasing pollination and seed set (Keto and Namai, 1987). Insight into the characteristics of florets that control the reliability of self-pollination may help breeders to improve the tolerance of florets to such temperature stresses (Matsui *et al*., 2000b).

Materials and Methods

The experimental material consisted of four medicinal landraces of rice having superior grain quality with tall stature and low yielder (Navara, Kavuni, Veeradangan and Kathanellu). and two improved semi-dwarf

high yielding varieties with medium grain quality (ADT 43, TPS4) along with selected F_2 segregants employed for three replication in Randomized Block Design by adopting a spacing of 30 x 10 cm at Department of Plant Breeding and Genetics, Agricultural College and Research Institute, Madurai during Kharif 2012-2013. All the agronomical practices and plant protection measures were followed as per recommendations. Genetics of floral traits was worked out through standard methodologies.

Microscopic observations

The dissection of spikelets was done using a stereomicroscope and images were taken with DP70 digital camera floral traits were measurement using biowizard 10.0 version software. Using stereomicroscope to measure (length and breadth of anther, stigma, style and ovary) and correlated based on mean data. In each variety, 15 florets on primary branches were sampled about 3 h after anthesis. Florets closed about 1 h after they began to open. Spikelets collected with minimum disturbance were used to record the floral traits. Three florets are selected at random to measured the length and breadth of the anther, stigma, style, ovary using standard procedure. The length and breadth of the stigma was measured from tip of the stigma to the base of both stigma branches and the mean was taken for analysis. The area was calculated using length and breadth of floral traits was measured and expressed in mm^2. Measurements were repeated more than three times for each land race and segregating generations of rice variety compared with standard check during the flowering period.

Result and Discussion

The estimates on genotypic co-efficient of variation, phenotypic co-efficient of variation, heritability and genetic advance as per cent of mean for the traits under study are furnished (Table 1). Genetic variability analyses revealed narrow difference between phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) for all the characters indicating the reliability of PCV as a measure of GCV. In general the PCV were higher than the corresponding GCV (Table 3) and GCV provides a mean to study the genetic variability generated in floral traits.

High value of phenotypic coefficient of variation and genotypic coefficient of variation show that the genotypes exhibit much variation among themselves with respect to these characters. Further high phenotypic coefficient of variation and genotypic coefficient of variation for any characters indicated more scope for selection. The wide variation was observed for the characters stigma length (0.685 to 0.933 mm) and percentage of pollen fertility (86.83 to 94.81 %).

The values for genotypic co-efficients of variation ranged from pollen fertility (2.47 %) to stigma breadth (33.12 %). The high GCV was observed for stigma breadth (33.12 mm) and ovary breadth (20.99 mm). Moderate GCV was observed for style length (18.83 mm), anther breadth (17.91 mm), ovary length (17.77 mm), anther length (10.55 mm) and style breadth (10.14 mm). The lowest GCV was recorded for stigma length (9.29 mm) and pollen fertility (2.47 %).

The values for PCV ranged from pollen fertility (3.15 %) to stigma breadth (33.23 mm). The highest magnitude of phenotypic co-efficient of variation was observed for stigma breadth (33.23 mm) and ovary breadth (21.17 mm). Moderate PCV was observed for style length (18.93 mm), anther breadth (17.98 mm), ovary length (17.96 mm), anther length (10.75 mm) and style breadth (10.52 mm). The lowest PCV was recorded for stigma length (9.64 mm) and pollen fertility (3.15 %). Those results are in conformity with those of by Sheeba *et al*., 2006.

High heritability coupled with low genetic advance, low heritability with high genetic advance or low heritability and low genetic advance offer less scope for selection, as they were more influenced by environment and accounted for non-additive gene effects. High heritability coupled with high genetic advance is indicative of greater proportion of additive genetic variance and consequently a high genetic gain is expected from selection (Singh and Rai 1981). The characters having high heritability with low genetic advance as per cent of mean appeared to be controlled by non-additive gene action and selection for such characters may not be effective (Singh and Singh 2007). The genotypes recorded high heritability values for all the characters under study. The presence of high heritability indicates that those characters are least influenced by environment.

As heritability in broad sense includes both additive and epistatic gene effects, it will be reliable only if accompanied by high genetic advance. Genetic advance as per cent of mean ranged from pollen fertility (3.99 %) to stigma breadth (67.99 mm) studies are presented in . Stigma breadth (67.99 mm) recorded the highest genetic advance. Moderate genetic advance was recorded by ovary breadth (42.89 mm), style breadth (38.57 mm), anther breadth (36.72 mm) and ovary length (36.24 mm). The lowest genetic advance was recorded by anther length (21.36 mm), style breadth (20.12 mm), stigma length (18.44 mm) and pollen fertility (3.99 %). High genetic advance indicated that these characters are governed by additive genes and selection will be rewarding for improvement of these traits. According to Jhonson *et al*. (1995), high heritability and genetic advance for a character would indicate the predominance of additive gene action such trait is likely to respond effectively to phenotypic selection. Among the lines for floral traits studied the cultivated high yielding varieties (ADT 43, TPS 4) and the medicinal land races (Kavuni, Navara, Kathanellu and Veeradangan) along with selected F_2 segregants (ADT 43 x Navara and TPS 4 x Kathanellu) had the high significant mean performance and genetic variability. The present results also suggest that length and breadth of anther, stigma, style and ovary is related to increase the number of pollen grains deposited on the stigmata ultimately to increase the productivity. The genetic improvement in rice floral traits is possible through selection exercised for reliable self pollination those characters which showed high values of phenotypic coefficient of variation and genotypic coefficient of

variation, heritability and genetic advance. However, characters predominantly controlled by additive gene action would be amenable to conventional breeding methods (Roy *et al*. 2012). This will provide an opportunity to select better recombinants for various characters and thereby creating large variability for these characters to increase the yield in the future generations.

Table 1. Estimates of genetic variability for floral traits in rice

Traits	Range	Mean	GCV (%)	PCV (%)	h^2 (%) (broad sense)	GA as per cent of mean
Anther length (mm)	1.145-1.591	1.3747	10.55	10.75	96.42	21.36
Anther breadth (mm)	0.270-0.454	0.2055	17.91	17.98	99.13	36.72
Stigma length (mm)	0.685-0.933	0.8204	9.29	9.64	92.32	18.44
Stigma breadth (mm)	0.100-0.278	0.2055	33.12	33.23	98.32	67.99
Style length (mm)	0.336-0.589	0.4466	18.83	18.93	98.89	38.57
Style breadth (mm)	0.073-0.094	0.0822	10.14	10.52	92.84	20.12
Ovary length (mm)	0.323-0.533	0.432	17.77	17.96	97.97	36.24
Ovary breadth (mm)	0.073-0.334	0.2766	20.99	21.17	98.38	42.89
Pollen fertility (%)	86.83-94.81	91.2767	2.47	3.15	61.60	3.99

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PILOT STUDY ON USAGE OF RO REJECTS IN AGRICULTURE BY SCREENING SELECTED VARIETIES FOR ITS SALINITY TOLERANCE

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Abstract

Land and water are the two most important natural resources for agricultural development and economic advancement of any country. With worldwide concerns about water scarcity, agriculture is under pressure to improve water management and explore available options to match supply and demand. In the coastal areas of India, scarcity of drinking water is acute as freshwater aquifers are not available at suitable depths and surface / ground water is highly saline. Desalination is a technical option to increase the availability of freshwater both in coastal areas with limited resources and in areas where brackish waters – such as saline groundwater, drainage water and treated wastewater – are available. Water desalination is a well-established technology mainly for drinking-water supply in water scarce regions. However the major issue with these plants, are its rejects (ie, water water – which is highly concentrated with salts and other inorganic materials). Using these rejected waste water, as irrigation water for agriculture will be a viable alternative in water scarce areas. With this background a study has been conducted to screen selected crops for its feasibility to use saline water for irrigation. In this study, we screened nine different varieties of rice and few selected crops [(Vigna unguiculata (Cow Pea – Climbing Variety), Vigna unguiculata (Cow Pea – Bush Variety), Pisum sativum (Long pea)] for the salt tolerance using different salinity level. Germination studies revealed that all the nine varieties survived upto 2500 ppm salinity solution and at 5000 and 10,000 ppm the germination rate differed significantly between the varieties. Among the varieties Kala, Tirchy I, Vysakh, Seeraka Samba performed better in terms of its plant morphological and its biochemical parameters in relation to salinity tolerance. Salinity caused significant effect on plant height, leaf area, MSI and RWC. Salt tolerance is required for the sustained plant growth in the soil, since salinity injury in rice is caused by both osmotic imbalance and accumulation of chloride (Cl) ions. Future studies on this aspects will greatly contribute to the evaluation and breeding of salt tolerant *varieties*.

Key words : Salinity, RO Rejects, Rice varieties, RWC, MSI

Introduction

Water conservation is becoming increasingly vital as the population growth and drought conditions become more widespread and impactful. Agriculture has been affecting a major cause of water scarcity, since non-availability of sufficient water for irrigation. So now, RO Rejects from RO plants (for purification of water for drinking) can help farmers in coastal areas by irrigating RO Rejects to various salt tolerant crops. Using water efficiently can significantly impact farmers' profits and contribute to the global mission to preserve water. With a low per capita availability of land and water in India compared to other countries, enhancing agricultural productivity has become essential to meet the demands for ever growing population. Due to various reasons both these resources are being constantly under pressure and require a holistic approach to sustain the productivity of agricultural crops. Agriculture is by far the largest (81 %) water consumer in India and hence more efficient use of water in agriculture needs to be the top most priority. Water input per unit irrigated area will have to be reduced in response to water scarcity and environmental concerns. With worldwide concerns about water scarcity, agriculture is under pressure to improve water management and explore available options to match supply and demand. In the coastal areas of India, scarcity of drinking water is acute as freshwater aquifers are not available at suitable depths and surface / ground water is highly saline. Households are mainly dependent on rainwater harvesting, pond sand filters and pond water for drinking purposes. In such areas supplying of fresh drinking water using desalinization plants will be boon to the coastal communities. Desalination is a technical option to increase the availability of freshwater both in coastal areas with limited resources and in areas where brackish waters – such as saline groundwater, drainage water and treated wastewater – are available. Desalinated water can also be crucial in emergency situations where water sources have been polluted by saline incursions. Water desalination is a well-established technology mainly for drinking-water supply in water scarce regions. However the major issue with these plants, are its rejects (ie, water water – which is highly concentrated with salts and other inorganic materials). Using these rejected waste water, as irrigation water for agriculture will be a viable alternative in water scarce areas. With this background a study has been conducted to screen selected crops for its feasibility to use saline water for irrigation.

Objectives

- Identifying the suitable salt tolerant crops based on screening and pot culture experiment
- To assess the influence of saline water (RO Rejects) irrigation on plants and sustainability in crop production

Materials And Methods

The experiment was carried out at Land and Water Management (Agriculture) laboratory at CWRDM. The treatments tried are Normal Irrigation water (Tap water as control), Saline water of 1000 ppm, 2500 ppm, 5000 ppm and 10,000 ppm, respectively. Saline water solutions are prepared from NaCl salts as per the standard procedures for the required concentrations. Nine varieties of rice (viz., Uma, RNR, Kala, Tirchy I, Vysakh,

Seeraka Samba, C Ponni, Jyothi) were used for screening the salinity tolerance. The moisture content of seeds was determined on an oven-dry basis, and a representative sample of seeds from the same group was used in this study.

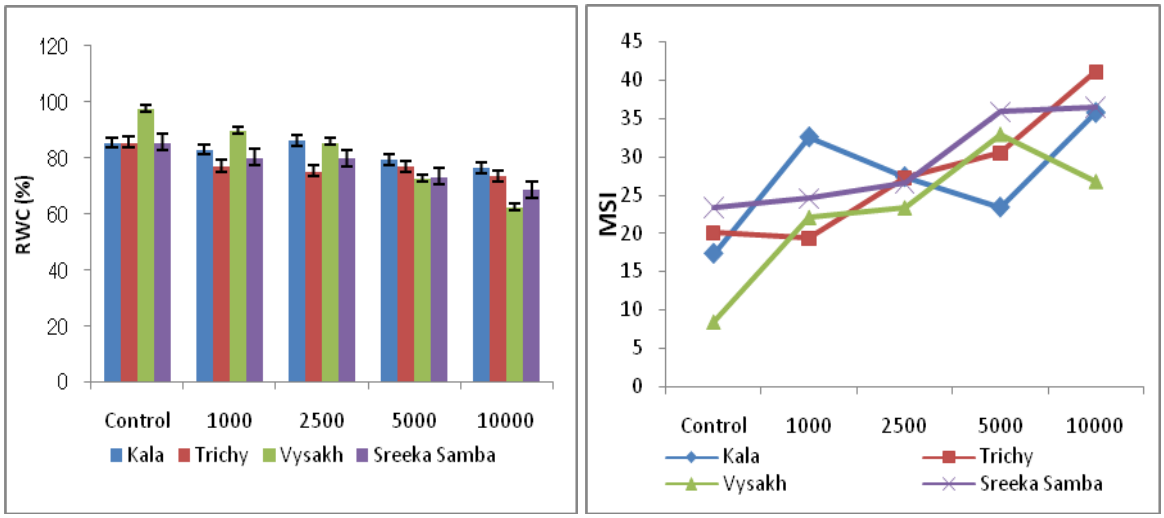
In addition, three varities of pea seeds (Vigna unguiculata (Cow Pea – Climbing Variety), Vigna unguiculata (Cow Pea – Bush Variety), Pisum sativum (Long pea) were also used for screening experiment of salinity tolerance. The seeds were germinated in petriplates and then transplanted to trays (the roots being passed through holes beneath the trays) which were dipped in different concentrations of saline solutions as that of the case in rice.The experiment was carried out under laboratory conditions. Petri dishes with tissue paper and tissue paper alone were used as germinators. The petri dishes were examined each day and the numbers of seeds germinated i.e. with testa split and root showing were noted. Similarly another set of pot culture experiment was conducted with the same varieties.

In the pot culture experiment, crop biometric characteristics such as Plant height, Number of leaves, leaf length, leaf width were measured by adopting standard procedures. In addition, Relative Water Content (RWC), Membrane Stability Index (MSI), and Chlorophyll were estimated using standard procedures. Statistical analysis was accomplished by means of average values, standard error and t-test (treatments *vs.* control) using IN STAT software V. 3.36.

Results and discussions

Germination studies revealed that all the nine varieties survived upto 2500 ppm salinity solution and at 5000 and 10,000 ppm the germination rate differed significantly between the varieties. Among the varieties Kala, Tirchy I, Vysakh, Seeraka Samba showed better performance and germinated upto 10000 ppm of salinity and found statistically significant. Similarly in the pot culture experiment also these varieties performed better when compared to other varieties in plant biometric parameters. Salt tolerance is required for the sustained plant growth in the soil, since salinity (NaCl) injury in rice is caused by both osmotic imbalance and accumulation of chloride (Cl) ions. Present study revealed that there is distinct behaviour of rice varieties.

Fig.1. Influence of Salinity on RWC and MSI for different rice varieties



The study revealed that shoot and root length, and biomass of rice (*Oryza sativa*) were reduced with increasing levels of NaCl (Abbas et al. 2013). Similar results were obtained in other experiment also. In this also, salinity caused significant effect on plant height, leaf area, MSI and RWC (Table 1).

MSI reduced in a manner of imposing period as well as severity under stress. Salinity results in malfunctioning of the cellular membranes by increasing their permeability to ions and electrolytes. However, few varieties such as Kala, Trichy, *Vyshak* and Sreeka samba could maintain its relative water content (RWC) and reduce membrane injury which is measured as membrane stability index (MSI) (Fig.1). Earlier results with different rice varieties suggest that it could maintain lower shoot Na^+ accumulation and lower shoot Na^+/k^+ ratio under high salinity (Asch et al., 2000; Hakim et al., 2010). The possible tolerance mechanisms of these varieties may be the enhanced level of osmoprotectants such as sugars, amino acids (proline), and glycine betaine, could assist to overcome the salinity stress as higher proline content in rice genotypes under salinity stress.

Table 1. Data Collection from salinity experiment using rice varities

Variety	Concentration (ppm)	Plant Height	No.of leaves	No.of Tillers	No. of Panicles	Leaf length	Leaf width	MSI (%)	RWC (%)
Uma	Control	44.5	36	7	0	55.9	1.2	60.00	73.47
	1000	3.5	24	4	0	63.9	1.1	50.00	84.15
	2500	34.5	28	4	0	62.5	1.3	35.56	94.00
	5000	36.7	28	5	0	49.8	1.2	40.55	77.05
	10000	31.5	33	4	0	53.3	1	35.18	68.92
RNR	Control	44.9	19	4	0	50.7	1.2	63.83	83.87
	1000	42.5	18	3	0	59.4	1.3	62.00	89.81
	2500	53.9	28	4	0	48.8	1.6	71.08	85.57
	5000	60.5	40	6	0	63.8	2	55.98	82.76
	10000	52.5	37	4	0	52.9	1.7	44.25	81.48
Kala	Control	50	21	6	0	89.5	1.5	38.10	85.58
	1000	40.8	16	6	0	74.9	1.4	32.50	67.16
	2500	39.5	19	4	0	70.5	0.8	17.31	75.34
	5000	49.3	23	5	0	96.7	1.6	23.33	80.00
	10000	45.5	18	3	0	89.4	13	35.69	73.56
TRICHY	Control	90.2	24	5	0	709	1.3	20.00	85.54
	1000	96.4	27	3	0	66.3	1.2	19.35	73.08
	2500	87.7	43	9	0	57	1.3	27.27	86.17
	5000	87.2	21	4	0	86.7	1.2	30.43	79.37
	10000	87	29	4	0	57.4	1.2	41.05	76.52
Vysakh	Control	112.5	13	3	3	46.8	1.2	8.47	77.61
	1000	108.3	12	3	3	50.5	1.2	92.05	79.59
	2500	106.5	25	5	3	30.3	1	93.29	75.49
	5000	112.5	18	3	3	40.8	1.5	72.82	62.67
	10000	110.5	16	3	3	47.1	1	96.72	62.32
Seeraka Samba	Control	50.2	28	5	0	53.5	1.3	33.29	85.51
	1000	48.6	26	4	0	74.8	1.1	24.51	80.16
	2500	38.5	19	4	0	68.8	0.6	12.66	69.81
	5000	76.5	26	5	0	41.8	1.5	35.82	63.37
	10000	51.3	29	5	0	68.5	1.2	21.92	48.61
C Ponni	Control	67.1	24	5	2	37.5	1.2	60.57	79.45
	1000	80.2	35	8	2	43.8	1.1	50.00	82.14
	2500	71.6	26	5	4	22.9	0.9	19.13	60.00
	5000	63.6	38	6	3	38.8	1.3	26.95	52.00
	10000	49.3	43	8	1	37.5	1.2	28.37	53.01
Jyothi	Control	72.3	25	6	3	49.1	1.2	54.00	80.39
	1000	73.2	20	5	3	42	1.2	25.83	76.79
	2500	73.1	20	4	3	30.7	1.3	42.19	80.00
	5000	74.5	29	5	1	35.4	1.3	31.45	85.71
	10000	68.3	18	4	3	39.7	1.2	12.24	67.50

Conclusion

In this study, we screened different varieties of rice and few selected crops for the salt tolerance using different salinity level. Among the varieties Kala, Tirchy I, Vysakh, Seeraka Samba performed better in terms of its plant morphological and its biochemical parameters. Future studies on this aspects will greatly contribute to the evaluation and breeding of salt tolerant varieties.

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EFFECT OF FOLIC ACID SUPPLEMENTATION ON HAEMATOLOGY, SERUM ENZYMES AND HORMONE PROFILE IN GESTATING AND LACTATING SOWS

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Abstract

The effect of dietary folic acid supplementationon haematology, serum enzymes and hormone profile in gestating and lactating sows was studied.Eighteen crossbred (Landrace X Desi) sows were randomly distributed into three groups of six sows each immediately after insemination in a completely randomized design (CRD). Sows in the control (T_0)group was fed with basal diet (folic acid at1.3 mg/kg), whereas, T_1 and T_2 groups were fed the basal diet supplemented with folic acid at 15 mg/kg during gestation and lactation.

The concentration of Hb and RBC count were higher ($P<0.01$) at the end of gestation (114d) and lactating (21 d) period than in the same sows at the beginning of study (0 d). The PCV (%) of folic acid supplemented (T_1 and T_2) groups as compared to control (T_0) group was higher ($P<0.01$) while the platelet and WBC counts were comparableamong the groups.

The serum enzymeconcentration viz., ALT, AST, ALP, LDH and CK revealed no significant ($P>0.05$) effect of folic acid supplementation. The serum T_3andT_4 concentrationwas higher ($P<0.01$) on 0 d (at the time of AI), farrowing (114d) than at end of lactation period (21std) in the same sows. The serum $T_3$and T_4 hormone was comparable whereas cortisol (ng/ml) level of sows was significantly reduced ($P<0.05$) in folic acid supplemented groups compared to control. Thus, based on the results concerning supplementation of folic acid to sows at 15mg/kg diet did not affect physiological parameters and reduce stress during gestation and lactation .

Keywords: Folic acid, Haematology, Serum enzymes, Serum hormones,Sows

Introduction

India's pig population is around 9.06 million and it contributes 1.7% of country's total livestock (DAHDF, 2019). Pigs are usually raised by the economically weaker section of the society and it provides animal protein for consumption, critical source of cash income and manure for cropping (Chauhan*et al*., 2016)
Modern system of pig rearing, higher litter size at birth and rapid post-weaning growth rate increases the requirement of few macro and micronutrients. Folic acid is one such micronutrient needed in considerable amount for the growth and development of conceptuses and placental structures (Pond and Houpt, 1978). Folic acid (B_9) is recognized as a factor of great importance in the control of sows' prolificacy (Matte *et al*., 1984). Gestational and lactational folic acid supplementation influence the transfer of folates from the sow (maternal) to the fetus and to the suckling piglet via colostrum and milk (Barkow*et al*., 2001). Further, some of the negative effects of elevated homocysteine (HCY) concentrations such as intrauterine growth retardation (IUGR) and oxidative stress can be reversed by feeding supplementary folic acid (Liu *et al*., 2012). The present study hypothesized that the response of maternal folic acid supplementation on progeny would also depend on the stage of gestation and lactation. Thus the present study was conducted to know about the precise time of folic acid supplementation in maternal diet by assessing the haematology, serum enzymes and serum hormone profile in gestating and lactating sows.

Materials and Methods

Experimental site, animals and housing

In a CRD, eighteen healthy Landllycrossbred sows (Landrace × Desi) were randomly distributed into three groups of six each after insemination and kept inpens under standard management conditions and were vaccinated and dewormed as per the recommended schedule.

Experimental design and dietary treatments

The sows in the control (T_0) group were fed with basal diet (folic acid at 1.3 mg/kg) as per NRC (1998)whereas, sows in T_1 and T_2 groups were fed the basal diet supplemented with folic acid (MB Vet Chem, Navi Mumbai, India.) at 15 mg/kg throughout the gestationand also during lactation respectively.The basal diet (mash feed) was prepared using crushed maize, wheat bran, de-oiled soybean meal, mineral mixture, and sodium chloride (Table 1).
All the pregnant sows were fed once daily (9:30 AM) at an allowance of 2.5 kg/d during gestation (0 to 84 ds) or 3.0 kg/d (85 to 114 ds)along with free access to clean drinking water. After farrowing lactation diet (42 ds i.e., till weaning) was fed to sows to a total of 2.5 kg plus 0.3 kg for every piglet (Table 1).

Blood collection and analyses

Prior to feeding and watering, blood samples were collected from 18 sows (6 sows/treatment) on 0 d and 114th-d post-insemination and 21 d of lactation from cranial vena cava. About 10 ml of blood was collected, 2 ml of was taken into a vacationer tube with anticoagulant (EDTA) for analysis of haematocrit profile and the remaining blood was used for serum collection and serum was stored at -20ºC until further analysis.The general haematocrit profile (haemoglobin, PCV, RBC, WBC and platelet count) was estimated with Haematology analyzer by Clindiag system B.V.B.A (Cat. # HA-22/20/Vet). The serum enzymes viz.,aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and creatine kinase (CK)were estimated colorimetrically using commercial diagnostic kits (Coral Clinical Systems, India) using spectrophotometer model UV-2601, Lambomed, USA. The serum hormones

such as T_3, T_4 and cortisol were estimated using ELISA kits supplied by LDN® immunoassay and services, Germany.

Statistical analysis

The data were subjected to one-way analysis of variance using SPSS (version 20.0) and the difference among the treatment groups was compared by Tukey's test and probability values $P < 0.05$ were considered significant.

Results and discussion

General hematological parameters, serum enzyme and hormone profiles are the best indicators of animal well-being and health status.Monitoring sows hematological and biochemical changes during gestation and lactation period are important. The concentration of Hb (g/dl) and RBC count (10^{12}/L) were increased ($P<0.01$) in gestating (114d) and lactating (21 d) sows as compared to non-gestating sows (0 d). There was a significant treatment ($P<0.01$) and period ($P<0.05$) increase in the PCV (%) of folic acid supplemented (T_1 and T_2) groups as compared to control (T_0) group (Table 2). The haematocrit profile in this study was within the normal reference range of swine (Carr, 1998). Similar to the present observations, Zanjani*et al*. (1974) noted a significant improvement in the level of Hb and RBC at the time of farrowing that could be due to faster foetal development during the last phase of gestation with a concomitant increase in oxygen demand, that triggers the endocrine system to release erythropoietin, the primary erythropoiesis regulator of foetus and adults. Contrary to this, Calvo *et al*. (1989) have observed significantly decreased haemoglobin level during the first half of gestation, with the lowest values in the 60 d (two months) after insemination. Throughout the experimental period, the platelet and WBC counts (10^9/L) were comparable among the groups (Table 2).In agreement with the results, Matte *et al*. (1990) found that intramuscular injection of folic acid to pigs had no effect on the haematological status.

The serum enzymes viz., AST, ALT, ALP, LDH and CK values did not differ significantly ($P>0.05$) and were within the normal physiological reference range for pigs (Table 2).Yao *et al*. (2013) reportedthat AST and ALT enzyme activities were not altered in pigs fed either 5 or 10 mg/kg folic acid. In another study conducted in rats, Roncales*et al*. (2004) observed that dietary folic acid (40 mg/kg diet) supplementation did not alter serum AST and ALT enzymes.

Adrenal glands and thyroid are important for animals to regulate stress. Thyroid gland secretes triiodothyronine (T_3) and thyroxine (T_4) hormones. Adrenal cortex cells synthesize cortisol, a steroid hormone which regulates the metabolism of glucose, fat, protein, salt and water. During stress conditions, the cortisol content of the animals immediately increases. The plasma cortisol concentration changes have been used as an indicator of stress in pigs (Hicks *et al*., 1998; Sutherland *et al*., 2009; Song *et al*., 2011). Higher ($P<0.01$) serum T_3 andT_4 level was noticed on 0 d (at the time of AI), followed by a d of farrowing (114^{th}d) and lowest at lactation period (21^{st}d). No significant ($P>0.05$) supplementation effects were observed on serum T_3 and T_4 (hormone (Table 4). All the above mentioned hormonal changes were related to sows different physiological stages (non-pregnant, pregnant and lactation). The thyroid hormone results are in agreement with Iveta *et al*. (2011) and Schone*et al*. (1997). Iveta *et al*. (2011) observed that the serum T_3 and T_4 levels were decreased ($p<0.05$)during 3-4 weeks postpartum compared to the hormone levels of sows in various reproductive stages.Schone*et al*. (1997).Further, variations of thyroid hormone (T_3 and T_4) levels according to age have been described in several species including pigs (Iveta *et al*., 2011). Significant ($P<0.05$) treatment effect was noticed in cortisol (ng/ml) level. Significantly highest cortisol level (65.85±6.14ng/ml) was observed in T_0 group as compared to other groups T_1 (49.20±5.69ng/ml) and T_2 (47.52±5.57ng/ml). Further, across the experimental period significant ($P<0.01$) period effect was observed on T_3, T_4 and cortisol hormone levels (Table 4). Thus, it is evident that folic acid supplementation during gestation and lactation was beneficial in terms of reducing oxidative stress. Folic acid reduces the cortisol level by accelerating the activity of the GABAergic system. GABA decreases the secretion of corticotrophin-releasing hormone (corticoliberin), which stimulates a series of successive hormonal changes, leading to the secretion of cortisol by the adrenal cortex (Stachowicz and Lebiedzinska, 2016).

Based on the results evinced in this study, folic acid supplementation during gestation and lactation is beneficial in terms of reducing oxidative stress. The cortisol levels in sows decreased due to folic acid supplementation and cortisol being a good indicator of stress, folic acid supplementation (at15 mg/kg diet) during gestation and also during lactation was beneficial to ameliorate the oxidative stress during gestation and lactation.

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Table 1. Ingredient composition and chemical composition of basal diets (as-fed basis)

Ingredients (%)	Pregnancy period		Lactation period	
	Control diet (T_0)	Folic acid supplemented diet (T_1& T_2)	Control diet (T_0& T_1)	Folic acid supplemented diet (T_2)
Crushed maize	73	73	65	65
De-oiled soya bean meal	12	12	22	22
Wheat bran	10	10	8	8
Oil / fat	2	2	2	2
Calcite	0.5	0.5	0.6	0.6
Di calcium phosphate	0.5	0.5	0.3	0.3
Mineral and vitamin mixture[†]	1.5	1.5	1.5	1.5
L-Lysine	0	0	0.2	0.2
Sodium chloride	0.5	0.5	0.4	0.4
Folic acid (mg/kg diet)	1.3	15	1.3	15
Nutrient composition (As fed basis)				
Digestible energy (kcal/kg) [‡]	3394	3394	3413	3413
Crude protein (%)[§]	12.74	12.74	16.18	16.18
Crude fibre (%)[§]	3.83	3.83	4.58	4.58
Calcium (%)[§]	0.75	0.75	0.77	0.77
Total phosphorus (%)[§]	0.64	0.64	0.63	0.62

Each 1kg contains: vitamin A 20,00,000 IU; vitamin D_3 4,00,000 IU; vitamin B_2 0.8 g; vitamin E 0.3 g; vitamin K 0.4 g; vitamin B_{12} 2.4 mg; calcium pantothenate 0.1 mg; niacin 4 g; choline chloride 60 g; calcium 0.28 g; manganese 11 g; iodine 0.4 g; iron 3 g; zinc 6 g; copper 0.8 g; cobalt 0.18 g; phosphorus 80 g.[‡]Calculated values as fed basis. [§]Analyzed values as fed basis.

Table 2. Effect of dietary folic acid supplementation on hematological and serum enzyme profilein gestating and lactating sows

Parameter	Treatments[†]			Significance[‡]		
	T_0	T_1	T_2	T	P	T*P
Hb (g/dl)	14.38±0.26	14.93±0.35	14.74±0.46	0.323	<0.001	0.283
PCV (%)	40.14^{B}±0.71	42.05^{A}±0.86	42.67^{A}±1.10	0.029	<0.001	0.449
RBC (10^{12}/L)	6.55±0.17	6.70±0.14	6.93±0.18	0.178	<0.001	0.747
Platelet (10^{9}/L)	259.72±8.50	258.67±7.29	257.39±6.82	0.978	0.397	0.982
WBC (10^{9}/L)	13.99±0.75	13.70±0.46	13.12±0.44	0.559	0.211	0.979
Aspartate aminotransferase (IU/L)	41.48±1.90	41.23±1.80	41.86±1.42	0.971	0.930	0.998
Alanine aminotransferase (IU/L)	41.27±1.37	41.30±1.21	41.49±1.24	0.992	0.951	0.999
Alkaline phosphatase (IU/L)	48.06±1.51	51.58±1.56	50.94±1.32	0.357	0.779	0.793
Lactate dehydrogenase (IU/L)	411.81±11.53	411.32±6.49	412.13±5.53	0.998	0.992	1.000
Creatine kinase (IU/L)	258.86±12.00	258.46±8.74	261.83±14.65	0.980	0.987	1.000

T_0, basal diet; T_1 and T_2, basal diet supplemented with FA at 15mg/kg feed throughout the gestation and also during lactation period, respectively

[XY]Means bearing different superscripts in a row differs significantly (P≤0.05) and (P≤0.01)

[‡]Significant effects of dietary treatments (T), period of blood collection (P) and their interaction (PxT).

Table 3. Effect of dietary folic acid supplementation on serum hormone profile in gestating and lactating sows

Treatment†	Ds post-insemination		Lactation	Treatment	Statistical significance		
	0 d	114 d	21st d	mean	T	P	T*P
			T_3 (ng/ml)				
T_0	1.21±0.158	0.87±0.068	0.45±0.088	0.84±0.096	0.938	<0.001	0.999
T_1	1.16±0.093	0.87±0.071	0.45±0.091	0.82±0.085			
T_2	1.14±0.097	0.85±0.056	0.45±0.129	0.81±0.088			
Period mean	1.17^{X}±0.07	0.86^{Y}±0.04	0.45^{Z}±0.06				
			T_4 (nmol/L)				
T_0	51.78±4.28	50.84±2.48	16.71±0.36	39.77±4.25	0.998	<0.001	0.999
T_1	52.57±2.39	50.74±2.48	16.41±1.00	39.91±4.19			
T_2	52.55±3.68	50.26±2.22	16.79±0.61	39.87±4.19			
Period mean	52.30^{X}±1.92	50.61^{X}±1.30	16.63^{Y}±0.39				
			Cortisol (ng/ml)				
T_0	37.16±7.11	80.93±4.78	79.47±7.96	65.85^{A}±6.14	0.028	0.001	0.327
T_1	38.76±11.27	50.18±11.76	58.65±5.04	49.20^{B}±5.69			
T_2	38.06±10.62	49.44±8.98	55.06±9.63	47.52^{B}±5.57			
Period mean	37.99^{Y}±5.34	60.18^{X}±6.03	64.39^{X}±4.96				

$^{AB/XY}$Means bearing different superscripts within a column (AB) or row (XY) differs significantly (P≤0.05) and (P≤0.01)

PRIMING CANE NODE FOR ACCELERATING GERMINATION IN TROPICAL SUGARCANE VARIETY (CoC 24)

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Abstract

Sugarcane is an important cash crop and plays an important role in the country's Agricultural economy. But day by day due to various reasons sugarcane productivity is going down, on the other side, production cost is increasing heavily due to increase in fertilizer cost, labour cost, electricity charges, etc. Deteriorating soil health is also one of the concerns of low cane yields. Since the possibility of increasing the cane area is very less in view ofemerging low input high value crops, the estimated aim can only be achieved by enhancing the cane productivity and improving its sugar recovery simultaneously. In this view, the development of package of agro-technologies is the only master key to unlock the potential of sugarcane yield. However, the combination of ecological sustainability with technological development is essential to maintain the high level of cane productivity without disturbing the soil environment. India has been bestowed with certain key environments, i.e., bright sunshine all the year, diversified soil types, vast array of climatic changeability and a large collection of sugarcane varieties adapted for particular agro-ecological niches. Following are the agrotechnologies for sustainable sugarcane based cropping systems suggested for better yield and production of sugarcane by improving germination to obtain early bulking of the crop (Yadav *et al*., 2021). Within 5-6 days of priming, the buds start sprouting. This technique also maintains a uniform crop stand. With favorable growth conditions, this technology guarantees 25 per cent higher sugarcane production over the conventional method. Information on priming cane node for accelerating germination in sugarcane is lacking under tropical climate and hence, the present investigation was undertaken.

Methodology

The field experiments were conducted for two years at Sugarcane Research Station, Cuddalore. The clone CoC-25 was selected as test variety the soil of the experimental site is sandy loam with pH of 7.4, organic carbon (0.42 %), bulk density (1.41 g cc^{-1}) and infiltration rate (1.37 cm h^{-1}). The initial nutrient status of the soil is 181.0: 23.4: 232.5 NPK kg ha^{-1}. The following are the treatments viz., T_1 - Un-primed cane node; T_2 - Treating cane node in hot water at 50° C for 2 hours; T_3 - Treating cane node in hot water (50° C) urea solution (3 %) for 2 hours;T_4 - Priming cane node with cattle dung, cattle urine and water in 1:2:5 ratio;T_5 - Conventional 3 bud sett planting and T_6 – Primed and sprouted cane node (Incubated for four days after priming). Analysis of variance was performed for cane yield, yield attributes and juice quality parameters following a randomized block design (Gomez and Gomez 1984). All the recommended package of practices except fertilizer treatments were adopted during the experimentation.

Results

Among the six treatments, sugarcane planting with Primed and sprouted cane node (Incubated for four days after priming) (T_6) recorded significantly the maximum germination of 69.82, 73.56 and 86.89 per cent at 20, 30 and 40 days after planting. There was no germination up to 10 DAP. Primed and sprouted cane node (Incubated for four days after priming) (T_6) significantly recorded the higher shoot count of 99,250 ha^{-1} and 2,02,350 ha^{-1} on 60 and 90 days after planting and maximum of 1,70,250 ha^{-1} at 150 DAP. Primed and sprouted cane node (Incubated for four days after priming) (T_6) significantly recorded the higher millable cane population of 1,28,240 ha^{-1}. The same treatment has also recorded the maximum cane length, cane diameter and individual cane weight of 284.06 cm, 2.89 cm and 1.63 kg and it was on par with the primed and sprouted cane node (Incubated for four days after priming) which recorded 277.13 cm, 2.78 cm and 1.59 kg respectively. Numerically higher CCS(%) was recorded with the treatment T_5 (12.52 %).

Table 1. Effect of priming cane node for accelerating germination on growth and yield characteristics of sugarcane

Treatments	Germination (%)				Shoot counting ('000/ha)		
	20 DAP	30 DAP	40 DAP	60 DAP	90 DAP	120 DAP	150 DAP
T_1	42.50	57.23	68.45	53.22	105.30	98.25	91.25
T_2	46.85	58.98	72.63	64.58	159.85	151.25	143.26
T_3	54.20	63.56	76.24	69.25	165.23	152.00	147.82
T_4	59.50	67.70	78.52	78.56	173.56	163.51	151.23
T_5	57.95	63.25	77.25	78.51	180.23	175.25	168.25
T_6	69.82	73.56	86.89	99.25	202.35	182.35	170.25
CD (P=0.05)	3.01	3.24	3.60	2.85	7.50	6.11	6.55

Table 2. Effect of priming cane node for accelerating germination on yield parameters and yield of sugarcane

Treatments	Millable cane ('000/ha)	Cane length (cm)	Cane diameter (cm)	Individual cane weight (kg)	CCS (%)	Cane Yield (t/ha)	Sugar Yield (t/ha)
T_1	78.20	234.53	2.11	1.12	11.28	89.7	10.12
T_2	84.28	248.40	2.45	1.31	12.18	97.85	11.92
T_3	89.51	251.10	2.60	1.43	12.02	111.3	13.38
T_4	94.40	276.25	2.70	1.50	12.32	132.5	16.32
T_5	101.51	277.13	2.78	1.59	12.43	141.2	17.55
T_6	128.24	284.06	2.89	1.63	12.52	148.4	18.58
CD (P=0.05)	8.72	12.15	0.11	0.05	NS	6.11	0.62

Conclusion

Planting of sugarcane with Primed and sprouted cane node (Incubated for four days after priming) significantly recorded all the growth, quality and yield parameters of sugarcane.

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ENHANCING SOURCE - SINK PARTITIONING EFFICIENCY OF GROUNDNUT (*Arachis hypogaea* L.) BY ARRESTING LATE FORMED FARMERS

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Abstract

Field experiments were conducted for two consecutive years during Kharif 2017 and Kharif 2018 to study the effect of arresting late formed flowers on yield improvement in groundnut at Regional Research Station, Vridhachalam. The experiment was laid out in strip plot with four treatments viz., T_1 - Control (without any spray), T_2 - Mepiquat chloride @125 ppm on 60 DAS, T_3 - NAA @ 200 ppm on 60 DAS and T_4 - NAA @ 300 ppm on 60 DAS. Among the different treatments foliar application of NAA@ 200 ppm at 60 DAS recorded higher number matured pods (21.7) and pod yield (2591 kg/ha) with 11.5 % yield increase over control.

Keywords: Groundnut, late formed flowers, growth regulators, pod yield

Introduction

Groundnut (*Arachis hypogaea* L.) is an important oilseed leguminous crop grown in India and it is the 13th most important food crop and 4th most important oilseed crop of the world. In India 82 per cent of the total production of groundnut is concentrated in five states. Gujarat is the major producer of groundnut contributing 34.8 per cent of total production followed by Rajasthan (Harisudan and Subrahmaniyan, 2020). Groundnut acreage in the country is fluctuating over the years and from the last two decades the area had declined from 83 lakh ha to 47 lakh ha. Due importance on yield improvement has to be given to sustain the area under groundnut cultivation. Further the groundnut production constraint has to be addressed. With regard to growth habitat, like many other legumes, groundnut has indeterminate growth habit where growth and development of reproduction and vegetative phase overlap with each other which result in low yield due to competition for various metabolites including photosynthates. In addition another constraint in low pod yield is the longer duration of flowering extending up to 120 days, most of which abscise causing low efficiency. Apparently, the flowers produced during the initial 3 weeks after the commencement of flowering would have a better chance of developing into mature pods since these establish relatively more vigourous sink. The late formed flowers may or may not develop pods and if they develop pods, it fails to attain the pod shape on drying or shriveling (Usha Parmar *et al*., 1989).

Groundnut manifests the problems of diversity in maturing pod due to flower abscission and immature fruit development. Studies on crop growth regulation is a pre-requisite to enhance the source to sink partitioning efficiency in groundnut where only 30 % of the total pegs develop into mature pods with available biomass. The role of phenolics to determine the level of inhibitors and promoters and physiological manifestations appearing due to exogenous applications of long chain alcohols in different crop species have been reported. Hence, it is inevitable to induce production of maximum number of flowers at the early reproductive stages leading to better availability of potential sinks during early span of reproductive phase. Keep these aspects in view field investigation was carried out to study the effect of arresting late formed flowers on yield improvement in groundnut.

Materials and methods

Field experiment was conducted over 2 years during Kharif 2017 and Kharif 2018 at Regional Research Station, Tamil Nadu Agricultural University, Vridhachalam (11° 30 ‘ N, 79° 26’ E, 42.67 m altitude) to study the effect of arresting late formed flowers on yield improvement in groundnut. The experiment was conducted in Strip plots of size 1000 m^2 each which are non replicated. The treatments involves T_1 - Control (without any spray), T_2 - Mepiquat chloride @125 ppm on 60 DAS, T_3 - NAA @ 200 ppm on 60 DAS and T_4 - NAA @ 300 ppm on 60 DAS. The crop was maintained by following recommended package of practices (Crop Production Guide - 2012 Dept. of Agriculture) uniformly for all treatments. The crop was observed for flower initiation and daily production. Flower initiation was observed on 25th day after sowing (DAS). The total number of flowers produced per plant was determined by recording the daily flower count from the day flowering initiated, in five tagged plants from each replication. The flower counts were continued until 90 per cent of the plants in the plots ceased to flower. The synchrony in flowering was assessed by determining the minimum number of days required for the production of the first forty flowers during the early flowering period. The pegging percentage and pod-setting ratio were computed by using the formula suggested by Subrahmaniyan *et al*. (2008a). Standard sampling procedures were adopted to classify the pods into juvenile (J). potential mature (PM) and mature pods (M). i The pods which failed to maintain the pod shape on drying (shrivelled) were designated as juvenile pods. The potential mature pods are those which retained l their shape on drying, and had a length of more than 1.5 cm. At harvest the pods were grouped in normal way as mature and I immature pods.

Results and discussion

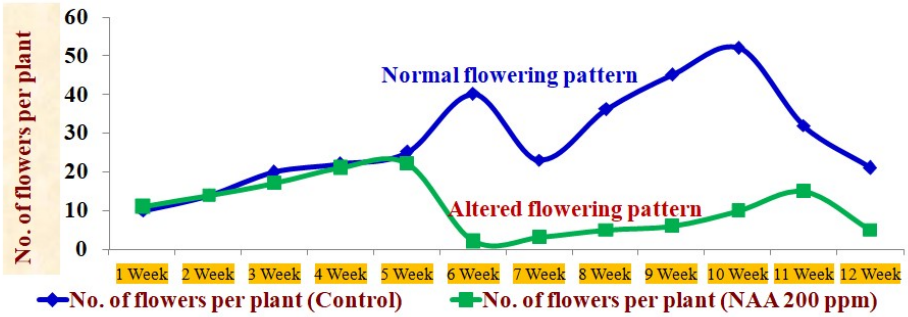
Significant difference in yield attributes and pod yield of groundnut was observed by arresting late formed flowers (Table 1). Among the different treatments foliar application of NAA @ 200 ppm at 60 DAS

recorded higher number of pegs/plant (48.4) with a higher peg to pod ratio of 28.7, higher matured pods (21.7). The findings are in conformity with the results of Mondal *et al*., (2011b) observed that increases in leaf chlorophyll and nitrogen content of mungbean at the time of early growth development period which help in early formed flowers getting more available assimilates then later formed flowers. These advantages in producing high number of pod set thereby high yield. That is the reason, the higher rates of flower generation within 10 to 15 days after flowering revealed higher number of mature pod and seed yields. Further, earlier formed flowers had a higher pod set than the latter may be due to most of the sugar produced by leaf is utilized in filling the pods that occurs at proximal position of raceme (Spollen *et al*., 1986a).

Table 1. Effect of growth regulator on yield attributes and pod yield of groundnut

Treatments	No. of pegs/plant	Peg to pod ratio	Peg to pod conversion (%)	Shellin g(%)	100 kernel weight (g)	No. of matured pods/plant	No. of immature pods/plant	Pod yield (kg/ha)
T_1	45.9	21.1	2.2 :1	71.7	37.9	16.2	9.5	2292
T_2	46.8	22.7	2.1 : 1	73.6	38.2	20.2	7.3	2305
T_3	48.4	28.7	1.7 : 1	77.6	38.4	21.7	6.5	2591
T_4	44.0	20.5	2.1 : 1	72.4	38.0	17.7	8.2	2290

Fig.1. Arresting late formed flowers to improve seed yield in groundnut



Flowering is arrested from 6th week by the foliar application of of NAA@ 200 ppm at 60 DAS (Fig 1). Similarly higher pod yield (2591 kg/ha) with 11.5 % yield increase over control was obtained by foliar application of NAA@ 200 ppm at 60 DAS. The findings are in conformity with Vinothini *et al*., 2018.

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INFLUENCE OF HEAT UNITS (GROWING DEGREE DAYS) ON GROWTH AND YIELD ATTRIBUTES AND YIELD OF GROUNDNUT

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Abstract

An field experiment was conducted at experimental farm of Agricultural College and Research Institute, Eachangkottai during Margazhi pattam 2019 on Effects of sowing window and its heat units on the performance of groundnut to find out the most suitable sowing windows for sowing of groundnut and to study the relationship between meteorological parameters and different dates of sowing affects on yield of groundnut. The experiment was conducted in Randomized Block Design. The groundnut VRI-2 was sown at different sowing windows of 05.01.2019, 12.01.2019, 19.01.2019, 26.01.2019 and 02.02.2019 and harvest was taken during 18.04.2019, 25.04.2019, 30.04.2019, 06.05.2019 and 12.05.2019 respectively. Various biometric observations (plant height, number of branches, dry matter, number of pods) and yield attributes and yield of groundnut VRI-2 was recorded. The heat unit concept of GDD also worked out for individual sowing windows. Among the sowing dates, 5th January 2019 recorded essential GDD of 1651.3°C and higher growth attributes, yield attributes and yield (2370 kg/ha).

Key words: Groundnut, growing degree days, yield

Introduction

Groundnut (*Arachis hypogaea* L.) is an important oilseed crop grown under rainfed conditions. It is a very sensitive crop to climatic variations, especially rainfall, temperature and radiation (Banik *et al*., 2009). In India, about 75 per cent of the groundnut area lies in a low to moderate rainfall zone (parts of peninsular region and western and central regions) with a short period of distribution (90-120 days). Rate of plant growth and development is dependent upon temperature surrounding the plant and each species has a specific temperature range represented by a minimum, maximum and optimum. The number of days required for cultivars to reach maturity depends primarily on location, date of sowing and temperature. Due to variations in daily minimum and maximum temperatures from year to year and between location, number of days from planting to physiological maturity varies and, is not a good predictor of crop development (Hatfield *et al*., 2011). Meteorological indices viz. growing degree days (GDD), helio thermal unit (HTU), and photo-thermal unit (PTU) based on air temperature are used to describe changes in phenological behavior and growth parameters (Girijesh *et al*., 2011; Prakash *et al*., 2015). Plants have a definite heat requirement before they attain certain phenophases. The optimum time is mainly dependent on prevailing agro-climatic conditions of an area besides the variety grown. Hence the present study was undertaken to find out the optimum sowing window and the amount of heat units required to change their phonological development for groundnut.

Materials and methods

The field experiment was conducted at experimental farm of Agricultural College and Research Institute, Eachangkottai during Markazhi pattam (January 2019) in randomized block design with four replication. The groundnut variety VRI 2 was sown at different sowing windows of 05-01-2019, 12-01-2019, 19-01-2019, 26.01.2019 and 02.02.2019 and harvest was taken during 18-04-2019, 25.04.2019, 30-04-2019, 06-05-2019 and 12.05.2019 respectively. Nutrients were applied @ 25:50:75 kg NPK ha $^{-1}$ in the form of urea, SSP and MOP, respectively along with 10 t of farm yard manure. Gypsum was applied during the time of ear thing up @ 500 kg ha^{-1}. The seeds were sown at a depth of 5 cm with 30 x 15 cm spacing. The data on the parameters like plant height, leaf area index, dry matter production , number of pods and yield of ground nut VRI 2. Daily observations on maximum and minimum air temperature were recorded at meteorological observatory, and the weather data were used for the analysis. Growing Degree Days (GDD) is defined as the sum over the growing season of a crop of the difference between the daily temperature and a reference temperature. GDD was expressed in terms of ^{0}C day. The growing degree days (GDD) was worked out by considering the base temperature of 10^{0}C (Patel *et al*. 1999). The total growing degree days (GDD) for different phonological phases were determined by the following formula

Accumulated GDD = Σ [(T_{max} + T_{min})/2]- T_b (^{0}C day)

Where,

GDD = Growing degree days

T_{max} = Daily maximum temperature (^{0}C)

T_{min} = Daily minimum temperature (^{0}C)

T_b = Base temperature (10 ^{0}C)

Results and Discussion

Growth attributes

Growing degree days and photothermal units are widely used indices for describing the phenological responses of crop to temperature.The requirement of GDD and PTU for completion of different phenophase for different groundnut Growing degree days and photothermal units are widely used

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Growing degree days and photothermal units are widely used indices for describing the phenological responses of crop to temperature. The requirement of GDD and PTU for completion of different phenophase for different groundnut varieties were worked out and presented in

The weather parameters such as rainfall and sunshine hours played a critical role on the crop growth, which in turn decides the crop yield. The groundnut VRI-2 was sown at different sowing windows of 05.01.2019, 12.01.2019, 19.01.2019, 26.01.2019 and 02.02.2019 and harvest was taken during 18.04.2019, 25.04.2019, 30.04.2019, 06.05.2019 and 12.05.2019 respectively. During the cropping period, the first sowing window (05.01.2019) recorded 177.5 HU to attain germination phase, 552.5 HU for germination to flowering phase and 944.3 HU for flowering to harvest with a total HU of 1653.3. The first sowing window recorded higher total heat units (HUA) of 1651.3 than other sowing windows. Higher plant height at 30 DAS (19.6cm), 60 DAS (43.2cm) and 90 DAS (62.4cm), leaf area index at all the growth stages and dry matter production at all the growth stages 30 DAS (795 kg/ha), 60 DAS (2300 kg/ha) and 90 DAS. (8200 kg/ha) were recorded in the first sowing window of 05.01.2019. This might be due to the better sunlight for longer duration which produce more photosynthates, increase in the number of branches and increased the total dry matter per plant (Meena *et al*., 2015). Lower plant height, leaf area index and dry matter production was registered in the all the growths stages in sowing windows of 02.02.2019, which might be duto the short day conditions of the crop (Table 2).

Yield attributes and yield

The maximum number of 26.2 pods plant $^{-1}$ was recorded in first sowing window followed by second, third sowing window at 18 and 17, respectively.The higher grain yield of 2370 kg/ha was obtained from the first sowing window (05.01.19) followed by second and third sowing which recorded 2050 and 2000 kg/ ha, respectively. The yield increase might be due to accumulation of more heat unit in the first sowing window than the other time of sowing. Increase in the growth parameter provides better translocation of photosynthates to the sink and thereby, increases the pod yield. Thus, variation in the growth parameters varies the pod yield. Naik *et al*., (2018) also obtained similar results. The crop sown during 5th January recorded significantly higher pod yield which was due to the favorable weather conditions that prevailed during crop growth period and similar findings were reported by Canavar and Kaynak (2008) and Bala *et al*. (2011).(Table 2)

Table 1. Effect of Heat Units at different Phonological Stage and yield of groundnut

Treatments	Sowing to germination	Germination to Flowering	Flowering to Harvest	Total (HUA)
T_1 (05.01.2019)	177.5	529.5	944.3	1651.3
T_2 (12.01.2019)	187.75	534.5	861.3	1583.5
T_3 (19.01.2019)	200.75	511	839.5	1551.25
T_4 (26.01.2019)	206.5	547.5	811.5	1565.5
T_5 (02.02.2019)	214.5	546.25	795.5	155
C.D (P=0.05				

Table 2.Effect of Heat Units on growth and yield attributes and yield of groundnut

Treatments	**Plant height (cm)**			**Leaf area index**			**Dry matter production (kg/ha)**			**No of pods/ plant**	**Yield (kg/ha)**
	30 DAS	**60 DAS**	**90 DAS**	**30 DAS**	**60 DAS**	**90 DAS**	**30 DAS**	**60 DAS**	**90 DAS**		
T_1	19.6	43.2	62.4	0.150	1.90	2.91	795	2570	8200	26.3	2370
T_2	18.6	37.3	53.2	0.130	1.45	2.70	718	2320	7350	24.2	2050
T_3	18.4	36.2	52.2	0.125	0.98	2.50	690	2260	6900	23.5	2000
T_4	15.1	31.3	46.5	0.112	0.71	2.35	654	2100	6700	22.2	1770
T_5	14.1	23.2	42.8	0.05	0.66	1.60	598	1950	4355	19.9	1230
S.Ed	1.10	2.27	3.27	0.01	0.090	0.160	43.37	103.5	355.5	3.6	125.1
C.D	3.58	7.35	10.6	0.03	0.290	0.520	141.3	224.5	772.1	10.8	271.4

Conclusion

Thus, it can be concluded that, the first sowing (05.01.2019) recorded essential GDD of 1651.3°C and higher growth and yield attributes and yield of groundnut (2370 kg/ha). which can translocate photosynthates to the sink and produce more number of pods than other date of sowing.

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EVALUATION OF NEW GENERATION FUNGICIDES AGAINST SESAME FOLIAR DISEASES UNDER FIELD CONDITIONS

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Abstract

Sesame (Sesamum indicum L.) is also known as sesamum, til, gingelly, simsin, gergelim and it is the most ancient oilseed crop in the world, regarded as 'Queen of Oilseeds', the quality of its oil being of high nutritional and therapeutic value. The field experiment was conducted in a plot size of 2.4x 3.0 m with eight treatments and three replications in RBD using the susceptible variety VRI-1 for the management of foliar diseases of sesame. Seed treatment (ST) with T. viride @ 10 g/kg + furrow application of T. viride (2.5 kg/ha enriched in 100 kg of FYM) @ 250 kg/ha is common for T_1- T_6.Five systemic fungicides were selected for the field experiment. The fungicides viz., Myclobutanil @ 1 g/l, hexaconazole 4% + zineb 68% @ 2 g/l, cymoxanil 8%+ mancozeb 64% @ 2g/l, trifloxistrobin 25% + tebuconazole 50% @ 0.5 g/l, hexaconazole 5%+ captan 70% @ 2g/l were evaluated against foliar diseases of sesame under field condition. The results of the field experiment revealed that T_1 - seed treatment with T. viride @ 10 g/kg + furrow application of T. viride (2.5 kg/ha enriched in 100 kg of FYM) @ 250 kg/ha + foliar spray of myclobutanil @ 1 g/l was effective in managing the foliar diseases of sesame which recorded minimum Alternaria leaf spot (16.8 PDI) and powdery mildew (15.9 PDI) with higher yield of 648 kg/ha. This was followed by foliar spray of trifloxistrobin 25% + tebuconazole 50% @ 0.5 g/l which recorded Alternaria leaf spot (17.2 PDI) and powdery mildew (16.4 PDI) with yield of 614 kg/ha. In control, the maximum Alternaria leaf spot (31.4 PDI) and powdery mildew (42.6 PDI) disease intensity with minimum yield of 462 kg/ha was recorded.

Key words: Integrated management, Powdery mildew, Alternaria leaf spot, Sesame

Introduction

Sesame (Sesamum indicum L.) is also known as sesamum, til, gingelly, simsin, gergelim and it is the most ancient oilseed crop in the world, regarded as 'Queen of Oilseeds', the quality of its oil being of high nutritional and therapeutic value. In the Indian traditional medicine, Ayurveda, sesame is the major ingredient in many of the health rejuvenating formulations and massage oils contain sesame oil as the major ingredient. India is the largest sesame growing country in the world, with an area of 1.76 m.ha and production of 0.75 m.t. But productivity wise it is among the lowest with 384 kg/ha. One of the important biotic reasons for poor yield is diseases. In Tamil Nadu, the sesame crop is mainly affected by Alternaria leaf blight, powdery mildew and dry root rot and phyllody diseases during both karif and rabi.

Materials and Methods

The field experiment was conducted in a plot size of 2.4x 3.0 m with eight treatments and three replications in RBD using the susceptible variety VRI-1 for the management of foliar diseases of sesame. Seed treatment (ST) with T. viride @ 10 g/kg + furrow application of T. viride (2.5 kg/ha enriched in 100 kg of FYM) @ 250 kg/ha is common for T_1- T_6.Five systemic fungicides were selected for the field experiment. The fungicides viz., Myclobutanil @ 1 g/l, hexaconazole 4% + zineb 68% @ 2 g/l, cymoxanil 8%+ mancozeb 64% @ 2g/l, trifloxistrobin 25% + tebuconazole 50% @ 0.5 g/l, hexaconazole 5%+ captan 70% @ 2g/l were evaluated against foliar diseases of sesame under field condition.

Two spraying of the fungicides were carried out, first at the time of initiation of the disease and second at 15 days after first spraying. In control plots only water was sprayed. The disease rating was done by 0 to 5 scale and average disease severity index based on percentage leaf area affected was calculated. The per cent disease intensity (PDI) was worked out.

Scale	Per cent leaf area infection
0	No infection
1	1-10% leaf area infected
2	11-25% leaf area infected
3	26- 50% leaf area infected
4	51-70% leaf area infected
5	71-100 % leaf area infected

Disease intensity of foliar disease viz., Alternaria leaf spot and powdery mildew was observed at 75 days after sowing using 0-5 disease rating scale by random selection of 25 plants per plot. The seed yield also recorded for each treatment and the data were statistically analysed.

Per cent Disease Index (PDI)

$$PDI = \frac{\text{Sum of numerical ratings}}{\text{Total number of plants observed x Maximum grade}} \times 100$$

The results of the field experiment revealed that T_1 - seed treatment with T. viride @ 10 g/kg + furrow application of T. viride (2.5 kg/ha enriched in 100 kg of FYM) @ 250 kg/ha + foliar spray of myclobutanil @ 1

g/l was effective in managing the foliar diseases of sesame which recorded minimum Alternaria leaf spot (16.8 PDI) and powdery mildew (15.9 PDI) with higher yield of 648 kg/ha. This was followed by foliar spray of trifloxistrobin 25% + tebuconazole 50% @ 0.5 g/l which recorded Alternaria leaf spot(17.2 PDI) and powdery mildew (16.4 PDI) with yield of 614 kg/ha. In control, the maximum Alternaria leaf spot (31.4 PDI) and powdery mildew (42.6 PDI) disease intensity with minimum yield of 462 kg/ha was recorded. Meena and Ratnoo, (2014) observed that mancozeb and hexaconazole were the best fungicides for control of all the three species of A.alternata, A.macrospora and A.gossypina. Singh and Majumdar (2002) reported propiconazole was the most effective fungicide in controlling fruit rot of pomegranate caused by Alternaria alternata.

Table. 1. Integrated management of foliar diseases of sesame

S. No	Treatments	Alternaria leaf spot (PDI)	Powdery mildew (PDI)	Yield (kg/ha)
T_1	Spray of Myclobutanil @ 1 g/l	16.8 (23.4)	15.9 (22.7)	648
T_2	Spray of hexaconazole 4% + zineb 68% @ 2 g/l	18.4 (25.1)	25.6 (28.4)	528
T_3	Spray of cymoxanil 8%+ mancozeb 64% @ 2g/l	19.7 (26.1)	21.5 (27.6)	541
T_4	Spray of trifloxistrobin 25% + tebuconazole 50% @ 0.5 g/l	17.2 (24.5)	16.4 (23.4)	614
T_5	Spray of hexaconazole 5%+ captan 70% @ 2g/l	21.6 (27.4)	27.4 (34.6)	516
T_6	Spray of Trichodermaviride @ 0.4%	27.9 (29.7)	31.7 (33.8)	497
T_7	Spray of carbendazim 12% + mancozeb 63% 75WP @ 2g/l (Treated check)	17.8 (24.6)	18.3 (24.9)	584
T_8	Untreated check	31.4 (33.6)	42.6 (36.4)	462
SEd		2.43	1.82	21.6
CD(P=0.05)		5.16	3.91	43.4

*Mean of three replications

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DIRECT AND RESIDUAL EFFECT OF SULPHUR APPLICATION IN RICE (*ORYZA SATIVA* L.)-BROWN SARSON (*BRASSICA COMPESTRIES* L.) CROPPING SYSTEM

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Abstract

Significant positive effects of 60 kg ha^{-1} of sulphur at par with 40 kg sulphur ha^{-1}against 20 kg sulphur ha^{-1}as manifested on yield and yield parameters of rice were further carried over to succeeding brown sarson crop. Rice yield in *kharif* 2018 and 2019 (6.71 and 6.86 t ha^{-1} respectively) obtained at 60 kg ha^{-1}of sulphur dose was significantly higher over 20 kg sulphur ha^{-1} (6.07 and 6.15 t/ha respectively). Similarly number of panicles m^{-2}, spikelets panicle^{-1}, grains penicle^{-1} and 1000-grain weight (g) at 60 kg sulphur ha^{-1} significantly increased over 20 kg sulphur ha^{-1} from 301.69 to 310.89, 108.11 to 114.85, and 90.96 to 96.10 and from 19.91 to 21.78 on pooled basis in respective manner. Increase in seed and straw yield of brown sarson at residual effect of 60 kg sulphur ha^{-1} over 20 kg sulphur ha^{-1} was from 0.94 to 1.21 tonnes ha^{-1} and 4.89 to 5.92 tonnes ha^{-1} in respective manner on pooled basis. The increase in oil content (%) recorded with sulphur at 60 kg ha^{-1} over sulphur at 20 kg ha^{-1} was 12.43.and 16.10 in 2008-09 and 2009-10 respectively. Sulphur content and uptake showed significant increase with sulphur application upto 40 kg sulphur ha^{-1} during both the years. For higher grain yields in rice-brown sarson cropping system sulphur need to be applied at 40 kg S ha^{-1} for rice along with recommended package of NPK for succeeding brown sarson crop in Kashmir valley

Keywords*:* rice-brown sarson, residual effect, sulphur, yield

Introduction

Sulphur is important for growth and development of all crops as it is related to amino acids like cystein, cystine and methionine and activity of photolytic enzymes. Poor crop production as a result of acute sulfur deficiency has frequently been reported by many scientists in different regions of India (Khan, 2000). The positive influence of residual effect of S on growth, uptake of nutrients and yield of many crops were reported by many authors in sugarcane ratoon crop, sunflower, greengram and groundnut-rice cropping sequences. In India rice occupies an area of 45 million hectare with production of 99.37 millions tonnes and with an average productivity of 2.20 tonnes ha^{-1} as against the world average of 3.92 t/ha (Anonymous, 2009). Annual production of rice from Jammu & Kashmir state is 562.4 thousand tonnes from an area of 263.01 thousand hectares (Anonymous, 2009a). In Brown sarson India ranks 2nd on area basis after China but stands at the 4th position in terms of production, contributing around 11% of the world's total production. India has nearly 6.19 million hectares under mustard with an average production of 7.37 million tonnes annually (Agricultural Statistics, 2009). The Kashmir valley has an estimated area of 64290 hectares under mustard cultivation. The production of mustard is 413000 quintals, and the oil production is 22,000 metric tonnes against the required oil consumption of 52,000 metric tones (Anonymous, 2008). Thus, 30,000 metric tonnes of oil for cooking purposes is to be fetched from outside the valley, the main source being Punjab. Hence the present investigation was taken up to study the effect of S application on growth and yield of rice and on subsequent brown sarson.

Material and Methods

The experiment was conducted at Agronomy farm of Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar, India (34°5′ N latitude and 74°5′ E longitude) during 2018-19 and 2019-20. Soil was silty clay loam in texture, low in available nitrogen and medium in available phosphorus, potassium and sulphur. The soil pH (6.80); electrical conductivity (0.28 dSm^{-1}); organic carbon (0.90 %); available N (268.0 kg ha^{-1}); P (15.4 kg ha^{-1}); available K (270.60 kg ha^{-1}); S (9.20 kg ha^{-1}); and bulk density (1.15 Mg m^{-3}) were recorded from upper 0-15 cm layer of soil. The area is designated as temperate zone. The experimental site receives 800 mm of rainfall annually. The daily average minimum air temperature during rice growth (June–september) was 13.4 °C and maximum temperature was 28.78 °C. Healthy 32 days old seedlings were transplanted (2 seedlings hill^{-1}) in the third week of June with a hill spacing of 15 cm x 15 cm. The experiment comprises of three treatments of sulphur as 20, 40 and 60 kg S ha^{-1} with three replications and a net plot size of 12 m^2. The S is complemented through application of gypsum as per the treatment schedule. In both the years the panicles m^{-2}, spikelets panicle^{-1}, grains panicle^{-1}, 1000 grain weight(g), grain and straw yield (t ha^{-1}) were recorded treatment wise. Sulphur uptake (kgha^{-1}) in rice, grains and straw samples were determined to calculate sulphur uptake. After the harvest of main crop of rice residual crop of brown sarson (*cv*. KS.101) was grown. Brown sarson received 60 kg N ha^{-1}, 30 kg P ha^{-1} and 20 kg K ha^{-1} with no sulphur application. Before and after harvest of each crop, soil samples from all plots were taken and analyzed for residual available sulphur status.

Result and Discussion

Yield attributes

The beneficial effect of added S influencing the spikelets panicle^{-1}, grains panicle^{-1}, 1000 grain weight (g), was observed in the present study with S 40 kg ha^{-1} and S 60 kg ha^{-1} recording on par values but has

significant increase over 20 kg S ha^{-1} during both the years (Table1). The increased yield attributes in the present experiments might be the cause of increased protein metabolism due to S application These results are in conformity with the findings of Duraisingh, 2001 *et al.* who reported that among the yield attributes' number of filled grains as well as percentage of filling were significantly affected by application of sulphur at 40 kg ha^{-1}.

Yield

The improvement in yield with increasing levels of sulphur from 20 to 60 kg ha^{-1} was found to be 11.09 % however, significant response was found only up to 40 kg S ha^{-1} (Table 2). Sing *et al.* (2002) also reported that application of sulphur at 30 kg and 45 kg ha^{-1} significantly increased rice grain and straw yield over control. Increase in yield due to sulphur fertilizer application could be attributed to its important role in the synthesis of proteins and S-containing amino acids as well as enhanced photosynthetic activity of the plant by increased chlorophyll synthesis. S fertilization also resulted in an increased uptake of nutrients *viz*., N, P and S thus resulting in higher yield. The higher magnitude of grain yield response indicates greater contribution of S in grain production. The similar trend of increase in grain yield due to S addition was also established by many authors (Sakal, *et al.* 2000 and Singh *et al.* 1997). Straw yield also followed the same trend as that of grain yield where the application of S increased the rice straw yield from 7.78 to 8.32 t ha^{-1} up to 60 kg S ha^{-1} on pooled basis (Table 2). The increase in straw yield due to S fertilization was mainly because of the stimulatory effect of applied S in the synthesis of chloroplast and activation of ferridoxin photosynthetic process. The results are in line with that of Misra 2003 who reported the involvement of sulphur in metabolic processes of plants and its role in protein and hormone synthesis. Sulphur application increased harvest index due to conversion of photosynthates in to grain.

Nutrients Uptake

The uptake of S in rice-brown sarson increased with increasing levels of S upto 60 kg S ha^{-1} and the significant increase was upto 40 kg S ha^{-1}(Table 3). This seems to be associated with increased S availability from applied S with a concomitant increase in S concentration and dry matter production. The results are in line with Narendranath, (2005) who reported that uptake of S by maize grain and stalk increased significantly with increasing levels of S which seems to be associated with increased S availability from applied S with a concomitant increase in S concentration and dry matter production. Increasing levels of S progressively enhanced the N uptake by rice (Table 3). Increase in N uptake may be attributed to increase in N concentration of plant and dry matter yield due to rising S levels. Such synergistic relationship between N and S has been reported by Jena *et al*. (2006). Application of S progressively increased the total P uptake up to 60 kg S ha^{-1} during both the years which might be due to better root development and beneficial effect on P uptake induced by the sulphur application Dwivedi *et al*. (2002).

Residual effect of sulphur on Brown sarson

In the present investigation, sulphur applied to proceeding *i.e.* rice crop positively affected the seed yield of succeeding brown sarson crop showing, therefore, significant residual effect (Table 4). Plots carrying residual effect of 60 kg S ha^{-1} which was statistically at par with 40 kg S ha^{-1} recorded significantly higher seed yield over 20 kg S ha^{-1}. Such beneficial effect of residual sulphur has also been demonstrated by Jaggi and Raina (2008). The highest oil content and straw yield were recorded in residual sulphur at 60 kg ha^{-1}. However, it was statistically on par with residual sulphur at 40 kg/ $^{-1}$ while as sulphur at 20 kg ha^{-1} recorded the lowest oil content and straw yield during both the years (Table 4). The increase in oil content and straw yield of brown sarson due to S fertilization was mainly because of the stimulatory effect of residual S in the synthesis of chloroplast and activation of ferridoxin photosynthetic process, its involvement in metabolic processes and its role in protein and hormone synthesis. The results were in agreement with that of Misra (2003) and Sakal *et al*. (2000). Harvest index increased progressively from plots carrying over residual effect of sulphur from 20 kg ha^{-1} to 60 kg/ha but the effect was not significant, this can be attributed due to conversion of photosynthates in to grain.

A review of the data on observed residual available sulphur (hereafter called as residual sulphur) indicate that at the end of three crops a balance of 10.32 kgs of available sulphur was found (observed) against the calculated value of 135.85 kg/ha in plots received 40 kg S ha^{-1} during two years. In plots receiving sulphur at the rate of 120 and 80 kg/ha (in the two years), though the observed residual sulphur increased upto 60 kg S ha^{-1} (Table 5). With higher levels of sulphur application the status of observed residual sulphur showed loss which might be due to immobilization of applied sulphur into organic sulphur (Aulakh *et al*. 2002). Our results further demonstrate the role of soil microbial biomass as sink and/or source of sulphur to maintain equilibrium between the demand for and supply of sulphur. The other losses were possibly through uptake by weeds, leaching, erosion and through run-off. It is therefore, suggested that to increase the efficiency of applied sulphur and save it against various types of losses; the same should be applied in 2 or 3 splits and plots be kept free of weeds during the crop growth and also during the vacant interval between two crops.

Conclusion

This study reveals significant positive direct and residual effects of sulphur upto its dose of 40 kg/ha in rice (*Oryza sativa*) – brown sarson (*Brassica compestries*) cropping sequence as reflected by yield, sulphur uptake and other yield parameters. To get optimum yield and earning, sulphur may be applied 40 kg/ha in rice crop. Even after so much of sulphur removals and losses during the period of 3 crops, there is noticeable build up of available sulphur in response to its addition at different rates. Raising second crop to utilize residual sulphur, applying sulphur in 2 or 3 splits; and keeping the fields free of weeds are some of the measures suggested to

increase sulphur use efficiency in rice (*Oryza sativa*) – brown sarson (*Brassica compestries*) cropping system in Kashmir valley.

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Table 1. Effect of sulphur (kg ha^{-1}) application on Panicles m^{-2}, spikelets panicle^{-1}, grains panicle^{-1} and 1000-grain weight (g) of rice.

Treatments	Yield attributes											
	Panicles m^{-2}			Spikelets panicles^{-1}			Grains panicles^{-1}			1000-grain weight (g)		
	2008	2009	Pooled	2008	2009	Pooled	2008	2009	Pooled	2008	2009	Pooled
Sulphur levels												
20	301.24	302.14	301.69	106.51	109.71	108.11	91.14	90.71	90.92	19.66	20.16	19.91
40	307.54	308.44	307.99	110.78	113.99	112.38	94.98	94.39	94.70	21.29	21.70	21.54
60	310.44	311.34	310.89	113.25	116.46	114.85	96.68	95.60	96.10	21.53	22.03	21.78
SE(m) ±	2.48	2.47	1.73	1.97	1.99	1.38	1.02	1.02	0.70	0.57	0.57	0.39
CD(P=0.05)	5.03	5.03	3.50	3.97	4.02	2.78	2.07	2.07	1.46	1.16	1.16	0.81

Table 2. Effect of sulphur (kg ha^{-1}) application on grain yield (t ha^{-1}), straw yield (t ha^{-1}) and harvest index of rice.

Treatments	Grain Yield (t ha^{-1})			Straw yield (t ha^{-1})			Harvest Index		
	2008	2009	Pooled	2008	2009	Pooled	2008	2009	Pooled
Sulphur levels (kg S /ha)									
20	6.07	6.15	6.11	7.73	7.82	7.78	44.62	44.65	44.64
40	6.67	6.75	6.72	8.05	8.14	8.09	45.04	45.06	45.05
60	6.71	6.86	6.79	8.27	8.36	8.32	45.11	45.14	45.12
SE(m) ±	0.55	0.53	0.38	0.66	0.65	0.46	0.10	0.09	0.06
CD(P=0.05)	1.13	1.11	0.78	1.33	1.32	0.93	0.21	0.20	0.14

Table 3. Effect of sulphur application on S, N and P uptake (kgha^{-1}) by rice-brown sarson cropping system.

Treatments	Grain (Pooled)			Straw (Pooled)		
	S-uptake	N-uptake	P-uptake	S-uptake	N-uptake	P-uptake
Sulphur levels (kg S /ha)						
20	12.70	83.47	23.12	16.43	54.77	14.18
40	14.73	92.39	25.96	18.71	59.39	15.97
60	15.13	94.53	25.98	19.23	61.05	16.38
SE(m) ±	0.38	1.83	0.60	0.39	0.91	0.85
CD(P=0.05)	0.77	3.71	1.33	0.79	1.84	1.73

Table 4. Residual effect of sulphur (kgha^{-1}) application on Seed yield (t/ha), straw yield (t/ha) and oil content (%) of brown sarson.

Treatments	Seed Yield (t ha^{-1})			Straw yield (t ha^{-1})			Oil content (%)		
	2008	2009	Pooled	2008	2009	Pooled	2008	2009	Pooled
Sulphur levels (kg S /ha)									
20	0.92	0.95	0.94	4.78	4.91	4.90	31.84	32.02	31.93
40	1.10	1.16	1.16	5.46	5.51	5.48	33.60	33.70	34.65
60	1.18	1.24	1.21	5.91	5.94	5.92	35.80	37.20	36.50
SE(m) ±	0.45	0.44	0.44	0.65	0.66	0.45	0.65	0.65	0.45
CD(P=0.05)	0.89	0.88	0.61	1.33	1.35	0.92	1.33	1.33	0.92

Table 5. Sulphur balance sheet.

Initial Soil available sulphur (kgha^{-1})	S (kg/ha) added In 2 crops of rice	Total available S (kg/ ha)	S (kg ha^{-1}) uptake by 3 crops	Observed residual S (kg ha^{-1})	Calculated residual S (kg ha^{-1})	Observed – calculated residual S (kg/ ha)
9.20	40	49.20	32.12	10.32	135.85	-125.53
9.20	80	89.20	39.53	13.10	80.47	-67.37
9.20	120	129.20	44.15	15.21	27.88	-12.67

CUSTOMISING ENZYME MIXTURE TO WHOLE COTTON SEED

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Abstract

A study was carried out to customize enzymes specific to whole cotton seed. Whole cotton seed was rich in crude protein (19.31± 0.17%) and ether extracts (14.73 ± 0.97%). The per cent glucan, xylan and arabinan in whole cotton seed were 17.49 ± 0.72, 17.45 ± 1.39 and 18.71 ± 1.29 % respectively. The experiment was carried out to fix the required enzyme level for whole cotton seed with six replications in each stage. The respective levels of *cellulase*, *xylanase*, *glucanase*, *pectinase* and *amylase* identified for whole cotton seed were 46.0, 28.0, 1.5, 2.0 and 9500 (U/g). In the last phase of the experiment, five treatments *viz.* selected level, two levels (5 % and 10 %) lower and two levels higher (5 % and 10 %) than the selected level, each with six replications in completely randomised design were tested to arrive at the "customized enzyme mixture" for whole cotton seed. Significantly ($p<0.05$) highest release of monomers per g of whole cotton seed was noticed on addition of various enzymes at selected level and was fixed as "Customized enzyme mixture for whole cotton seed". Customised enzyme mixture for whole cotton seed" was determined to be 46 U of *cellulase,* 49.6 U of *xylanase*, 46 U of *glucanase* and 42.2 U of *pectinase* and 9500 U of *amylase*.

Keywords: Customized enzyme mixture, Enzyme, Whole cotton seed

Introduction

The need to meet the feed requirements over the competition of food and feed between animals and human has led to the discovery of various newer additives. Under the category of new growth promoters; probiotics, prebiotics, synbiotics and enzymes are being tried to achieve the goal of high quality animal produce with a better economic efficiency.Enzymes have been used in animal feeds for the past two decades. The successes of feeding enzymes to mono gastric have been well documented as against the ruminants. Gaafar *et al*. (2010) in their study on efficiency of fibrolytic enzyme on high forage diets in ruminants concluded that using of high fiber total mixed ration improved productive performance and proved to be economically effective in buffaloes on enzyme supplementation. Several studies show that there is potential in supplementing exogenous fibrolytic enzymes to the ruminants (McAllister *et al.,* 2001).

However, in spite of the promising results in relation to growth rate and milk production due to fibrolytic enzyme supplementation, much variability is also noticed while including enzyme supplementation in ruminant diets. This variability may be attributed to various factors such as product formulation, under or over supplementation of enzymes, inappropriate method of supplementation, composition of the diet and other factors. The most important contributing factor as reviewed by many scientists is that the commercially available enzymes in the market for the ruminants are for the non-existent feed applications, which implies that the cock tail of enzyme mixtures are not substrate or feed specific (Beauchemin *et al.,* 2003). Hence a study was aimed to customise enzyme for some of the common feeds used by farmers for ruminant feeding. Considering the common feeding regimen in north east coastal areas of Tamilnadu and Puducherry, whole cotton seed was chosen for the study.

Cotton seed is high in protein, energy and fibre and is a good source of phosphorus and vitamin E. Cooke (2006) had extensively reviewed the usefulness of whole cotton seed as potential feed to the dairy cattle and its effectiveness as modulator of rumen volatile fatty acid production. Though, the presence of gossypol, a polyphenolic binaphthyl dialdehyde that can produce toxic effects in animals is a hindrance to its use as potential source of feed ingredient; still the unique physical characteristics of cottonseed, along with its nutritional value, have resulted in it being extensively used in diets for lactating dairy cattle (NCPA, 2011).

Materials and Methods

Proximate analyses and acid insoluble ash of six samples of whole cotton seed were carried out using standard procedures as per (AOAC, 2000) and the results were expressed on dry matter basis. Fibre fractions namely neutral detergent fibre (NDF), acid detergent fibre (ADF), hemicellulose, cellulose and lignin were estimated in six samples as per the method of Goering and Van Soest (1970). Estimation of total NSP, soluble NSP and insoluble NSP was carried out as per the procedure of Englyst (1989).

The pure enzymes chosen for the study *viz.,cellulase, xylanase, pectinase, glucanase* and *amylase* were procured from a commercial firm and assessed for their individual and associative activity. Activity of *cellulase, xylanase, pectinase and glucanase* was determined using di nitro salicylic acid (DNSA) reducing sugar method (Miller, 1959). Estimation of *amylase* activity was done based on the method of Smith and Roe (1949). One IU of *amylase* is defined as the amount of enzyme required to produce 10 per cent fall in the intensity of the blue colour of starch-iodine complex under the assay condition.

Quantity of exogenous enzymes (*cellulase, xylanase, glucanase, pectinase and amylase*) needed for maximum sugar release from whole cotton seed was determined using pure enzymes at a range starting from minimum to a higher level with broader interval initially. Later, the levels of the pure enzymes were narrowed

down and the exact level of enzymes needed was determined. Subsequently the effect due to combination of individual enzymes was studied by testing five levels, two levels (5% & 10%) lower and two levels (5% & 10%) higher than the enzyme supplemented level determined to evolve "Customised enzyme mixture to whole cotton seed".

Results and Discussion

The moisture, dry matter, crude protein (CP), crude fibre (CF), ether extractives (EE),total ash, nitrogen free extractives (NFE) and acid insoluble ash of whole cotton seed was 10.88±0.18, 89.12±0.18, 19.31±0.17, 34.19±0.39, 14.73±0.97,3.85±0.67,27.92±3.53 and0.31±0.04 per cent respectively. These results are marginally lesser when compared with that of Calhoun *et al.* (1995) who reported 23.0%, 20.8% and 17.0% ofCP, CF and ether extractives respectively. Anonymous (2009) have recorded the range of 21.8 to 34.2% for crude protein, 15.4 to 36.3% for ether extractives, 15.4 to 28.2% for crude fibre, 23 to 53.6 for Nitrogen free extractives and 3.8 to 5.0% for total ash. The average nutrient concentrations published by NRC (2001) for WCS (whole cotton seed) are 23.5% CP, 50.3% NDF, 40.1% ADF, and 19.3% EE. The lower crude protein, ether extractives and relatively higher fibre content in whole cotton seed recorded in this study may be due to variety grown in the study area. The high fibre concentrations provided by the lint and the hull fractions are desirable for maintaining effective fibre levels in the diet. The ability to maintain energy while increasing effective fibre levels makes whole cotton seed a unique feedstuff.

Table 1. Per cent fibre fractions (Mean* ± SE) of whole cotton seed used in this experiment (DMB)

S.No.	Fibre Fractions	Whole cotton seed
1.	NDF	57.11±0.71
2.	ADF	39.81±1.04
3.	Cellulose	30.37±1.17
4.	Hemicellulose	17.30±0.34
5.	ADL	9.44±0.18

NDF, ADF, Cellulose, Hemicellulose and ADL of whole cotton seed analysed in the present study were 57.11±0.71, 39.81±1.04, 30.37±1.17, 17.30±0.34 and 9.44±0.18 percent respectively. Whole cotton seed is rich in cellulose and its lignin content is also high. Myer (2012) reported 52% and 30% of NDF and ADF respectively in whole cotton seed which is lower than the values observed in this study. Anonymous (2009) have recorded the range of 40 to 54.8% for NDF and 29 to 40.1% for ADF. The relatively higher crude fibre content (34.19%) in whole cotton seed suggests the role of fibrolytic enzymes to enhance the nutritional potential.

The results of non-starch polysaccharide content (Table 2) of whole cotton seed showed higher soluble and a lesser insoluble NSP. The high content of cellulose in whole cotton seed is reflected in the high amount of total NSP and soluble NSP as against the lower content of insoluble NSP. It could be argued that the nature of cellulose present in whole cotton seed could be of the less highly ordered crystalline regions interspersed by more disordered amorphous regions. Thus, for the complete hydrolysis of cellulose, either concentrated acid or a complete *cellulase* system capable of attacking both amorphous and crystalline regions may be necessary(Sinitsyn *et al*., 1990).

Table 2. Per cent Non Starch Polysaccharide content (Mean* ± SE) of whole cotton seed (DMB)

Ingredient	Soluble NSP			Insoluble NSP			Total NSP		
	Glucan	Xylan	Arabinan	Glucan	Xylan	Arabinan	Glucan	Xylan	Arabinan
Whole cotton seed	13.39 ±1.32	11.69 ±1.07	14.10 ±1.96	3.70 ±1.39	5.09 ±1.07	4.63 ±2.19	17.49 ±0.72	17.45 ±1.39	18.71 ±1.29

*Mean of 6 samples each.

The enzyme activity of *cellulase, xylanase, pectinase* and *glucanase*purchased in the pure form from the open market was found to be 1660.67, 1410.67, 6000.00 and 830.33 U/g respectively. The *amylase* activity was 198250 IU/g. Based on this, the minimum activity of the levels of the enzymes required for maximum monomer release was arrived by incubating the feed ingredients at 42°C for 2 hours.It was found that the minimum activity levels of *cellulase*, *xylanase*, *glucanase*, *pectinase* and *amylase* required to hydrolyze whole cotton seed to release maximum level of monomers on incubating at 42 °C for 2 hours was 46.0, 28.0, 1.5, 2.0 and 9500 U/g respectively.

Enzyme preparations for ruminants are evaluated based on their capacity to degrade plant cell walls (Pendleton, 1998). These enzymes fall into general classification of *cellulases* and *xylanases*. The cell wall content of the plant refers to the crude fibre which contributes to maintenance of the rigidity of the structure involved. The crystalline regions of cellulose are rigid and not easily accessible to endo-acting *cellulase*s while the amorphous regions are easily attacked by dilute acid, endo*glucanase*s or exo*glucanase*s (Sinitsyn *et al*., 1990).

This explains the higher requirement of *cellulase* to hydrolyse per gram of whole cotton seed in order to facilitate complete hydrolysis of amorphous and crystalline regions.

Based on the nature of the polymers the enzyme requirement also varies. This is reflected in the findings of this study wherein it could be seen that more amount of *cellulase* is required when compared to *xylanase* as the levels of requirement found to be associated with the composition of cell wall fraction.The soluble starch content as indicated by the nitrogen free extractives is a measure for the amount of *amylase*required to be added to the feed which is in accordance as found in our study. Endo*glucanase*s are known to specifically cleave the internal β-1, 4 glycosidic bonds of amorphous, swollen and substituted celluloses as well as cello-oligosaccharides (Godana, 2007). These enzymes are generally inactive towards crystalline cellulose and cellobiose which could explain the lesser requirement for *glucanase* as against the *cellulase* required to hydrolyse whole cotton seed.

The effect due to combination of individual enzymes was studied by testing five levels, control, two levels (5% & 10%) lower and two levels (5% & 10%) higher than the enzyme supplemented level determined to evolve "Customized enzyme mixture to whole cotton seed". Carbohydrate monomers released per gram of whole cotton seed in various treatments due to different enzymes on incubating at 42°C for 2 hours is presented in table 3.

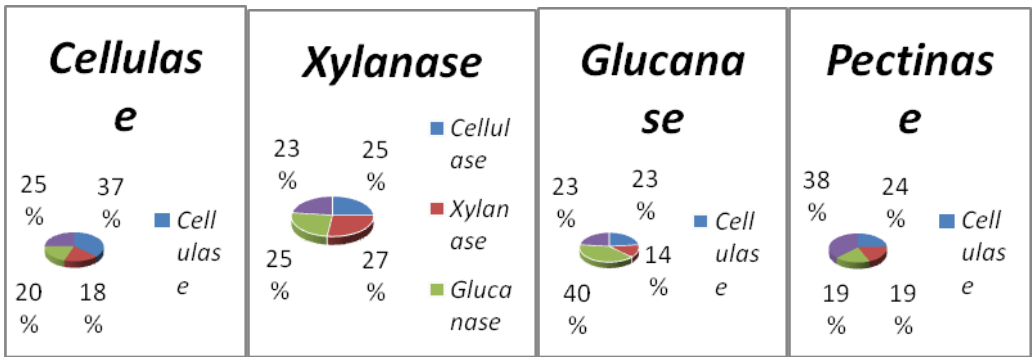
Table 3. Carbohydrate monomers released per gram of whole cotton seed in various treatments due to different enzymes on incubating at 42 °C for 2 hours

Enzyme	**Amount of Carbohydrate monomers released per gram of whole cotton**				
	Enzymes supplemented to whole cotton seed- level	**Lower Level**		**Higher level**	
	Control	5%	10%	5%	10%
Cellulase	7636.719^{b} ±755.65	5925.93^{a} ±375.97	5259.21^{a} ±543.47	4341.67^{a} ±478.09	4341.67^{a} ±483.45
Xylanase	8145.833^{b} ±806.03	6349.21^{a} ±402.82	5653.65^{b} ±584.23	4618.79^{a} ±508.61	5010.63^{a} ±522.91
Glucanase	7636.719^{b} ±755.65	5925.93^{a} ±375.97	5259.21^{a} ±543.47	4341.67^{a} ±478.09	4341.67^{a} ±483.45
Pectinase	7187.500^{b} ±711.19	5555.56^{a} ±352.47	4916.21^{a} ±508.02	4020.06^{a} ±442.68	4384.3^{a} ±457.55
Amylase	7636.719^{b} ±755.65	5797.1^{a} ±367.79	5259.21^{a} ±543.47	4341.67^{a} ±478.09	4632.47^{a} ±483.45

*Mean of 6 samples each. Mean values bearing different superscripts within a row differ significantly ($p<0.05$).

Significantly highest ($p<0.05$) release of monomers per gram of whole cotton seed on addition of the various enzymes were noticed in control group, which was the level in the "Enzymes supplemented to whole cotton seed". Therefore this level of combination of enzymes was the required level for whole cotton seed and be referred as "Customized enzyme mixture for whole cotton seed".

Further Enzyme activity assay indicated that each enzyme exhibited different levels of associated enzyme activities among them as indicated in the picture given below.



Though *xylanase* was predominant in *xylanase* it also showed activities of *cellulase* and *glucanase* that was almost equal in their rate of reaction. Similarly *pectinase* had associated activities of *xylanase* and *glucanase* that was also equal in their rate of reaction. However, it was observed that the *xylanase* had fairly good activity of other enzymes *viz.,cellulase, pectinase* and *glucanase,* which means supplementing *xylanase* in the feed will also provide fair amount of *cellulase, pectinase* and *glucanase.* Hence *xylanase* was chosen tosupplement and the contribution of the other enzymes through *xylanase* was calculated to identify the quantum of enzyme (*xylanase*) supplementation to hydrolyse whole cotton seed.

Accordingly per gram of whole cotton seed requires 46 U of *cellulase,* 28U of *xylanase,* 1.5 U *glucanase* 2 U of *pectinase* and amylase 9500 Uand based on assay for associated enzyme activity, each gram of *xylanase* was found to contain 1310.18U of *cellulase,* 1410.67 U of *xylanase*, 1310.18 U *glucanase* and 1200.68U of *pectinase* at the proportion of 25% *cellulase*, 27% *xylanase,* 25% *glucanase* and23% *pectinase.* Thus, to provide "selected levels of enzymes for whole cotton seed", 0.035g of *xylanase* can be utilized.

Conclusion

The enzymes required for hydrolyzing one gram whole cotton seed were determined to be 46, 28, 1.5, 2.0 and 9500 U/g for *cellulase, xylanase, glucanase, pectinase* and *amylase* and are referred to as "selected levels of enzymes for whole cotton seed".The pure enzymes used in this study were purchased from open market, found that none of the enzymes were pure and exhibited associative activity of the other enzymes. The proportion of associative enzyme activity of *xylanase*in percentage were, *cellulase*-25%, *glucanase*-25%and *pectinase*-23% in addition to xylanase-27%. Considering the associated activities of other enzymes, the level of enzyme required for whole cotton seed need to be revised. For example, use of 0.035 g of *xylanase* sufficient to provide the enzyme activities of 46 U of *cellulase,* 49.6 U of *xylanase*, 46 U of *glucanase* and 42.2 U of *pectinase*which can be termed as "Customised enzyme mixture for whole cotton seed" along with the addition of 9500 U of *amylase*.

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STUDIES ON IMPACT OF ORGANIC SOURCES AS BASAL AND FOLIAR SPRAY FOR HIGHER PRODUCTIVITY AND SOIL HEALTH IN IRRIGATED FINGER MILLET

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Introduction

Finger millet (*Eleusine coracana*.) is a tropical small millet indigenous to India . It is a traditional long duration, hardy and drought resistant crop. Now a days, growing health consciousness among the consumers also creates demand for this type of nutri- cereals which are anti diabetic and antioxidant in nature (Sunitha *et al*., 2010). The continuous use of inorganic fertilizers under intensive cropping system has caused widespread deficiency of secondary and micronutrients in soil. At this juncture, a keen awareness has sprung on the adoption of "organic farming" as a remedy to cure the ills of chemical agriculture. Organic farming minimizes the use of external inputs and aims at optimization of crop productivity rather than its maximization through renewal and strengthening of ecological processes and functions of farm ecosystem (Shukla *et al* .,2011). Foliar fertilization is a simple and effective method of providing nutrients to crops. This study was programmed with the objective to evaluate the basal application of organic sources and foliar nutrition on growth and yield parameters of finger millet. Application of foliar spray of organic nutrients along with chemical fertilizers would be a sound proposition in the input management leading to better yields. Also, use of organic manures and organic sprays and biofertilizers minimize cost of inorganic fertilizers, thereby act as a boom to farmers by making the waste into wealth and maintain the soil health and fertility.

Methodology

A field experiment was conducted at Centre of Excellence in Millets, Athiyandal, Thiruvannamalai during *kharif,* 2015-16 to evolve a suitable organic sources of nutrient with foliar application. Soil at the experimental site was classified as sandy clay loam . The experiment was conducted with four organic sources fertilizer and five different nutrients as organic foliar sprays under split plot design with three replications. Application of organic sources such as M_1-Farm yard manure @ 12.5 t ha^{-1}, M_2 - Vermi compost (5 t ha^{-1}) M_3 - Farm yard manure (6.5 t/ha) + sun hemp (6 t/ha) and M_4 - Inorganic source of recommended dose of fertilizer (40 : 20 :20 NPK kg/ha) as a control. Application of organic foliar sprays such as S_1-3 % Panchakavya, S_2 - 3 % Vermiwash , S_3- 3 % Jeevamruth , S_4- 5 % Coconut water and S_5 - Water spray a control

RESULTS AND DISCUSSION

Yield parameters

Yield attributes (Table 1.) of ragi was significantly influenced by the different nutrient sources and foliar spray levels to ragi . Yield attributes *viz*., no. of fingers/ear head (7.8 fingers/plant) , finger length (9.8 cm) and test weight (3.04) were maximum at Basal application of 6.5 t Farm yard manure + raising sunhemp & plough in situ on 45 DAS (B_1). Among the foliar spray treatments, F_1 (3 per cent panchakavya) had shown higher number of fingers/ear head (7.1 fingers/plant) , finger length (9.9 cm) and test weight (3.06). The interaction effects between main and sub plot treatments was found to be non significant.

Yield (Kg/ha)

The observations recorded on grain and straw yield are presented in Table 2. With regard to main plot treatments, Basal application of 6.5 t Farm yard manure + raising sunhemp & plough in situ on 45 DAS (B_1) was the best treatment than the other treatments during *kharif* season of 2015. The maximum grain yield of (2333 kg ha^{-1}) and straw yield (3559 kg ha^{-1}) was recorded during *kharif*, 2015 . The least grain yield was recorded with the treatment B2 (Vermi Compost @ 5 t ha^{-1}). Among the foliar spray treatments, F_1 (3 per cent panchakavya) had recorded higher grain yield (2223 kg/ha) and straw yield (3338 Kg/ha). Nutrient sources and foliar spray did not shown significant interaction effect on ragi yield

Conclusion

The results revealed that soil application of 6.5 t/ha of Farm yard manure + 6 t/ha of sunhemp significantly recorded higher grain yield (2333 Kg/ha) and straw yield (3559 kg/ha) of ragi. With respect to organic foliar sprays, 3 % Panchakavya enhanced the growth attributes, yield attributed like grain (2233 kg/ha) and straw yield (3338 kg/ha) respectively. Plant growth is also dependent on the rate of accumulation of dry matter. The dry matter accumulation may reflect on the economic yield in view of the fact that vegetative parts of the plant serve as a source where as grains are the sink. Increase in grain yield differed significantly due to different nutrient sources. Foliar application of organic source of nutrients at the flowering stage may improve the physiological efficiency and may play a significant role in raising the productivity of the crop. The improvement in iron and calcium content of finger millet with panchagavya spray might be ascribed to beneficial effects of panchagavya on crop quality.

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Table 1.Effect of different nutrient sources and organic foliar spray on the yield attributes of ragi during *kharif* 2015-2016

Treatments	No. of fingers / earhead					Finger length (cm)					1000 grain weight(g)				
	B_1	**B_2**	***B_3***	***B_4***	***Mean***	**B_1**	**B_2**	***B_3***	***B_4***	***Mean***	**B_1**	**B_2**	***B_3***	***B_4***	***Mean***
F_1	7.3	5.3	9.8	5.8	**7.1**	10.1	9.4	10.5	9.8	**9.9**	3.06	3.05	3.08	3.05	**3.06**
F_2	6.8	4.9	8.4	5.8	**6.5**	10.0	9.0	10.1	9.7	**9.7**	3.00	3.08	3.08	2.98	**3.04**
F_3	6.2	4.5	7.2	6.2	**6.0**	9.6	8.8	9.9	9.8	**9.5**	2.98	3.08	3.02	2.97	**3.01**
F_4	5.4	4.3	6.7	4.8	**5.3**	9.4	8.7	9.3	9.1	**9.1**	2.98	3.01	3.00	2.95	**2.98**
F_5	6.5	4.2	6.9	5.2	**5.7**	9.4	9.3	9.2	9.4	**9.3**	3.00	2.95	3.02	2.95	**2.98**
Mean	**6.4**	**4.6**	**7.8**	**5.6**	**6.1**	**9.7**	**9.0**	**9.8**	**9.6**	**9.5**	**3.00**	**3.03**	**3.04**	**2.98**	

	S.Ed	CD (0.05)	S.Ed	CD (0.05)	S.Ed	CD (0.05)
MAIN PLOT (M)	0.12	0.29	0.19	0.46	0.06	0.15
SUB-PLOT(S)	0.12	0.24	0.19	0.39	0.06	0.13
S X M	0.53	NS	0.39	NS	0.03	NS
M X S	0.68	NS	0.40	NS	0.08	NS

Table 2.Effect of different nutrient sources and organic foliar spray on yield of ragi during *kharif* 2015-2016

Treatments	Grain yield (kg/ha)					Straw yield (kg/ha)				
	B_1	**B_2**	***B_3***	***B_4***	***Mean***	**B_1**	**B_2**	***B_3***	***B_4***	***Mean***
F_1	2250	2032	2610	1999	**2223**	3450	3055	3799	3050	**3338**
F_2	2165	1990	2412	1987	**2139**	3391	2999	3698	3022	**3277**
F_3	2028	1898	2320	1945	**2048**	3211	2876	3595	3016	**3174**
F_4	1951	1675	2046	1768	**1860**	2987	2380	3208	2455	**2757**
F_5	2113	1812	2276	1911	**2028**	3199	2405	3499	3000	**3026**
Mean	**2101**	**1881**	**2333**	**1922**		**3248**	**2743**	**3559**	**2908**	

	S.Ed	CD (0.05)	S.Ed	CD (0.05)
MAIN PLOT (M)	81.4	203.2	114	286
SUB-PLOT(S)	96.4	197.3	147	301.4
S X M	192.9	NS	294.6	NS
M X S	190.8	NS	287.4	NS

EXPLORATION OF DIFFERENT CHITINOLYTIC BACTERIA AS CONSORTIUM AGAINST TIKKA LEAF SPOT DISEASE IN GROUNDNUT – AN INNOVATIVE APPROACH

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Abstract

Nutritionally groundnut is rich in minerals, vitamins, oil and proteins. Yield loss in groundnut is due to varied factors *viz.,* weeds, pest, bacteria, virus, and fungal diseases. The use of microorganism to control plant pathogen, known as biological control, is now in practice and has increasingly captured the attention of agricultural scientists as an alternative strategy for the management of plant diseases. Thus, it is important to explore new alternatives for disease control that reduces economic loss and have nil ill effects on human health. Chitinolytic microorganisms producing chitinase enzyme that have been used as biocontrol agents for several crops with promising result. Hence the present investigation was conducted to test the different chitinolytic microbes as a potential biocontrol agent against fungal disease of tikka leaf spot of groundnut. Chitinolytic bacteria (*Bacillus subtilis, Bacillus licheniformis* and *Pseudomonas fluorescens*) were isolated from the groundnut rhizosphere soil sample and efficient isolates were developed as consortium. Effect of seed treatments with the chitinolytic bacterial consortium, on the fungal disease tikka leaf spot of groundnut, (T_9 - *B. subtilis* (Bs_1) + *B. licheniformis* (Bl_3) + *P. fluorescens* (Pf_4) @ 10 ml/Kg of seed) recorded the maximum control of tikka leaf spot disease and increased the growth parameters of groundnut. The result of the present study has proved that application with a combination of the chitinolytic microbial consortium, *B. subtilis* (Bs_1) + *B. licheniformis* (Bl_3) + *P. fluorescens* (Pf_4) @ 10 ml/Kg of seed exhibited a general trend towards greater suppression of fungal disease tikka leaf spot in groundnut crop. In addition to disease control, better nutrient uptake, plant growth promotion, and enhanced crop yield was observed.

Keywords: Chitinolytic bacterial consortium, tikka leaf spot and biocontrol.

Inroduction

Groundnut (*Arachis hypogaea L.*) is a king of oil seed crop is popularly called as wonder nut and poor man's cashew nut. Groundnut plays a pivotal role in the oilseed economy of India (Thamaraikannan *et al*., 2009). It is primarily utilized as seed as they are rich source of edible oils containing fat (40-50%), protein (20-50%) and carbohydrate (10-20%). Besides, several other important dietary components are also present in groundnut such as calcium, magnesium, phosphorus, zinc, iron, potassium, niacin, folacin, vitamin E, riboflavin and thiamine (Fabra *et al.,* 2010). Groundnut crop is prone to infect by various diseases and more than 55 pathogen including fungi, bacteria and viruses have been reported to affect groundnut. The fungal disease of tikka leaf spot caused by *Cercospora personata*, the symptoms for this disease dark brown to almost black circular spots on the lower surface of leaflet. The disease cause possible yield loss is 10 to 50 per cent. The use of many common pesticides, fungicides cause serious health problems. Chitinolytic microorganisms have been used as bio control agents for several crops with promising result. These chitinolytic microbes produced chitinase enzyme have received special attention due to their role in the bio control of fungal pathogens (Mathivanan *et al*., 1998). A variety of pathogenic microorganisms contain chitin coats which provide protection against external factors. Chitinase have been employed to breakdown these protective coats and weaken the defense system of several pathogenic microorganisms and insects (Hamid *et al.,*2013). This research was designed and conducted to isolate and characterize the chitinolytic bacteria from groundnut rhizosphere soil and to formulate chitinolytic bacterial consortium against tikka leaf spot a fungal pathogen as an effective biocontrol agent.

Materials and methods

Isolation of Chitinolytic bacteria from groundnut rhizosphere soils

Chitinolytic bacteria were isolated from the rhizosphere soil samples collected from different groundnut growing areas of Tamil Nadu by serial dilution method on Nutrient agar medium, King's B medium for *Bacillus subtilis, Bacillus licheniformis* and *Pseudomonas fluorescens,* respectively by incubating at room temperature for 24 h. Colonies with characteristics of *Bacillus subtilis, Bacillus licheniformis* and *Pseudomonas fluorescens* were isolated individually and purified by streak plate method.

Survey on the fungal disease of tikka leaf spot of groundnut in Cuddalore district

A field survey was conducted to assess the extent of tikka leaf spot disease caused by *Cercospora personata* and their occurrence on groundnut in Cuddalore district of Tamil Nadu state. Ten location were selected for the study. The per cent disease index was worked out using the 0 to 9 scale according to "Phytopathometry" by Mayee and Datar (1986) as mentioned below.

Scale 0 – No symptoms on any plant
1 – 1% or less plants killed
3 – 1-10% plants killed
5 – 11-20% plants killed
7 – 21-50% plants killed
9 – 51 % or more plants killed

$$\text{Per cent Disease Index (PDI)} = \frac{\text{Sum of disease rating}}{\text{Total No. of plants observed}} \times \frac{100}{\text{Maximum grade used}}$$

Also, the infected plants showing the typical symptoms of tikka leaf spot due to infection with *Cercospora personata* were collected along with rhizosphere soil for isolation of the pathogens. The other information's regarding the soil type in which the crop is grown and the variety of groundnut cultivation was also recorded in the respective survey fields.

Preparation of culture filtrate of different chitinolytic bacteria

The effective chitinolytic bacteria *Bacillus subtilis, Bacillus licheniformis* and *Pseudomonas fluorescens* isolates were inoculated into Erlenmeyer flasks containing 50 ml of sterile Nutrient agar broth, King's B broth respectively and kept on a rotatory shaker at 100 rpm for 48 hours. Then the culture was filtered through bacteriological filter under vacuum and the filtrates thus obtained were used for the studies.

Effect of culture filtrates of different chitinolytic bacteria on the mycelial growth of *Cercospora personata* (Poisoned food technique)

The culture filtrates of different chitinolytic bacteria were separately incorporated into sterile PDA medium at 5,10 and 15 per cent by adding the calculated quantity of the culture filtrates to the medium by means of a sterile pipette. The PDA medium without the culture filtrate serves as control. The amended media were transferred to sterile Petri dishes separately @ 15 ml and allowed to solidify.

Each plate was inoculated at the center with a five-day old (9 mm) PDA culture disc of *C.personata* was measured when the mycelial growth fully covered the control plates.

Preparation of liquid formulation of Chitinolytic bacterial consortium

For the preparation of liquid formulation, the method suggested by Manikandan *et al.* (2010) was followed. The most effective isolate of *Bacillus subtilis* (Bs_1)*, Bacillus licheniformis* (Bl_3) and *Pseudomonas fluorescens* (Pf_4) was inoculated individually into respective broth and incubated at room temperature (28 ± 2˚C). Further, the respective broths were added with glycerol at 2 per cent level. After incubation period, the formulation was assessed for adequate CFU following serial dilution plating technique and the formulation thus prepared.

Seed treatment with different chitinolytic bacteria

Seed of groundnut were surface sterilized with two per cent sodium hypochlorite for 30 seconds, rinsed in sterile distilled water and dried overnight. Ten ml of chitinolytic consortium based formulated inoculums was taken in a Petri dish. To this, 100 mg of carboxy methyl cellulose (CMC) was added as an adhesive material. Seeds were soaked in chitinolytic consortium suspension for 2 hours and air dried overnight in a sterile Petri dish.

Results

The survey on tikka leaf spot disease incidence is presented in table 1. The different locations of Cuddalore district surveyed for groundnut tikka leaf spot incidence, Vadakuthu registered maximum incidence of disease (43.33%) followed by Adhinarayanapuram with (41.11%), Adhivaraganallur with (40.89%) and Muthandikuppam with (36.67%), Virupakshi (35.56%), Chinnakomatti (34.67%). The other locations *viz.,* Vegakollai (31.78%), Melpathi (27.78%) and Thondamanatham (25.56%) had moderate disease incidence, while the minimum tikka leaf spot incidence of (21.56%) was recorded in Annagramam.

The effect of culture filtrate of different Chitinolytic bacterial isolates on the mycelial growth of *Cercospora personata* (Poison food technique) assessed and presented in table - 2. In *Bacillus subtilis* isolate Bs_1 with 22.45,13.40 and 2.78 at 5,10 and 15 per cent concentration of the culture filtrate of different chitinolytic bacterial isolates respectively which was found to be the best against the maximum growth of 90 mm in the control in poison food technique. The least reduction of the growth was recorded by the isolate Bs_3 with 29.47, 18.45 and 4.78 mm at 5,10 and 15 per cent concentration of the culture filtrate respectively. *Bacillus licheniformis* Bl_3 with 22.65,12.78 and 2.45 mm at 5,10 and 15 per cent concentration of the culture filtrate of different chitinolytic bacterial isolates respectively which was found to be the best against the maximum growth of 90 mm in the control in poison food technique. The least reduction of the growth was recorded by the isolate Bl_4 with 28.47, 19.56 and 6.78 mm at 5,10 and 15 per cent concentration of the culture filtrate respectively. The mycelial growth of *C.personata* was found reduced with an increase in the concentration of culture filtrates of all the isolates of the different chitinolytic bacteria's tested and the reduction was significantly the maximum in the case of *Pseudomonas fluorescence* isolate Pf_4 with 21.62,12.86 and 1.65 mm at 5,10 and 15 per cent concentration of the culture filtrates

respectively as against the maximum growth of 90 mm in the control in poison food technique. This was followed by the isolate Pf_2 with 23.65,13.65 and 2.15 mm. The least was reduction of the growth was recorded by the isolate Pf_3 with 27.59,19.65 and 3.78 mm at 5,10 and 15 per cent concentration of the culture filtrate respectively. The three best isolates *Bacillus subtilis* (Bs_1) , *Bacillus licheniformis* (Bl_3) , *Pseudomonas fluorescence* (Pf_4) were selected for the consortium based formulations.

The effect of different treatments of Chitinolytic bacteria against tikka leaf spot disease of groundnut were assessed and presented in table - 3. The effect of seed treatment with chitinolytic bacteria either individually or as combination showed significant influence on the incidence of tikka leaf spot of groundnut when compared to control. Among the various treatments the treatment (T_9 - *B. subtilis* (Bs_1) + *B. licheniformis* (Bl_3) + *P. fluorescens* (Pf_4) @ 10 ml/Kg of seed) recorded the minimum tikka leaf spot incidence (11.70%) which was on par with that of treatment (T_2 - Carbendazim 50%WP as seed treatment @4g/Kg of seed (13.70 %). This was followed by the treatment with dual inoculation T_8 - *P. fluorescens* (Pf_4) + *B. subtilis* (Bs_1) @ 10 ml/Kg of seed (15.00 %), T_7 - *B. licheniformis* (Bl_3) + *P. fluorescens* (Pf_4) @ 10 ml/Kg of seed (17.80 %) and T_6 - *B. subtilis* (Bs_1) + *B. licheniformis* (Bl_3) @ 10 ml/Kg of seed (19.90). The individual treatment T_3, T_4 and T_5 also recorded 23.90 %, 23.00 % and 21.70 per cent tikka leaf spot incidence, respectively. The maximum of 26.90 per cent was recorded in treatment T_1 (control).

Table 1. Survey on the incidence of Groundnut tikka leaf spot disease in cuddalore district of tamil nadu

S. No.	Location	Soil type	Variety	Situation	Tikka leaf spot incidence (%)
1.	Virupakshi	Sandy loam	VRI-2	Rain fed	35.56^{d}
2.	Vegakollai	Clay loam	JL-24	Irrigated	31.78^{e}
3.	Annagramam	Clay loam	VRI-2	Irrigated	21.56^{h}
4.	Chinnakomatti	Sandy loam	Local	Rain fed	34.67^{d}
5.	Adhinarayanapuram	Clay loam	JL-24	Rain fed	41.11^{b}
6.	Adhivaraganallur	Sandy loam	Local	Rain fed	40.89^{b}
7.	Thondamanathan	Red sandy	VRI-2	Rain fed	25.56^{g}
8.	Melpathi	Clay loam	JL-24	Irrigated	27.78^{f}
9.	Muthandikuppam	Clay loam	Local	Irrigated	36.67^{c}
10.	Vadakuthu	Red sandy	VRI-2	Irrigated	43.33^{a}

Table 2. Effect of culture filtrate of different chitinolytic bacteria on the mycelial growth of *cercospora personata* (poison food technique)

S. No	Isolate Number	Mycelial Growth (mm) 5%	Per cent inhibition over control	10%	Per cent inhibition over control	15%	Per cent inhibition over control
1.	Bs_1	22.45^{a}	65.06	13.40^{a}	75.11	02.78^{a}	96.91
2.	Bs_2	26.14^{d}	60.96	17.58^{d}	70.47	04.65^{d}	94.83
3.	Bs_3	29.47^{e}	57.26	18.45^{e}	69.50	04.78^{e}	94.69
4.	Bs_4	25.36^{b}	61.82	14.95^{b}	73.39	03.48^{b}	96.23
5.	Bs_5	25.46^{c}	61.71	17.45^{c}	70.61	03.50^{c}	96.11
6.	Bl_1	23.78^{b}	63.58	13.45^{b}	75.06	02.89^{b}	96.79
7.	Bl_2	24.12^{c}	63.20	14.32^{c}	74.09	05.41^{b}	93.99
8.	Bl_3	22.65^{a}	64.83	12.78^{a}	75.80	02.45^{a}	97.28
9.	Bl_4	28.47^{e}	58.37	19.56^{e}	68.27	06.78^{e}	92.47
10.	Bl_5	26.45^{d}	60.61	17.89^{d}	70.12	05.78^{d}	93.58
11.	Pf_1	24.15^{d}	63.17	16.45^{d}	71.72	03.17^{d}	96.48
12.	Pf_2	23.65^{b}	63.72	13.65^{b}	74.83	02.15^{b}	97.61
13.	Pf_3	27.59^{e}	59.34	19.65^{e}	68.17	03.78^{e}	95.80
14.	Pf_4	21.62^{a}	65.98	12.86^{a}	75.71	01.65^{a}	98.17
15.	Pf_5	23.70^{c}	63.67	15.89^{c}	72.34	03.26^{c}	96.38
16.	Control	90	-	90	-	90	-

Means with same alphabets are statistically on par by Duncan's Multiple Range Test (DMRT) at 5% level.

Table 3. EFFECT OF SEED TREATMENT WITH CHITINOLYTIC BACTERIAL CONSORTIUM AGAINST TIKKA LEAF SPOT DISEASE INCIDENCE IN GROUNDNUT

Tr.No	Treatments	Tikka leaf spot incidence (%)				Mean
		25 DAS	50 DAS	75 DAS	At harvest	
T_1	Control	23.10^{h}	25.40^{g}	28.70^{f}	30.20^{f}	26.90^{h}
T_2	Carbendazim 50%WP as seed treatment @4g/Kg of seed	7.70^{a}	11.50^{a}	15.20^{a}	19.55^{a}	13.50^{b}
T_3	*Bacillus subtilis* (*Bs_1*) @ 10 ml/Kg of seed	19.10^{g}	22.20^{f}	25.10^{e}	29.20^{f}	23.90^{g}
T_4	*Bacillus licheniformis* (*Bl_3*) @ 10 ml/Kg of seed	17.90^{f}	21.30^{f}	24.60^{d}	28.10^{e}	23.00^{g}
T_5	*Pseudomonas fluorescens* (*Pf_4*) @ 10 ml/Kg of seed	15.20^{e}	20.60^{e}	23.20^{d}	27.60^{e}	21.70^{f}
T_6	*B. subtilis* (*Bs_1*) + *B. licheniformis* (*Bl_3*) @ 10 ml/Kg of seed	13.78^{d}	18.20^{d}	21.90^{d}	25.70^{d}	19.90^{e}
T_7	*B. licheniformis* (*Bl_3*) + *P. fluorescens* (*Pf_4*) @ 10 ml/Kg of seed	11.68^{c}	17.90^{c}	19.40^{c}	22.40^{c}	17.80^{d}
T_8	*P. fluorescens* (*Pf_4*) + *B. subtilis* (*Bs_1*) @ 10 ml/Kg of seed	8.10^{b}	14.60^{b}	16.50^{b}	20.70^{b}	15.00^{c}
T_9	*B. subtilis* (*Bs_1*) + *B. licheniformis* (*Bl_3*) + *P. fluorescens* (*Pf_4*) @ 10 ml/Kg of seed	6.30^{a}	10.75^{a}	13.40^{a}	16.30^{a}	11.70^{a}

Means with same alphabets are statistically on par by Duncan's Multiple Range Test (DMRT) at 5% level

Conculsion

In most research to date, bio control agents were applied singly to combat a Phyto pathogen. But the results of the present study have proved that application with combination of bio control agents *viz.,* B. *subtilis* (*Bs_1*) + *B. licheniformis* (*Bl_3*) + *P. fluorescens* (*Pf_4*) @ 10 ml/Kg of seed exhibited a general trend towards greater suppression of fungal disease in groundnut such as tikka leaf spot caused by *Cercospora personata.* Such enhanced suppression exerted by consortium of chitinolytic bacteria may be due to the combined action of different mechanisms and better performance of chitinolytic consortium in varied microclimate and seasons. In addition to disease control, better nutrient uptake, plant growth promotion and enhanced crop yield as observed in the present study adds another advantage over the use of fungicides in disease management strategies.

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REMOVAL OF ORGANIC POLLUTANTS FROM DOMESTIC WASTEWATER USING VERMIFILTRATION TECHNOLOGY

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Abstract
The study was conducted to treat domestic wastewater through vermifilter using earthworms *Eiseniafoetida*. The vermifilter with a volume of 158.4 L with 1500 earthworms was operated for 10 days continuously at 3 h hydraulic retention time, after the initial stabilization phase of one week. Significant organic pollutant reduction and coliform removal were observed during vermifiltration of domestic wastewater. The maximum biochemical oxygen demand, chemical oxygen demand, total suspended solids and total dissolved solids reduction were 96.0%, 87%, 90% and 80%, respectively. Significant removal of total coliforms was also observed during vermifiltration. The mean log value of total coliforms in influent was 210 MPN/100 ml and reduced to 20 MPN/100 ml with a log removal value (*K*) of 1.02.

Key words:Vermifilters, earthworms, biochemical oxygen demand, chemical oxygen demand, total suspended solids and total dissolved solids

Introduction

Sewage water management is a growing issue due to the present unsustainable practices of its disposal. The pathogen rich solid waste, sewage sludge will be produced from the sewage treatment plant is also deteriorating the situation. Vermifiltration is a biofilter with earthworms, where the earthworms digest the organic pollutants screened on the filter bed, and they passively aerate the system by burrowing action and removes pathogens (Arora and Saraswat, 2021).Sustainability can be achieved by vermifiltration system of the sewage water which produces soil enriching vermicompost and plant nutrient rich vermin water (Kumar and Ghosh, 2019). Further, sludge production will be evaded in this eco-friendly vermifiltration. The vermifiltered water is clean and disinfected enough to be reused for farm irrigation and in parks and gardens. Hence, experiments were undertaken to evaluate the vermifiltrationin removal of pollutants from domestic wastewater.

Materials and methods

The sewage was collected collected from A.D. Agricultural College and Research Institute, Trichy and analysed various physico-chemical properties as per the Standard Methods for the Examination of Water and Wastewater (APHA, 1992). Vermifilter was fabricated using acrylic sheet with a thickness of 2.5 mm with the dimensions of 60 cm × 60 cm × 88 cm. The vermin-bioreactor was filled with coarse gravel (4-5 cm) to a height of 6 cm, above that pepples (2-3 cm) was added to a height of 6 cm, followed by coarse sand of 8 cm. Above that garden soil was layered to a height of 24 cm (earthworm packing bed) where earthworms were added (1500 worms) and were allowed to acclimatize for about seven days before the experiments. After the stabilization phase, vermifilter was allowed to run for 10 days continuously with a constant HRT of 3 h. Wastewater from the drum flowed through the 0.5 inch polypropylene tube by gravity at a rate of 0.008 m^3/hr. The sewage percolated down through various layers in the vermifilter bed passing through the soil layer inhabited by earthworms, the sandy layer, the pepples, the gravel, and at the end was collected in a bucket at the bottom of the vermifilter bed. At two days interval, the treated sewage was collected from the outlet and analysed for pH, EC, BOD, TSS, TDS to assess the treatment efficiency of vermifiltration. The population of total coliforms was enumerated by the Most Probable Number (MPN) format as per the Standard Methods for the Examination of Water and Wastewater (APHA, 1992). The control biofilter bed, without earthworms was set as a comparison to evaluate the effect of earthworms as bio-filters. The control bio-filter bed was also prepared as an exact replica of the vermifiltration bed but had no earthworms added to it.

Results and discussion

Characteristics of domestic wastewater

The pH of the domestic wastewater was 8.35 and the EC was 1.26 dSm^{-1}. The total dissolved solids and total suspended solids were 420 to and 798 mg l^{-1} respectively. The total solids content was 1218 mg l^{-1}. The BOD and COD were 512 and 740 mg l^{-1} respectively. The total coliforms of domestic wastewater was 210 MPN/100ml. The Coliform bacteria (of which *Escherichia coli*is a member) are often associated with enteric pathogenic organisms and have been shown to be useful indicators of the presence of fecal contamination.

Treatment of domestic wastewater using vermifilter

The vermifilter was operated for 10 days continuously at 3 h hydraulic retention time, after the initial stabilization phase of one week. The earthworm production, growth, breed and survive in the moist environment is

very well was observed during the entire period of the experiment.The pH range of the feed was 8.16 to 8.48.The pH of treated effluent was 7.25 to7.74 in vermifilter and 7.86 to 8.20 in control. The neutralization of pH could be due to earthworm mediated rapid mineralization of organic fractions of wastewater. Earthworm activity caused an in-built pH buffering ability by decreasing the pH, hence neutralizing the sewage wastewater. The Electrical conductivity (EC) of feed ranged 1.18 to 1.39 dSm^{-1}. The EC of treated effluent was 0.22 to 0.36 dSm^{-1} in vermifilter and 0.57 to 0.78 dSm^{-1} in control. The total suspended solids (TSS) level of the feed was 404 to 430 mg L^{-1}. The TSS removal efficiency was stabilized at 87 - 90%. The TDS level of the feed was 705 to 740 mg L^{-1}.The TDS removal efficiency was 80 - 84% in vermifilter and in control was 52 - 55%. The reduction of TSSof sewage might be due to trapping of suspended solids on top of the vermifilter and processed by earthworms and fed to the soil microbes immobilized in the vermifilter. The TSS and TDS values remaining after vermibiofiltration of the sewage water were also acceptable for use of the treated sewage water for irrigation purposes i.e. TDS range of 118-120 mg L^{-1} and TSS range of 46-51 mg L^{-1}. However, values below 100 mg L^{-1} are still acceptable.

The BOD level of the feed was 506 to 520 mg L^{-1}. The BOD removal efficiency was stabilized at 95% approximately in vermifilter whereas in control only 63-68%. Since the earthworms are primarily accountable to biodegrade waste as compared to inorganic waste through enzyme as a biocatalysts to quicker the rate of biochemical reaction, BOD removal efficiency was found to be much better in vermifilter. The symbiotic and synergetic activity of earthworms and microorganism might be the reasons for the reduction of BOD from the sewage in vermifilter (Arora*et al.,* 2014).

The BOD values remaining after vermin-biofiltration of the sewage water was acceptable for use of the treated sewage water for irrigation purposes i.e., BOD range of 20-30 mg/l. On 4^{th} and 10^{th} day of the treatment, the influent and effluent samples were collected and coliform populations were enumerated and the resultant treated water from the vermifilter was well within the limits of the requirements of the irrigation water. The mean log value of total coliforms in influent was 210 MPN/100 ml and reduced to 20 MPN/100 ml with a log removal value (*K*) of 1.02.

Microbial activity in vermifilter

The microbial population viz., bacteria, fungi and actinomycetes was enumerated for the soil collected from the soil of both vermifilter and control bed on 10^{th} day of treatment process. The results showed that there is enhance in the population of the microorganisms in the vermifilter soil than control. The population of the Bacteria, fungi and actinomycetes in the vermin-bioreactor is more in number than control. The more number of microbial populations in vermi-bioreactor attributed the higher removal of pollutants from sewage.

Conclusion

Based on the results obtained from the experiments, it could be concluded that thevermifiltration technology was suitable for sewage treatment at low cost with environment friendly. The BOD, TSS, TDS and *E.coli* were reduced by 95%, 90% 84% and 90 % respectively at 2 h HRT. The organic matter of sewage were consumed by earthworms and convertedinto value added vermicompost.

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PARASITIC NEMATODES ASSOCIATED WITH VEGETABLE CROPS IN KAMMAPURAM BLOCK OF CUDDALORE DISTRICT, TAMIL NADU

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Abstract

Plant-parasitic nematodes are the major biotic stressor in crop cultivation. Correct identification of nematode species is essential for choosing the proper methods of control. The economic consequences of crop losses caused by nematodes come in many variations and are associated with a decrease in the crop quality and yield. The assessment of such losses and periodic updates of these estimates may be of great use to set research priorities.), The nematode genera viz., Hoplolaimusspp. and Rotylenchulus spp. were found to associated with all the vegetable crops. However, density was highly variable from field to field and with in the same locality. The Meloidogyne spp. is occurred in higher absolute density (64.58/100cc soil) when compared to other nematodes. The prominence value (3.86) also recorded higher for Meloidogyne spp. The ectoparasitic nematode, Tylenchorhynchus spp. recorded in higher density (27.71 /100 cc soil) next to root-knot nematode and found with the absolute frequency of 62.5 %. The scutellonema spp. recorded only in samples collected from elephant foot yam.The dominance of Meloidogyne sp. on vegetable crops was previously reported by other researchers, who observed that these nematode species are abundant in vegetable farming. RKN causing yield losses in essential vegetable crops, such as cucumber (85%), tomato (59%), zucchini (40%), watermelon 36%, and lettuce (29%).

Keywords: *Plant parasitic nematodes, Vegetable nematodes, Community analysis, Meloidogyne Spp.*

Introduction

Plant-parasitic nematodes are the major biotic stressor in crop cultivation. Correct identification of nematode species is essential for choosing the proper methods of control. The economic consequences of crop losses caused by nematodes come in many variations and are associated with a decrease in the crop quality and yield. The assessment of such losses and periodic updates of these estimates may be of great use to set research priorities. In addition to that, it can serve as a benchmark for policy planners (Kumar *et al.,* 2020).

Materials and methods

This work focuses on investigating the plant-parasitic nematodes that affect vegetable crops. The study took place in the Kammapuram block of Cuddalore district (Tamil Nadu) between May 2019 and May 2020 and involved 64 samples of soil and roots of eight different vegetable crops *viz*., chillies, tapioca, brinjal, snakegourd, gogra, bhendi, bittergourd and Elephant foot yam. These locations were selected based on their level of importance for vegetable production. In each location, there were three samples per crop randomly taken. A representative sample (100 cc soil + 10 g root) of each location was collected.

Nematodes were extracted separately from roots and soil the each collected sample. The roots were gently washed to remove as much soil as possible and then cut into pieces of about 0.6 cm. Nematodes were removed from the 10 - gram root sample using the modified Berman method for 48 h. The suspensions of nematodes were collected in beakers then the supernatant was poured into a 10 ml tube and mixed with hot (65 °C) 4% formalin. The tubes were kept in a refrigerator at 4 °C until nematodes were identified and their population density assessed. Endoparasitic nematodes were examined on a root tissues using a stereoscopic microscope (15 × magnification). Nematode genus identification was done based on the morphological characters. The density of the each plant parasitic nematodes (both root and soil) were counted in each samples. Community analysis of the genus was done by using Norton's formula.

Result and Discussion

Eleven genus of plant parasitic nematodes were found associated with eight vegetable crops in Kammapuram block (Table 1), The nematode genera viz., *Hoplolaimus spp.* and *Rotylenchulus spp.* were found to associated with all the vegetable crops. However, density was highly variable from field to field and with in the same locality. The *Meloidogyne spp.* is occurred in higher absolute density (64.58/100cc soil) when compared to other nematodes. The prominence value (3.86) also recorded higher for *Meloidogyne spp.* The ectoparasitic nematode, *Tylenchorhynchus spp*. recorded in higher density (27.71 /100 cc soil) next to root-knot nematode and

found with the absolute frequency of 62.5 %. The *scutellonema spp*. recorded only in samples collected from elephant foot yam.

The dominance of *Meloidogyne sp.* on vegetable crops was previously reported by other researchers, who observed that these nematode species are abundant in vegetable farming. RKN causing yield losses in essential vegetable crops, such as cucumber (85%), tomato (59%), zucchini (40%), watermelon 36%, and lettuce (29%) (Gullino *et al*., 2019)

Table 1. Occurrence of plant parasitic nematodes in vegetable crops in Kammapuram block of Cuddalore district

S.No	Nematode species	Absolute density	Relative density	Absolute frequency	Relative frequency	Prominence value
1	*Helicotylenchus spp.*	26.76	11.79	62	14.64	2.10
2	*Pratylenchus spp.*	21.88	9.64	37.5	8.85	1.34
3	*Rotylenchus spp.*	23.04	10.15	70.00	16.53	1.93
4	*xiphinima spp.*	13.93	6.16	43.75	10.33	0.92
5	*Hoplolaimus spp.*	23.37	10.29	46.00	10.86	1.56
6	*Longidorus spp.*	8.41	3.69	17.18	4.05	0.35
7	*Tylenchorhynchus spp.*	27.71	12.37	62.5	14.76	2.18
8	*Criconema spp.*	7.17	3.16	26.56	6.27	0.37
9	*Meloidogyne spp.*	64.58	28.45	35.93	8.4	3.86
10	*Radopholus spp.*	6.78	3.10	17.18	4.00	0.28
11	*Scutelonema spp*	3.37	1.15	4.68	1.1	0.07

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BREEDING SYSTEM AND POTENTIAL POLLINATORS OF *Tamarindus Indica* L.

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Abstract

Tamarind is a prominent, highly valued, multipurpose tree grown in different parts of the country to support the livelihood of the rural population. It is a cross-pollinated tree and the seedling progenies exhibit a higher level of variation in different vegetative, reproductive, qualitative and quantitative traits.The fertility and viability of pollen grains of tamarind trees were estimated and showed no significant differences between Tamarind Gemetic Resources. Wide range of insects, including bees, wasps, and butterflies were attracted to the tamarind flowers. The collected insects were identified and classified into seven different groups. Insects belonging to the Hymenoptera order have been recorded as the most common pollinators of the tamarind tree.

Key Words: Tamarind, Pollen, Pollinator, Insects and Viability

Introduction

Tamarindus indica L. is found throughout much of the tropics. It is grown unattended in backyards, roadsides or wastelands (Gunasena and Hughes, 2000). It is suited for avenue plantings in roadsides, in and around villages as multipurpose trees for agroforestry systems or as fire break for forest margins. The wood is used for making furniture, tool handles, charcoal and fuel wood. The leaves are an important source of food and herbal medicine and the edible pulp of ripe fruits are used as flavoring agent in soups, jams, chutneys, sauces, and juices (Isholaet *al.,* 1990). The fruit pulp is the richest natural source of tartaric acid (8 - 18 %). It is the main acidulant used in the preparation of foods in India and other Asian countries (Shankaracharya, 1998).Reproductive success of cross pollinated trees is determined by quantity and quality of the gametes and off springs produced.

Pollination is essential to life on earth and over millions of years, bees and other insects have played important role for ensuring food security and nutrition while also preserving biodiversity and vibrant ecosystems for plants and humans.Although pollinating insects are most likely a major determinant of floral evolution and diversification, other insects may have a negative impact on reproductive success. As a result, understanding the pollinators that transfer pollen from one flower to another without damaging the floral parts is critical.

Many tropical fruit trees are not the priority of massive international markets, but they are still valuable in local and national economies as they are harvested by rural populations for local consumption and small-scale commercialization. Priorities for the economic development of rural populations are perceived to be population management and improving the quality and consistency of fruit production (Bonkoungou *et al.,* 1998). However, many of these important fruit trees have been defined by a lack of information on pollination biology. The degree to which plants rely on pollen vectors for seed production is determined by their breeding systems. Plants that are dioecious or genetically self-incompatible, rely entirely on cross-pollination for seed production (Richards 1986). Similarly, *Tamarindus indica* is a self-incompatible tree that relies entirely on cross-pollination (Nagarajan *et al.,* 1998).

To comprehend the reproductive ecology of tamarind populations, we must first identify the potential pollinators and their diet to understand how they participate in pollination. The cross-pollination had a direct impact on yield in tamarind tree plantations; therefore, at least five different genetic variants should be included during establishing commercial Tamarind orchards (Nagarajan et al., 1998). However, the information on potential pollinators which pollinate tamarind flowers was scanty. Therefore the present study aimed to test pollen viability, fertility and identify potential pollinators of Tamarind at the Institute of Forest Genetics and Tree Breeding (IFGTB) in Coimbatore, Tamil Nadu.

Materials and Methods

Study Site

Five Tamarind trees at the age of 25 years located at different places on the Forest Campus campus, Coimbatore were selected and observed for two continuous flowering seasons during 2019 -2020. The geographic position of the study area is N 11°018'.77'' latitude, E 76°94'.71'' longitude and elevation 312 MSL. The climate of this area receives rains of both South West and North East monsoons. Annual Rainfall is 750 mm and temperature varies from 23ºC to 35 ºC.

Breeding System

Breeding system of Tamarind Trees were tested with four different hand pollination techniques.Controlled pollination experiments for understanding three breeding systems namely Autogamy, GeitonogamyXenogamy andApomixis were undertaken. For each treatment, hundred flowers,

i) Autogamy: The flower buds were bagged with paperbags without emasculation one day priorto anthesis.
ii) Geitonogamy: The buds were emasculated 6–8 h beforeanthesis, Hand pollinated using pollen from other flowers of the same tree and bagged.
iii) Xenogamy: The mature buds were emasculated, pollinatedusing pollen from different trees
iv) Apomixis :Mature buds were emasculated and baggedwithout pollination.

The pollination studies were conducted in randomized block design consisting of five treatments with three replications. The data pertaining to the fruit set percentage in different breeding methods was tabulated and statistically analyzed using statistical software SPSS. The pollination studies were examined across the five treatments using analysis of variance (ANOVA) and comparison between the treatment means was done at 5% probability level. The standard error of mean was calculated for each treatment using three replications and presented as ±SE.

Pollinator interaction

Flowering branches of each of the trees were tagged with aluminum plates. Flower visitors and their foraging time were observed on the selected branches from 6 a.m. to 6 p.m. The flower visitors observed to pollinate the flowers of Tamarind were collected. Collected insects were killed in a glasscontainer containing cotton saturated with CCl% and sent to Entomologist for identification. Diversity,abundance, timing of visitation, and behaviour of insects were recorded.Pollen grains adhering to the insects' bodies were observed by a Scanning Electron Microscope to examine the pollen transfer nature.

Results and Discussion

The breeding system operative in Tamarind were tested with different pollination systems like autogamy, geitonogamy, xenogamy and Apomixes. The statistical test showed significant size difference ($p = 0.05$) among different pollination methods. The initial fruit set under self-pollination varied 3.25 to 4.50 and all the fruits were dropped due to abortion, Significantly highest fruit set was recorded inXenogamy(85.25%), compared to autogamy. However,Interestingly, there was no fruit set observed in individual flowers emasculated and bagged with beforeanthesis to ApomixisThe species showed maximum fruit set in allogamy and open pollination compared to autogamy and autogamy.

A preliminary study of potential pollinators of tamarind flowers on a forest campus found that the tamarind flowers attracted a diverse range of pollinators, including bees, wasps, and butterflies. From 6 a.m. to 6 p.m., various insect species visited the flowers in search of food. The identified insect species were classified into seven groups (Table – 1). The bees (*Apisdorsata, A.florea, A. cerana*) are the first to visit the flowers and begin collecting pollen and nectar. Their activity begins at 6 a.m., gradually increases from 7.30 to 9 a.m., and then gradually decreases in the evening, eventually stopping around 5.30 p.m., or after sunset. In this study, bees of the genus Apis were the most frequent flower visitors. Each insect species visits the flowers at a different time.

Since the tamarind flowers are nototribic, the size and nectar probing nature of flower visitors plays an important role in pollen transfer. The Apis was the most noticeable visitors; they landed on any of the three petals and then probed for nectar. Pollen is deposited nototribicallyon the bee as it approaches the flowers to probe nectar. When they approached another flower, the stigma brushed up against the bees' dorsal side, allowing them to collect pollen. The occasional visits of Xylocopasps also influenced pollination, and due to its larger body size, it came into contact with both essential organs at the same time. Flowers were frequently detached from inflorescences due to their landing nature and body weight. The digger bee (*Anthophora cingulate*) was more active and spent less time on individual flowers, allowing it to visit multiple flowers. When compared to other pollinator groups, the Hymenoptera bees visited the flowers on a consistent and regular basis to collect nectar. The *Macroglossumstellatarum* (Hummingbird Hawk Moth) and Panarasps.passed through the flowers on occasion, but their probing did not appear to be for effective pollen transfer. They simply inserted their long proboscis into the flower and collected the nectar, but their proboscis was insufficient to contact any vital parts of the flower. *M. stellatarum* prefers to probe the nectar while gliding rather than landing on flowers. The probing behavior of the sunbird (Leptocomasps.) is similar to that of *M.stellatarum* and *Parnara*sps. The time of maximum pollination activity in the morning coincided with maximum pollen availability and peak period of stigma receptivity.

Table 1. List of flower visitors and foraging nature in*Tamarindusindica*L..

S.No	Order	Family	Species	Visiting time	Duration of Forage (sec)	Foraging Nature
1.	Hymenoptera	Apidae	Apisdorsata	6 am to 6 pm	7 – 10	p
			Apisflorea	6 am to 6 pm	7– 10	N&P
			Apiscerana	6 am to 6 pm	7– 10	N&P
			Xylocopasps.	No specific time	5 – 7	N&P
		Anthophoridae	Anthophoracingulata	No specific time	3 – 4	P
2.	Lepidoptera	Sphigidae	Macroglossum stellatarum	No specific time	5 – 6	P
3.	Holometabola	Hesperriidae	Parnarasps.	4 pm to 5.30 pm	8 – 9	N&P
4.	Passeriformes	Nectariniidae	Leptocomasps.	No specific time	3 – 4	N&P
5.	Rodentia	Sciuridae	Funambulus palmarum	No specific time	2 – 3	P

Conclusion

The Breeding system of Tamarind reveals that it is self incompatible and cross pollinated tree species.The insects in the group Hymenoptera were always present at the peak of anther dehiscence and abundance during foraging activity. Furthermore, the insects were found to be carrying a significant amount of pollen, indicating their role in pollen transfer. The present study's findings indicated that the identified potential pollinators, particularly the group Hymenoptera, are effective pollinators of the *Tamarindusindica*in the forest campus. Future research should conduct similar studies in different tamarind tree sampling sites with varying environmental conditions to determine if other physical constraints affect the presence and pollinating behavior of the identified insect pollinators. Other insect orders that could be potential pollinators should also be investigated.

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EFFECT OF SEED PELLETING WITH NUTRIENTS AND FOLIAR SPARY OF NUTRIENTS AND PLANT GROWTH REGULATOR ON GRAIN YIELD AND NUTRIENT UPTAKE OF IRRIGATED BLACKGRAM (*Vigna mungo* L.)

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Abstract

Pulses are important food crop and well known for its nutritive value. India is the largest producer and consumer of pulses in the world. Among various pulses blackgram (*Vigna mungo* L.) has its own importance. Nutrients play a pivotal role in increasing the seed yield in pulses. The lack of nutrients during the critical stages of crop growth leads to nutrient stress and leads to poor yield and productivity of the crop. The yield levels of pulses are influenced by nutrient uptake by the crop. In the present study a treatment combination of DAP 40 g kg^{-1} seed, DAP 40 g + KCl 10 g kg^{-1} seed, DAP 40 g + KCl 10 g + Gypsum 20 g kg^{-1} seed and control in main plot and foliar spray treatments *i.e.*, DAP 2%, DAP 2% + KCl 1%, DAP 2% + NAA 40 ppm, DAP 2% + KCl 1% + NAA 40 ppm at two(30 & 45 DAS) and three (30,45 & 60 DAS) times totally eight treatment in sub plot were evaluated and found that a treatment combination of DAP 40 g + KCl 10 g + Gypsum 20 g kg^{-1} seed and DAP 2 % + KCl 1 % + NAA (40 ppm) at 30, 45 & 60 DAS registered higher grain yield and NPK uptake by blackgram crop in both the seasons.

Keywords: *Blackgram, Seed pelleting, DAP, KCl, NAA*

Introduction

Pelleting of seeds with nutrients will give an initial boost for germinating seeds and growing seedlings (Kavitha *et al*., 2003). Hence the seed pelleting as pre - sowing treatment is a boon to the black gram to have better germination, establishment and early vigour of the seedlings for the effective utilization of resources. The lack of nutrients during the critical stages of crop growth leads to nutrient stress and leads to poor yield and productivity of the crop. Foliar application of major nutrients like DAP and KCl was found to be as good as soil application and satisfy the needs at critical stages. Foliar spray of K provides support to the crop to withstand moisture stress conditions by maintaining proper water status in the plants. . Foliar application of NAA reduced the flower drop and improved the growth, flower and fruit setting of Chickpea (Upathyay, 2002). Similarly the application of DAP and NAA as foliar spray significantly improved the yield attributing characters by reducing flower shedding resulted increased number of pods per plant, (Ravisankar *et al.*, 2003).

Materials and methods

Field experiments were conducted at AC&RI, Killikulam, India, , to study the effect of seed pelleting with nutrients and foliar spray of nutrients and plant growth regulator on growth and yield of irrigated blackgram. The laboratory study was conducted with four seed pelleting treatments *viz.* DAP 40 g kg^{-1} seed, DAP 40 g + KCl 10 g kg^{-1} seed, DAP 40 g + KCl 10 g + Gypsum 20 g kg^{-1} seed and replicated four times. The experiments were laid out in split plot design, replicated thrice. The treatments comprised of four seed pelleting treatments *viz.*, DAP 40 g kg^{-1} seed, DAP 40 g + KCl 10 g kg^{-1} seed, DAP 40 g + KCl 10 g + Gypsum 20 g kg^{-1} seed and control in main plot and foliar spray treatments *i.e.*, DAP 2%, DAP 2% + KCl 1%, DAP 2% + NAA 40 ppm, DAP 2% + KCl 1% + NAA 40 ppm at two(30 & 45 DAS) and three (30,45 & 60 DAS) times totally eight treatment in sub plot.

Results and discussion

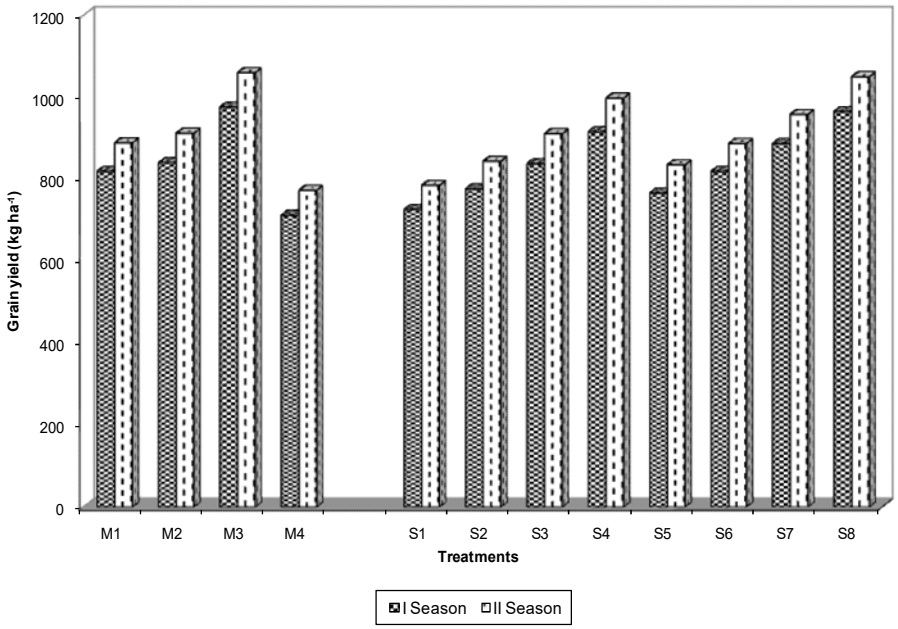
Grain yield

The grain yield of blackgram was significantly influenced by different seed pelleting and foliar spray treatments imposed in the present study.

Among the seed pelleting treatments, seed pelleting with DAP plus KCl and gypsum (M3) significantly produced higher grain yield of 976 and 1060 kg ha^{-1} during both the seasons respectively. This was followed by seed pelleting with DAP plus KCl (M2 – 840 and 912 kg ha^{-1} during both the seasons respectively), which was on par with seed pelleting with DAP alone (M1 – 819 and 889 kg ha^{-1} during both the seasons respectively) The lowest yield was contributed by the control without seed pelleting (712 and 773 kg ha^{-1} in both the seasons respectively).

The different foliar spray treatments also had significant influence on the yield. Among the foliar sprays, foliar spray combination of DAP plus KCl and NAA at 30, 45 and 60 DAS (S8) registered significantly the maximum yield of 965 and 1050 kg ha^{-1} respectively during both the seasons. This was on par with foliar spray combination of DAP plus KCl and NAA at 30 and 45 DAS (S4 – 916 and 998 kg ha^{-1}). The lowest yield was recorded under DAP foliar spray alone at 30 and 45 DAS (726 and 785 kg ha^{-1} during both the season respectively) and it was on par with S2 and S5 (Fig. 1).

Fig. 1. Influence of seed pelleting with nutrients and foliar spray of nutrients and plant growth regulator on grain yield (kg ha^{-1})



Nutrient uptake

Nitrogen uptake

The data on N uptake indicated that different seed pelleting practices significantly influenced the N uptake. Nitrogen uptake was the highest (58.45 and 66.15 kg ha^{-1}) under seed pelleting with DAP plus KCl and gypsum (M3). The lowest uptake was recorded by the control without seed pelleting (42.61 and 48.23 kg ha^{-1} respectively for both the seasons). Application of DAP plus KCl and NAA as foliar spray at 30, 45 and 60 DAS (S8) enhanced the N uptake in blackgram. The N uptake was higher than other treatments (57.78 and 65.72 kg ha^{-1} respectively during both the season) and this was on par with same foliar spray twice at 30 and 45 DAS (54.84 and 62.93 kg ha^{-1}). The minimum N uptake was registered by DAP foliar spray twice at 30 and 45 DAS and it was on par with DAP foliar spray at 30, 45 and 60 DAS. The interaction between seed pelleting and foliar spray was absent (Table 1).

Phosphorus uptake

The different seed pelleting treatments exhibited significant variation on P uptake. Maximum P uptake of 13.24 and 14.73 kg ha^{-1} was registered by seed pelleting with DAP plus KCl and gypsum, whereas the lowest P uptake was recorded under control (9.65 and 10.74 kg ha^{-1} respectively during both the seasons). Regarding the foliar spray treatments, the maximum P uptake was registered with foliar spraying of DAP plus KCl and NAA combination at 30, 45 and 60 DAS (13.09 and 15.06 kg ha^{-1} respectively during both the seasons). This was on par with treatment of same combination of spray at 30, and 45 DAS. The lowest P uptake was recorded under foliar spray of DAP twice at 30 and 45 DAS with the values of 9.84 and 11.08 kg ha^{-1} respectively during both the seasons and it was on par with (S2 and S5). The interaction between seed pelleting and foliar spray treatments was found non-significant (Table 1).

Potassium uptake

The higher K uptake of 28.65 and 33.39 kg ha^{-1} respectively during both the seasons was recorded under seed pelleting with DAP plus KCl and gypsum against the control (20.89 and 24.35 kg ha^{-1} respectively during both the seasons). The different foliar spray treatments also had significant influence on the K uptake of blackgram and was higher under foliar spray of DAP plus KCl and NAA at 30, 45 and 60 DAS (28.31 and 33.07 kg ha^{-1} respectively during both the seasons) and it was on par with the same spray combination twice at 30 and 45 DAS (S4) The interaction effect between seed pelleting and foliar spray treatments was not observed. Increased uptake of N, P and K under combined seed pelleting with DAP plus KCl and gypsum treatment was due to development of vigorous and healthy plants from the establishment stage itself and the healthy root system and the resultant DMP might have resulted in better uptake of nutrients and high analytical value. Application of foliar spray of DAP plus

KCl and NAA also enhanced the NPK uptake due to enhanced growth habit of crop ultimately the reason for effective absorption of the nutrients by the crop and more DMP which accounted for higher uptake (Table 1).

Table1. Influence of seed pelleting with nutrients and foliar spray of nutrients and plant growth regulator on nutrient uptake by blackgram (kg ha^{-1}).

Treatment		I season			II season		
Seed pelleting		N	P	K	N	P	K
M_1 -	DAP 40 g kg^{-1} seed	49.03	11.11	24.03	55.49	12.35	28.01
M_2 -	DAP 40 g + KCl 10 g kg^{-1} seed	50.27	11.39	24.64	56.90	12.67	28.72
M_3 -	DAP 40 g + KCl 10 g + Gypsum 20 g kg^{-1} seed	58.45	13.24	28.65	66.15	14.73	33.39
M4 -	Control	42.61	9.65	20.89	48.23	10.74	24.35
SEd		1.03	0.23	0.50	1.17	0.26	0.59
CD (0.05)		2.51	0.57	1.23	2.85	0.63	1.44
Foliar spray							
S_1 -	DAP 2 % at 30 & 45 DAS	43.44	9.84	21.37	49.84	11.08	25.41
S_2 -	DAP 2 % + KCl 1 % at 30 & 45 DAS	46.48	10.53	22.82	52.26	11.44	25.62
S_3 -	DAP 2 % + NAA (40 ppm) at 30 & 45 DAS	50.19	11.37	24.59	58.50	12.36	28.84
S_4 -	DAP 2 % + KCl 1 % + NAA (40 ppm) at 30 & 45 DAS	54.84	12.42	26.87	62.93	14.18	31.26
S_5 -	DAP 2 % at 30, 45 & 60 DAS	45.83	10.38	22.41	50.64	11.67	26.26
S_6 -	DAP 2 % + KCl 1 % at 30, 45 &60 DAS	49.04	11.11	24.03	54.28	12.20	28.67
S_7 -	DAP 2 % + NAA (40 ppm) at 30, 45 &60 DAS	53.12	12.03	26.03	59.34	13.31	29.80
S_8 -	DAP 2 % + KCl 1 % + NAA (40 ppm) at 30, 45 & 60 DAS	57.78	13.09	28.31	65.72	15.06	33.07
SEd		1.74	0.40	0.85	1.97	0.44	1.00
CD (0.05)		3.49	0.79	1.71	3.95	0.88	2.00

Interaction	**SEd**	**CD (0.05)**	**SEd**	**CD (0.05)**	**SEd**	**CD (0.05)**	**SEd**	**CD (0.05)**	**SEd**	**CD (0.05)**	**SEd**	**CD (0.05)**
M at S	3.42	NS	0.77	NS	1.68	NS	3.87	NS	0.86	NS	1.96	NS
S at M	3.49	NS	0.79	NS	1.71	NS	3.95	NS	0.88	NS	2.00	NS

Conclusion

Adoption of various seed pelleting and foliar spray treatments had significant impact on NPK uptake and followed similar trend as the trait of grain yield. The soil available N after harvest was more under the plots sown with unpelleted seeds and foliar spray of DAP 2% alone at 30 and 45 DAS. Available soil P and K also was higher under control (no pelleting) and foliar spray of DAP 2% alone at 30 and 45 DAS. Higher grain yield registered under seed pelleting with DAP 40 g plus KCl 10 g and gypsum 20 g kg^{-1} of seed. Under foliar spray, the above said characters were recorded higher with foliar spraying of DAP 2% plus KCl 1% and NAA (40 ppm) at 30, 45 and 60 DAS.

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A STUDY ON SALINITY AND DROUGHT STRESS ON GENOTYPES OF BLACKGRAM (*Vigna mungo L.)*

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Abstract

Pulses are most important crops as they have high nutritious value. In India, there are wide varieties of pulse crops which belong to Fabaceae family, which are grown throughout the year. Among the pulse crops black gram (*Vigna Mungo*) is the most common and can efficiently fix atmospheric nitrogen These crops undergoes several environmental stresses such as the biotic and abiotic stress. Among the environmental stresses the abiotic stress which is due to the non-living factors is more predominant among pulse crops. This causes various effects on plant metabolism. In the abiotic stressess, the drought stress and salinity is recurrent and unavoidable. During the stress condition, the plants produce lot of responses to overcome these stresses. One such response is the production of various anti-oxidative enzymes. In this study, two black gram varieties (CO6 and VBN8) are taken for analysing the anti-oxidative enzymes catalase and ascorbate peroxidase during abiotic stress. Salt and drought stress were artificially induced using the stress inducing agents such as sodium chloride (NaCl) and poly ethylene glycol (PEG) respectively in 25 days old plants. In the alternative days (1, 3 and 5) the plant samples were taken for the study Based on the studies, the black gram genotype VBN8 had high concentration of catalase and ascorbate peroxidase in salinity and drought condition as compared to the genotype CO6.

Keywords: *Black gram, Drought stress, Salt stress, Catalase, Ascorbate peroxidase*

Introduction

Black gram (*Vigna mungo*) originated in India, where it has been in cultivation from ancient times and is one of the highly prized pulses. It belongs to the family : Fabaceae, kingdom: Plantae, order: Fabales, Genus:Vigna, species : *V. mungo*. Mung bean seeds are rich in nutrients including essential amino acids, fatty acids, fibers , minerals and vitamins (Nair *et al*., 2013; FAO, 2016; Fulle, 2007). Pulses were grown under rainfed condition in soils of low fertility and poor nutrient management (Harisudan *et al*., 2009). Stress in plants refers to external conditions that adversely affect growth, development or productivity of plants. Plant stress can be divided into two primary categories namely abiotic stress and biotic stress. Abiotic stress imposed on plants by environment may be either physical or chemical, while as biotic stress exposed to the crop plants is a biological unit like diseases, insects, etc (Verma *et al*., 2013). Plants are encountered by number of abiotic stresses which impact on the crop productivity worldwide. These abiotic stresses are interconnected with each other and may occur in the form of osmotic stress, malfunction of ion distribution and plant cell homeostasis. The abiotic stresses occurring in plants include heat, drought, Salinity, cold etc (Audil Gull *et al*., 2019). In these abiotic stress the drought and salinity is more recurrent. The crops are highly responsive to environmental and abiotic factors which can affect its yield (Harisudan, 2020).

Drought is an emerging threat for agriculture plant production of majority of the crops including mungbean. It is continuously expanding its dimensions at global level due to rapid and drastic changes in climatic conditions, limited water sources and uneven rainfall (Fahad *et al*., 2017). Drought is caused due to no water or less water availability and it vary according to seasons and areas. Soil moisture stress will affect survival of different types of propagules differently (Harisudan *et al*., 2010). Salinity depends upon the quantity of salt concentration in the soil and in water supplied during irrigation (Parul Parihar *et al.,* 2014). It is mainly due to high accumulation of magnesium (Mg), calcium (Ca), sodium (Na). Salinity stress influences all stages of mungbean growth, physiology and development throughout its life cycle (Sehrawat *et al.,* 2019). Though salt ions are vital for plant growth, excess salt concentrations can reduce the water potential of the soil and also its accumulation can lead to cell shrinkage (Munns, 2002). Drought and salinity stress in mungbean affects every developmental stage of a plant and also the response of the plant to this stress is dependent on several factors such as variety, concentration of the stress inducing agents and at which stage of the growth the stress is applied. Plants exhibit various mechanisms against these stress factors. One wide range of response is production of various anti-oxidative enzymes to overcome these stresses. The enzymatic components of the antioxidative defence system comprise of several antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), guaiacol Peroxidase (GPX), ascorbate peroxidase (APX), Monodehydroascorbate reductase (MDHAR), Dehydroascorbate reductase (DHAR), and glutathione Reductase (GR). These enzymes operate in different sub cellular compartments and respond when cells are exposed to oxidative stress. Under normal circumstances, the concentrations of freeradicals(oxygen) remain low because of the activity of protective enzymes. These reactive free radicals are constantly generated in living organisms through biological reactions (Baskaran and Muruganandam, 2017)

In this study the NaCl and PEG was used to induce the stress in 25 days old plants that were maintained in the green house. Then the best genotype that shows more resistance to the drought and saline condition was identified by comparing the enzyme activity of the genotypes under the stressed condition

Materials and Methods

Field Plant materials

Black gram varieties (CO6 and VBN 8) was used for the study and these seeds were procured from the Department of Pulses, Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu, India-641003.

The plants were grown in pots in a green house at the facility available at PSG College of Arts and Science, Coimbatore, Tamil Nadu, India. After 25th day, salt and drought stress were induced.

Stress induction

Black gram varieties (Co6 & VBN8) were grown in pots and maintained in green house. The stress was induced by providing two different concentrations (50mM and 100mM) of NaCl and PEG to the 25 days old plants. About 40-50ml of prepared concentration of NaCl and PEG was sprayed per day to induce the stress in the plants. The stress was given in the alternative days (1^{st}, 3^{rd} and 5^{th} day) and the reading was taken in the following day.

Preparation of Plant Extract- stress induced samples

For estimating the activities of enzymes, 0.5g of leaves were collected and grinded in mortar and pestle with 5.0 ml of 100mM potassium phosphate buffer at pH 7.0 under ice cold conditions. The homogenate was centrifuged at 15,000rpm for about 20 minutes. The supernatant was taken for the further analysis. The samples were collected from day 1, day 3 and day 5.

Measurement of Catalase Activity

Activity of catalase was determined according to the modified method of Aebi (1983).The rate of decomposition of hydrogen peroxide was followed by decrease in absorbance at 240 nm. 100mM of phosphate buffer and 150mMof hydrogen peroxide were prepared. Then the reaction mixture was prepared it contains 1.5 ml phosphate buffer, 1.2 ml hydrogen peroxideand 300µl of enzyme extract. One unit of enzyme activity is calculated as the amount of enzyme required to liberate half the peroxide oxygen from hydrogen peroxide.

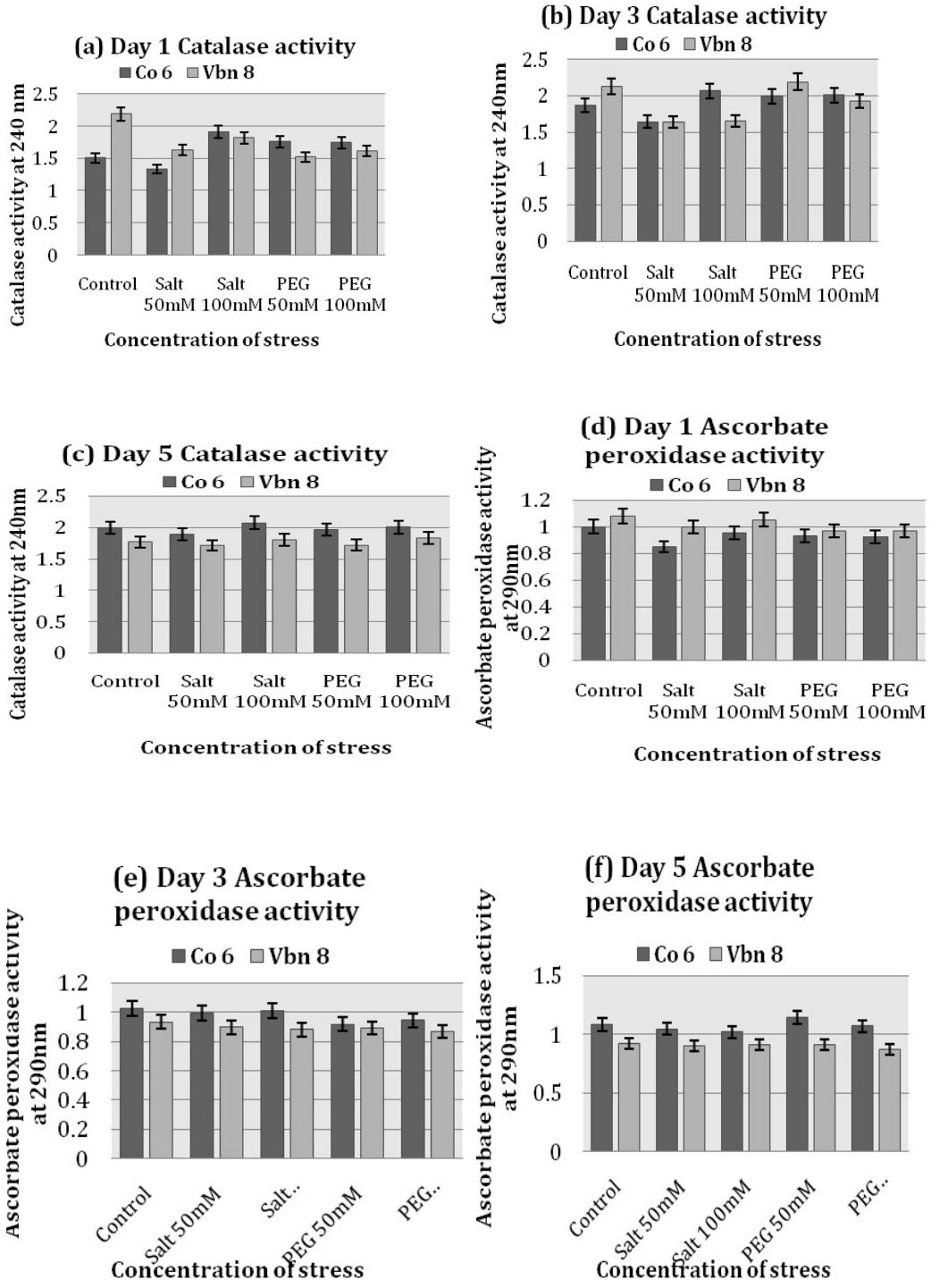
Measurement of Ascorbate Peroxidase (APX) :

Ascorbate peroxidase activity was estimated by the method of Nakano and Asada. This enzyme is specific for plants and it catalyzes the reduction of hydrogen peroxide using ascorbate as a substrate. The prepared reaction mixture contains 1.5 ml phosphate buffer, 300µl ascorbate, 600µl of hydrogen peroxidase and 600µl enzyme extract and the absorbance was measured at 290nm. For the reaction mixture 100mM of phosphate buffer and 5.0mM of ascorbate and 0.5mM of hydrogen peroxide were prepared. One unit of enzyme activity was calculated as the amount of enzyme required to oxidize 1.0µM of ascorbate/min/g FW. One mole of hydrogen peroxide oxidizes one mole of ascorbate to produce one mole of dehydro ascorbate. The rate of oxidation of ascorbate was followed by decrease in absorbance at 290nm.

Results

The study was conducted in an attempt to investigate the salt and drought tolerance potential in the two black gram genotypes Co6 & VBN8. The stress was given once and catalase and ascorbate peroxidase were assessed for day 1, day 3, day 5. The enzyme activity was initially higher in both the black gram genotypes Co6 & VBN8. But during the 5th day the enzyme activity is reduced. Prolonged stress condition will decrease the activity of the enzymes. In the stressed condition the black gram genotype Co6 showed low resistance when compared to the VBN8. The black gram genotype VBN8 showed increase in ascorbate peroxidase activity during severe drought condition to combat the stress condition. But the enzyme activity was lower than the control during saline condition. While comparing both the genotypes Co 6&VBN8,the black gram genotype VBN8 responded well and showed more resistance towards the drought and salinity condition.

Fig 1. In the graph the different concentration of stress factors 50mM and 100mM of NaCl and PEG along with control were taken in X axis and their mean value of enzyme activity were taken in Y axis. The figure a, b, & c represents the catalase enzyme activity and the figure d, e, f represent the ascorbate peroxidase enzyme activity for the day 1, 3 and 5 including the control



Discussion

In this study we compared and identified the black gram genotype that showed more resistant to the drought and salinity by comparing the statistical analysis of the two genotypes that were used for the analysis. The environmental stresses are the major problem to the plants and the response of the plants to these kinds of stresses is a complex reactions. The pulse crops such as mungbean are highly sensitive towards the various abiotic and biotic stress factors prevailing in the environment. Exposure to these stresses cause abnormal changes in physiological processes and metabolism of plants. This may results in alterations at biochemical and molecular level including expression, suppression or over-expression of normal genes along with stress responsive genes, transcription factors, osmolytes, proteins, peptides and enzymes of different mechanisms or signalling pathways responsible for stress tolerance (Nair *et al*., 2019). The plants produce several enzymes such as catalase and ascorbate peroxidase to overcome the stresses.Catalases are the principal scavenging enzymes which can directly dismutate hydrogen peroxide and is indispensable for ROS detoxification during stress (Van Breusegem *et al*., 2001).Overproduction of reactive oxygen species (ROS) under salinity stress damages the intracellular plant machinery resulting in oxidative stress. This may cause activation of programmed cell death in stressed cells (Hasanuzzaman *et al*., 2012; Nair *et al*., 2019). Evidences also suggest that when the synthesis of OH and H_2O_2 increases there will be a rapid reaction with RNA and Protein levels causing the differential expression (Scandalios 2005; Shull *et al*., 1991).

There are several studies that done in mung bean. In a study the mungbean showed that the vegetative growth stage is more sensitive towards salinity than the reproductive stage which determines that the occurrence or absence of reproductive phase in life cycle of the plant. Hence reproductive stage of mungbean is less affected in comparison to vegetative growth Phase (Sehrawat *et al.,* 2013; 2014a; 2014b). In another study on mitigating the adverse effect of drought in rainfed black gram, (Muthuvel *et al*., 1985) reported that farmyard manure application significantly increased the soil moisture status besides addition of nutrients. Application of farmyard manure along with inorganic fertilizers increased the black gram yield by 8.6 per cent over control. The comparative studies between the green gram (*Vigna radiata*) and the black gram (*Vigna Mungo*) also showed that the *Vigna mungo* is better in drought tolerance than *Vigna radiata* (Baroowa *et al.*, 2012). Conclusively it may be said that, these major abiotic stress causes adverse effects in *Vigna* species, which is the essential important crop with high nutritive value, so it is necessary to identify and develop the tolerant variety such as genetically improved mung bean genotypes that will show higher resistance towards these stresses

Conclusion

Black gram genotype VBN 8 had high concentration of catalase and ascorbate peroxidase in salinity and drought condition as compared to the genotype CO 6.

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PERFORMANCE ANALYSIS OF ELITE LOCAL ECOTYPES OF COCONUT FOR SPECIFIC TRAITS IN EAST COAST REGION OF TAMIL NADU

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Abstract

The conservation and utilization of indigenous coconut accessions or germplasms has been undertaken worldwide due to is economic magnitude. The aim of present study was designed to evaluate the five Tamil Nadu (a southern India state) elite indigenous coconut accessions for high nut yield and nut quality. Experiments were conducted in Coconut Research Station, Tamil Nadu Agricultural University, Veppankulam, Thanjavur district, Tamil Nadu, India, during the years 2006 – 2020 for five accessions along with.two checks (Kerakeralam and Aliyarnagar Tall) under ICAR-AICRP on Palms programme. Among the five local genotypes conserved and evaluated, IC 599265 (Selection from Kasangadu local ECT) recorded higher annual nut yield (82 nuts/palm/year) with desirable nut quality characters like higher dehusked nut weight (620g/nut), higher kernel (286 g/nut) and copra content (160g/nut) followed by IC59924 (Selection from Adirampattinam local ECT) which was collected from coastal eco system. IC 599263 (Selection from Thambikkottai local ECT) beared dwarf stature (650 cm/palm) as special trait.

Key words: Elite local genotypes, morphological and yield, nut quality parameters.

Introduction

Coconut (*Cocos nucifera*.L) is a only tropical palm in which all the parts are offering multiple uses and hence it's called as "Kalpavriksha" meant tree of heaven. It is believed to have originated in South East Asia (Indonesia, Malaysia and Philippines) or Miconesia (Harries et al.,1977). Evaluation and characterization of conserved accessions or germplasm lines in coconut repository is a prerequisite to identify the particular indigenous or exotic accessions possess imperative features, which will become useful in coconut breeding (Perera and Dissanayaka, 2014).

Coconut exhibits a huge variability in nut production ranging from 30 to 400 nuts / palm / year depending on environmental conditions and cultivars (Iyer and Rao *et al*., 1981) The copra obtained by drying the kernel of the coconut is the richest source of vegetable oil containing 65 to 70 per cent oil. Coconut is currently grown in nearly eighty countries spread along the tropical belt about 10 million families rely on coconut as either main source of income and stable food. Breeders dealing with coconut palms are aware of the significant difference in performance of coconut varieties from location to location and from year to year (Natarajan *et al*., 2010).

According to Fisher (1918) the continuous variation exhibited by quantitative traits with which the plant breeder includes heritable and non-heritable component. The choice of potential palms as one of the donor parent depends upon variability and proper selection for the desirable traits. The larger the variability in the material more will be the scope for improvement. Studies on the yield and nut traits in coconut germplasm are meager. This effort was made to document the diversity of morphology, yield and nut quality characters.

Materials and methods

A field study was conducted at Coconut Research Station, Veppankulam since 2006 to till date to evaluate elite local genotypes for yield and nut quality characters. Five genotypes (selection from East Coast Tall) along with two check variety (Kera Keralam and ALRCN1) were used for the study. The passport data of the elite coconut genotypes conserved and evaluated at CRS, Veppankulam were summarized in Table 1. The genotypes were 14 years old. The experiment was laid out in a Randomized Block Design with four replications with each genotype representing four palms per replication. The palms are planted at a distance of 7.5 m x 7.5 m. Recommended package of practices were followed for all the genotypes [6]. Observations were recorded from all five palms representing each genotype in each replication on vegetative, reproductive and nut characters with mean values were calculated. The yield of nuts per palm was recorded periodically at each harvest for five years from 2015 to 2020 and pooled to get nut yield per palm per year. Data was subjected to statistical analysis as per the standard procedures (Panse and Sukhatme, 2007)

Results and discussion

In the present investigation, significant differences were observed on palm height, palm girth, annual nut yield, dehusked nut weight, kernel weight and copra content (Table 2 and 3). Since harvesting is a big menace and difficult task among the tree climbers, dwarf stature is the critical trait which makes more attraction among the coconut growers which paves to extend the area. Accordingly, IC 599263 an elite genotype collected from the coastal area of Thambikkottai exhibited lesser palm height (649.33 cm) and palm girth (136.33 cm) at 14 years of planting. In contrast the maximum palm height was observed in IC 599266 of 726.67 cm, which was collected from

Kallikadu. The maximum number of leaves was recorded (34.92) in IC 599265, followed by IC 599264 (33.10). The number of leaves is an important character since it decides the ability of the leaf to support the bunches in the axis and also increases the photosynthetic efficiency. Similar results were also reported by (Jerard,2002; Jayalakshmy and Sree Rengasamy, 2002; Basavaraju *et al*., 2011; Suchitra, 2014).

The maximum number of inflorescence per palm per year was recorded by IC 599265 (13.16) followed by IC 599264 (13.02) compared to minimum values recorded in IC 599267 (11.17). Significantly highest pooled nut yield per palm was recorded by IC599265 (82 nuts) followed by IC 599264 (80 nuts). The genotype IC 599265 recorded higher dehusked nut weight (620 g / nut) followed by IC 599265 (538 g / nut). Kernel weight was more in IC 599265 (286 g/nut) with higher copra content (160 g/nut). Similar trends of increase in dehusked nut weight, kernel and shell weight were reported by Jeyabose et al. (2008) and Rachel *et al*. (2010). The higher annual nut yield may be due to the increased production of inflorescence per palm per year and number of functional leaves produced per year, which might have contributed higher photosynthetic accumulation towards the reproductive phase. Variation in nut characters might be due to both genetic factors and environmental factors, including available soil moisture and nutrient status (Selvaraj Vijai KS, Maheswarappa, 2016). Elite genotype collected from Kasangadu (IC 599265) is considered to be more suitable for further crop improvement programme as one of the donor parent at Coconut Research Station, Veppankulam in future.

Fig. 1. Performance of coconut genotypes for nut quality characters

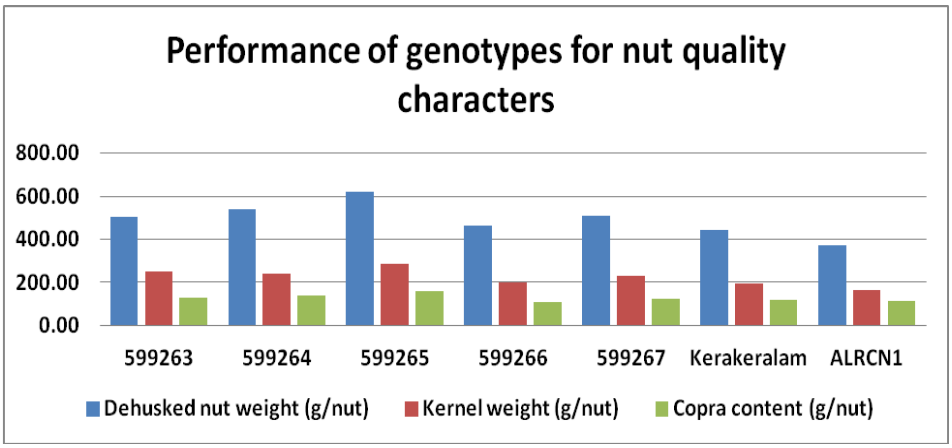

Table 2. Performance of elite coconut genotypes for vegetative characters.

Sl. No.	Genotypes (IC numbers)	Palm height (cm)	Palm girth (cm)	Number of leaves
1.	599263	649.33	136.33	32.72
2.	599264	684.33	162.67	33.10
3.	599265	726.67	152.67	34.92
4.	599266	676.00	174.00	32.69
5.	599267	731.33	158.67	31.19
6.	Kerakeralam	706.67	142.33	30.22
7.	ALRCN1	712.67	170.67	31.37
	S.EM =	3.07	2.14	0.21
	SE.d =	4.34	3.03	0.30
	CV =	1.09	3.38	1.64
	CD(5%) =	9.11	6.37	0.65
	Significance	S	S	S

Table 3. Performance of elite coconut genotypes for reproductive and nut characters.

Sl. No.	Genotypes (IC numbers)	Palm height (cm)	Palm girth (cm)	Annual nut yield (nuts/palm)	Dehusked nut weight (g/nut)	Kernel weight (g/nut)	Copra content (g/nut)
1	599263	649.33	136.33	72.33	505.00	248.67	128.67
2	599264	684.33	162.67	79.67	538.33	241.67	139.33
3	599265	726.67	152.67	81.67	620.00	286.00	159.67
4	599266	676.00	174.00	74.00	462.00	202.00	108.33
5	599267	731.33	158.67	68.67	511.33	232.67	125.33
6	Kerakeralam	706.67	142.33	70.33	445.00	196.33	116.33
7	ALRCN1	712.67	170.67	75.00	370.67	162.00	113.33
	S.EM =	3.07	2.14	1.17	2.66	0.70	1.21
	SE.d =	4.34	3.03	1.66	3.76	0.99	1.70
	CV =	1.09	3.38	5.33	1.33	0.77	2.34
	CD(5%) =	9.11	6.37	3.49	7.89	2.09	3.58
	Significance	S	S	S	S	S	S

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Table 1. Passport data of the elite coconut genotypes conserved and evaluated at CRS, Veppankulam

S.N	IC number	Sample Type	Sampling Method	Habitat	*Site of Collection*				*Latitude*	*Longitude*	*Altitude*	*Remarks for eliteness*
					Village	**Mandal**	**District**	**State**				
1.	599263	Seed nut	Random	Inland	Thambikkottai	Muthupet	Thiruvarur	T.N	10.40N	79.48E	1.82m	Slightly salt tolerant, dwarf with average yielding and high kernel thickness (1.3cm).
2.	599264	Seed nut	Random	Coastal	Adhirampattinam	Pattukkottai	Thanjavur	T.N	10.35N	79.40E	0.91m	Highly tolerant to salt and comes up well in saline sodic soil, higher whole nut weight (1325gm).
3.	599265	Seed nut	Random	Inland	Kasangadu	Pattukkotai	Thanjavur	T.N	10.38 N	79.27 E	99.39m	High yielding and medium statured plant.
4.	599266	Seed nut	Random	Irrigated	Kallikadu	Pattukkottai	Thanjavur	T.N	10.40 N	79.29 E	4.85m	Moderately tolerant to drought with intermediate nature of growth habit, higher kernel weight of 326.5gm and high copra content (168.0g).
5.	599267	Seed nut	Random	Irrigated	Thamarankkottai	Pattukkottai	Thanjavur	T.N	10.23 N	79.24 E	4.85m	High yielding (135 nuts/palm/year) with round shaped nuts.

EVALUATION OF ANNUAL MORINGA BASED INTERCROPPING SYSTEMS FOR HIGHER PRODUCTIVITY AND PROFITABILITY

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Abstract

Field experiments were conducted during 2010-14 at Horticultural College and Research Institute, Periyakulam to study the influence of intercrops on growth and yield of annual moringa (PKM 1). The intercrops viz., sweet potato, bhendi, chillies and vegetable cow pea were raised with 100:60 per cent (Pure crop: intercrop ratio). From the above study, it could be concluded that growing of chillies in the interspace of moringa (PKM 1) during first half year and vegetable cow pea after harvest of chillies as intercrop increased the moringa equivalent yield (37,909 kg/ha) than sole cropping of annual moringa. Growing bhendi in the interspace of annual moringa (PKM 1) during the first half year and vegetable cow pea after harvest of bhendi also increased the moringa equivalent yield (36,302 kg/ha). Higher B:C ratio was observed under sole moringa (9.81) followed by moringa + chillies (9.39).

Key words: Moringa, equivalent yield, intercropping

Introduction

Moringa has been cultivated in Tamil Nadu, Karnataka, Kerala and Andhra Pradesh. In Tamil Nadu it is being cultivated on commercial scale in about 7000 ha at Moolanur, Aravakurichi and Oddanchatram region. Oddanchatram and Aravakurichi are the major market centers for trade. Extremes of weather conditions that prevail in Northern States during Kharif as well as Rabi seasons do not favour the cultivation of Moringa. Hence, drumsticks are being transported from TN, AP and Karnataka to Northern states. In high density multi-species cropping system in old coconut garden in Tamil Nadu annual moringa have been profitability cultivated (Marimuthu *et al.,* 2001). In temperate hills of Sikkim, apple orchards are generally poor, so the major returns of farmers are from the intercrops like moringa scattered on farmlands in different crop combinations (Singh *et al.,*1991). Annual moringa is cultivated as mixed crop in chilli and brinjal field is also reported by Kader mohideen and Shanmugavelu (1982). Hence, an experiment was conducted to study the influence of intercrops on growth and yield of annual moringa.

Materials and methods

Field experiments were conducted during 2010-14 at Western block of Horticultural College and Research Institute, Periyakulam. The soil of the experimental site was sandy loam in texture, medium in available nitrogen (332.2 kg/ha), available phosphorus (19.3 kg/ha) and available potash (193.3 kg/ha). The experiment comprised of six intercropping systems along with sole cropping of sweet potato, bhendi, chillies and vegetable cow pea. The treatments viz., T_1 - Sole Annual Moringa (cv. PKM1), T_2 - Annual Moringa + Sweet potato(local type), T_3 - Annual Moringa+ Bhendi(cv. Arka anamika), T_4 - Annual Moringa+ Chillies(cv. PKM1), T_5 - Annual Moringa + Sweet potato- Vegetable cowpea(cv. PKM1), T_6 - Annual Moringa+ Bhendi - Vegetable cowpea, T_7 - Annual Moringa+ Chillies - Vegetable cowpea, T_8 - Annual Sole crop of Sweet potato, T_9 - Sole crop of Bhendi, T_{10} - Sole crop of Chillies, T_{11} - Sole crop of Vegetable cowpea were taken for the study. The experiment was laid out in randomized block design with 3 replications. The annual moringa variety "PKM 1", Sweet potato (local), Bhendi (Arka anamica), chillies (PKM 1) and vegetable cowpea (local) has been used for the experiment. Annual moringa was sown at 2.5 m x 2.5 m spacing. The seed germination was taken place 12 days after sowing. Pinching of terminal bud was done at a height of 75 cm. The intra row spacing of sweet potato, bhendi, chillies and vegetable cow pea was followed as per recommendation. The recommended dose of 100:100:50 kg NPK/ha was used and no additional dose of fertilizers was used for intercrops. For comparison, the yield of all intercrops were converted into moringa equivalent yield on price basis (Tomar and Tiwari, 1990).

Results and Discussion

Pod length and number of pods/tree was unaffected due to intercropping system in both the years. Yield attributes, number of panicles per tree, number of pods per panicle and pod yield of moringa was decreased due to intercropping system. The reduction in yield attributes and pod yield was higher under moringa + sweet potato – vegetable cow pea intercropping system. Not much reduction in pod yield was observed under bhendi, chilli intercropping system. Moringa + sweet potato-vegetable cow pea intercropping system reduced pod yield to significant extent.

Productivity

Moringa intercropped with bhendi, chillies gave significatnly higher moringa equivalent yield (kg/ha) compared with sole cropping of moringa in both the years. Among the intercropping systems, moringa + chillies –

vegetable cow pea (T_7) showed significantly higher moringa equivalent yield. However, signficantly lower value of moringa equivalent yield was obtained under moringa + bhendi. Wheareas, moringa + bhendi-vegetable cowpea (T_6) recorded higher moringa equivalent yield (36302 kg/ha) than moringa + sweet potato (35,838 kg/ha). The highest moringa equivalent yield was owing to better production of component crops.

Conclusion

Intercropping of chillies var.PKM 1 with annual moringa (var.PKM 1) in the first season followed by second season intercrop of vegetable cowpea in additive series (T_7) resulted in higher moringa equivalent yield (37909 kg/ha) than the sole crop of moringa alone (33,625 kg/ha). Growing of bhendi in the interspace of annual moringa (PKM 1) during the first half year and vegetable cowpea after the harvest of bhendi also increased the productivity (36,302 kg/ha).

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Table 1. Yield parameters of Annual Moringa (var.PKM1) as influenced by various intercropping treatments

Treatments	No. of flowers/ panicle		No. of panicles/ tree		No. of Pods per panicle		Pod length (cm)		Pod girth (cm)		No. of Pods /Tree		Pod yield (kg/Tree /year)	
	I crop (2010-2011)	II crop (2013-2014)	I crop (2010-2011)	II crop (2013-2014)	I crop (2010-2011)	II crop (2013-2014)	I crop (2010-2011)	II crop (2013-2014)	I crop (2010-2011)	II crop (2013-2014)	I crop (2010-2011)	II crop (2013-2014)	I crop (2010-2011)	II crop (2013-2014)
T_1- Sole moringa	52.0	49.8	21.8	44.1	5.4	4.30	62.7	64.4	4.9	5.0	211.0	101.6	18.7	12.0
T_2- Moringa + Sweet potato	41.0	43.3	35.4	41.8	3.6	4.00	53.4	62.4	5.1	4.6	196.0	105.4	16.7	11.4
T_3- Moringa + Bhendi	46.5	40.0	33.8	40.3	5.2	3.90	54.4	62.2	4.9	4.5	203.0	103.7	17.2	11.3
T_4- Moringa + Chillies	31.0	44.6	37.0	43.5	4.6	4.10	56.5	63.0	5.1	4.9	205.0	102.2	17.3	11.7
T_5 – Moringa + Sweet potato-vegetable cowpea	33.0	38.9	29.7	39.0	3.4	3.70	55.7	61.0	5.0	4.4	199.0	104.5	16.2	10.9
T_6- Moringa + bhendi-Vegetable cowpea	38.0	51.4	46.7	46.2	4.1	4.80	62.0	65.1	5.3	5.1	211.0	101.9	18.7	12.2
T_7- Moringa + Chillies-Vegetable Cowpea	41.0	55.2	47.0	48.1	3.8	5.20	55.8	66.4	5.1	5.3	221.5	106.3	18.2	13.1
SEd	0.9	0.7	0.8	0.7	0.1	0.07	0.3	2.7	0.02	0.2	0.1	10.1	0.1	0.6
CD (p= 0.05)	1.9	1.5	1.8	1.5	0.2	0.14	0.6	NS	0.05	0.4	0.3	NS	0.2	NS

Table 2. Moringa Equivalent Yield (kg/ha) of different intercropping systems (Pooled data of two years)

Treatments	Economic yields of crops(kg/ha)					Moringa equivalent yield of the cropping system (kg/ha)	Total Cost of Cultivation (Rs/ha)	Gross Income (Rs/ha)	Net Income (Rs/ha)	Benefit Cost Ratio
	Sweet potato	Bhendi	Chillies	Vegetable cowpea	Moringa					
T_1 -Sole moringa	-	-	-	-	33625	33625	34270	336250	301980	9.81
T_2- Moringa + sweet potato	10236	-	-	-	30720	35838	37154	343450	306296	9.24
T_3 -Moringa + bhendi	-	1848	-	-	31352	32830	37956	328310	290354	8.65
T_4- Moringa + chillies	-	-	2069	-	31784	34888	37154	348870	311716	9.39
T_5 –Moringa + sweet potato-vegetable cowpea	9059	-	-	1049	29735	35103	43278	340370	297092	7.86
T_6- Moringa + bhendi-vegetable cowpea	-	1836	-	961	34064	36302	44080	363020	318940	8.24
T_7- Moringa + chillies-vegetable cowpea	-	-	1937	1093	34129	37909	43278	379090	335812	8.76
T_8 - Sole sweet potato	10153	-	-	-	-	5077	24734	40250	15516	1.63
T_9 - Sole bhendi	-	1373	-	-	-	1098	8910	10980	2070	1.23
T_{10} -Sole chillies	-	-	1730	-	-	2595	21273	25960	4687	1.22
T_{11} - Sole vegetable cowpea	-	-	-	1496	-	1197	8403	11980	3577	1.43
SEd						1150				
CD(p=0.05)						2399				

STUDYING PHYSIOLOGICAL PARAMETERS GOVERNING DROUGHT TOLERANCE IN PIPELINE RICE CULTURES

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Abstract

Yield reduction of rice is a severe problem due to the advent of increasing water scarcity and efficiency is relatively low. Physiological attributes and yield performance of high yielding (HYV) rice cultivars need to be assessed by minimizing water loss. Therefore, a laboratory experiment was conducted in Agricultural College and Research Institute, Eachangkottai to investigate the impact of cultivars using six drought tolerant cultures (TM 12061, TM12077, TM 12012, TM14035 and TM 16017) with check varieties (TKM 12, Anna 4, Senthuram and Vandhana). During screening, water deficit stress is artificially induced by desires strengths of polyethylene glycol 6000. Polyethylene glycol has been used to stimulate water stress effects in plants (Swapna and Shylaraj, 2017). The experiment was laid out in complete randomized design (CRBD) with four levels of drought stress and four replications. The laboratory experiment revealed thatgermination count decreased with decrease in water potential (-ψw) or increase in osmotic potential of growing medium (-ψs). Under controlled condition all the genotypes attained 100% germination and the check varieties has 97-98% of germination. With decreased water potential TM 12077 showed better germination percentage.Shoot length and root length also recorded higher in the culture TM 12061 almost in all the water potential levels.maximum seedling vigour was found in the culture TM 12061 followed by TM 12077 in -0.6 MPa water potential. The positive adaptive responses of these rice varieties towards drought stress can be used in the genetic improvement of rice drought resistance breeding program

Introduction

In recent years, agriculture is facing two major challenges that include enhancement of food production sustainably to feed a growing world population and this increment needs to be accomplished under conditions of increasing scarcity of water resources. Drought is the major abiotic stress factor that limits rice production worldwide. Drought is particularly more frequent in South and Southeast Asia and Sub-Saharan Africa. India faced severe drought in 2002 and 2009, which ultimately caused a drastic reduction in rice production. In 2002, the total rice production was declined by 21.50 million tons due to drought, whereas, in 2009, rice production was declined by approximately 10.02 million tons (Directorate of Economics Statistics, 2009; Vikram et al, 2011). The world's farmers have to produce about 60% more rice than at present to meet up the food demands of the expected world population by 2025 (Fageria, 2007).Physiological basis of yield gap among high yielding rice cultivars has not been studied extensively. Such information is vital for identifying the physiological and morphological traits to support the selection and breeding of high yielding rice cultivars. Efforts are few to address the growth, physiological responses and yield of rice (Oryza sativa L.) to water stress under tropical environment (Zain et al., 2014). Therefore, in this study, an attempt has been made to assess the rice genotype tolerant to drought condition.

Materials and methods

Lab experiment was conducted at Agricultural College and Research Institute, Eachangkottai using six drought tolerant cultures (TM 12061, TM12077, TM 12012, TM14035 and TM 16017) with check varieties (TKM 12, Anna 4, Senthuram and Vandhana). Rice cultures and check varieties were collected from Rice Research Station, Tirur. Seeds of each genotypes were surface sterilized with 70% ethanol solution for five minutes. The seeds were then washed three times with sterilized distilled water. Seed germination test were performed by evenly distributing the seeds on a 10 cm diameter sterilized petridish with layers of germination paper. Each pertidish was moistened with 10 ml distilled water or uniform amounts of desired osmotic solutions to mimic drought stress. Three sets of such petridishes used for each cultures. Twenty seeds of each culture were allowed to germinate in petridish. The number of seeds that germinated was counted daily from day 2 upto day 14. Seedling length, germination percentage and vigour index were measured. During screening, water deficit stress is artificially induced by desires strengths of polyethylene glycol 6000. Polyethylene glycol has been used to stimulate water stress effects in plants (Swapna and Shylaraj, 2017). The experiment was laid out in complete randomized design (CRBD) with four levels of drought stress and four replications.

Distilled water was used as a control (0 MPa) and osmotic potentials -0.2, -0.4,-0.6 and -1.0 MPa were created by adding PEG 6000 at 4,8,s10 and 14 g per 100 ml distilled water.

Results and discussion

Germination percentage of the rice cultures presented in the Table.1 and the data revealed that germination count decreased with decrease in water potential (-ψw) or increase in osmotic potential of growing medium (-ψs).

Under controlled condition all the genotypes attained 100% germination and the check varieties has 97-98% of germination. With decreased water potential TM 12077 showed better germination percentage.Shoot length of different rice cultures presented in the Table.2. Shoot length and root length also recorded higher in the culture TM 12077 almost in all the water potential levels.

Table 1: Germination percentage (%) of different rice genotypes under varied PEG levels

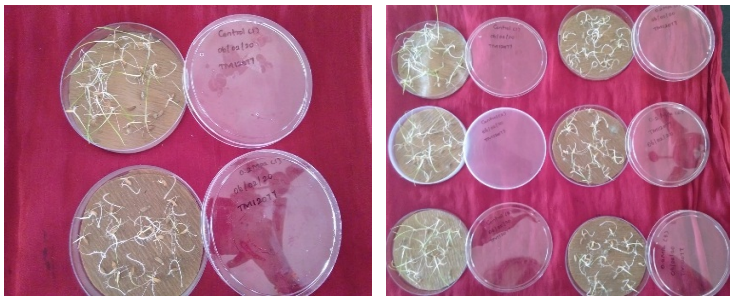
Genotypes	Control	0.2 MPa	0.4 MPa	0.6 MPa	1.0 MPa
TM 12061	100.0	91.8	85.4	60.7	30.8
TM 12077	100.0	90.5	84.7	48.0	24.1
TM 12012	100.0	88.4	84.9	55.4	28.4
TM 14035	100.0	89.4	83.9	41.4	26.7
TM 16017	100.0	89.7	81.4	43.6	27.0
TKM (R)12	98.0	80.5	71.4	21.4	0.0
Anna 4	97.0	84.6	79.4	38.9	14.8
Senthuram	98.0	88.7	79.4	45.8	18.4
Vandhana	98.0	89.1	79.0	37.4	15.7
SEd	0.44	0.42	0.39	0.36	0.34
CD (0.05)	0.92	0.88	0.85	0.82	0.81

Table 2: Shoot length (cm) of different rice genotypes under varied PEG levels

Genotypes	Control	0.2 MPa	0.4 MPa	0.6 MPa	1.0 MPa
TM 12061	12.8	10.0	8.0	6.7	2.5
TM 12077	14.9	10.4	8.7	7.2	3.2
TM 12012	14.1	10.4	8.1	6.8	2.3
TM 14035	14.0	10.6	8.2	7.0	2.8
TM 16017	13.4	9.0	8.2	6.5	2.5
TKM (R)12	11.4	8.5	5.5	2.3	0.0
Anna 4	11.2	10.1	6.7	4.2	1.9
Senthuram	12.7	10.4	6.6	4.7	1.0
Vandhana	12.0	10.0	6.1	5.6	0.6
SEd	0.20	0.18	0.16	0.14	0.13
CD (0.05)	0.40	0.37	0.35	0.30	0.28

Table 3: Root length (cm) of different rice genotypes under varied PEG levels

Genotypes	Control	0.2 MPa	0.4 MPa	0.6 MPa	1.0 MPa
TM 12061	19.6	17.25	15.50	11.20	8.85
TM 12077	23.70**	18.20	16.70	11.00	8.23
TM 12012	20.45	17.45	15.73	8.85	7.60
TM 14035	16.80	13.40	11.90	9.00	7.25
TM 16017	22.50	17.50	15.45	8.15	7.10
TKM (R)12	12.40	10.95	9.85	5.00	0.00
Anna 4	19.75	17.80	16.25	11.80	8.25
Senthuram	12.70	10.40	9.75	7.95	7.50
Vandhana	18.75	16.80	10.25	7.80	7.25
SEd	0.18	0.185	0.12	0.114	0.11
CD (0.05)	0.37	0.34	0.26	0.22	0.20



TM 12077 (Control and -0.2MPa) **TM 12077 @ different PEG conc.**

Conclusion

The morphological parameters recorded invitro using PEG 6000 in rice cultures and land races were found to have a positive correlation of superior root and shoot performance under the stress condition. Moreover, increased germination percentage, shoot length and root length towards their degrees of tolerance under drought stress with respect to the drought tolerant check variety Anna 4. Outcome of this study, it is clear that the rice cultures TM 12077, TM 16017 and 22.50 can be recommended as the most drought tolerant genotypes. This work is a step towards genetic improvement for drought tolerance in rice.

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A STUDY ON EXPORT POTENTIAL OF ORGANIC COCONUT BY USING MARKOV CHAIN MODEL IN COIMBATORE DISTRICT OF TAMIL NADU

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Abstract

The study estimated the Export Potential of Organic Coconut by using Markov Chain Model in Coimbatore District of Tamil Nadu.For finding the domestic potential - 30farmers who got certification from the TNOCD (Tamil Nadu organic seed certification Department) were selected randomly.The information regarding - quantities of organic produce exported from Tamil Nadu was gathered using the Interview schedule.It is comparatively advantage to export organic coconut to all the six countries.It is evident that Malaysiahas the most stable market when compared to other countries.The projected export share of Indian organic coconut to United Kingdom has increased from 12.92 per cent to 13.12 per cent.It was found that theprice fixing mechanism for organic produce is not good was the major constrains faced by the farmers.

Key words

Export potential, Organic coconut, Markov Chain Model, Nominal protection coefficient, Constraints

Introduction

Organic farming is gaining gradual movement across the world. Growing awareness of health and environmental issues in Agriculture has demanded production of organic food which is emerging as an attractive source of rural income generation. India is bestowed with lot of potential to produce all varieties of organic products due to its various agro climatic regions. This holds promise for the organic producers to tap the market which is growing steadily in the domestic market related to the export market. The Government of India has implemented the National Programme for Organic Production (NPOP). The national programme involves the accreditation programme for Certification Bodies, standards for organic production, promotion of organic farming etc.

Sample size

For finding the domestic potential - 30farmers who got certification from the TNOCD (Tamil Nadu organic seed certification Department) were selected randomly.For knowing the export potential, export details were collected from the exporter using the interview schedule.Using the Interview schedule the information regarding - quantities of organic produce exported from Tamil Nadu, countries to which they are exported, what is the constrains in organic produce exports are obtained.

Materials and Methods

Nominal protection coefficient:

Formula:

$NPC = Pd/Pw = Pw (1+t)/Pw$

Pd is domestic price of a product

Pw is world price of a product

t is tariff rate

Inference:

If NPC < 1, protected that is we can export our organic produce to the foreign countries.

if NPC >1, un protected , its not profitable to export our produce.

Markov Chain Model -Assumptions:

(a) The probability of an outcome on the t^{th} trial depends only on outcome of the preceding trial, and

(b) This probability is constant for all time periods (Lee et al., 1970).

$$E_{jt} = E_{jt}E_{i\,t-1}\,P_{ij} + e_{jt}$$

The structural change in export of organic produces was examined by estimating the transition probability using Markov-chain model. The data on quantity of organic produce exported to various countries can be taken and analysed in Markov chain analysis. To get the transition probability matrix, this LP was solved using LINGO (version 10) Package.

Garrett's Scoring technique (for constrains)

The respondents were asked to rank the problems in production, processing and marketing. In the Garrett's ranking technique these ranks were converted into percent position by using the formula

Percentage position =100 (Rij – 0.5) /Nj

Where, Rij = Rank given for i th factor by j th individual

Nj = Number of factors ranked by j th individual

Result and Discussion

Table No 1.Nominal protection coefficient

Sl.no	Countries	Domestic price / Tender Coconut	World price / Tender Coconut	NPC
1	United state	25	250	0.1
2	Malaysia	25	275	0.090
3	Singapore	25	260	0.096
4	United kingdom	25	280	0.089
5	Dubai	25	290	0.086
6	Thailand	25	295	0.084

Inference

It is comparatively advantage to export organic coconut to all the six countries like United States, Malaysia, Dubai, Thailand, United Kingdom and Singapore.

Table No 2. Transition probability matrix

2013-14 to 2017-18	United kingdom	Singapore	Malaysia	United States	Dubai	Thailand
United kingdom	0.0000	0.0000	0.1706	0.8294	0.0000	0.0000
Singapore	0.0000	0.0000	0.0000	0.2884	0.0000	0.7116
Malaysia	0.0000	0.6555	0.2695	0.0000	0.0000	0.0751
United States	0.0440	0.0000	0.0000	0.5140	0.1617	0.7548
Dubai	0.1030	0.1293	0.5023	0.0000	0.2653	0.0000
Thailand	0.4511	0.1144	0.0000	0.0000	0.4345	0.0000

Inference

The probability of retaining the previous market share (gain or loss) was interpreted by studying the diagonal elements of transitional matrix. The row elements in the transitional probability matrix provide the information on the extent of loss in trade, on account of competing countries. The column elements indicate the probability of gain in volume of trade from other competing countries and the diagonal element indicates probability of retention of the previous year's trade volume by the respective country. The matrix gives a broad indication of the change in the direction of trade of organic coconut from India. It is evident from Transition probability matrix**,** that Malaysia was the most stable market among the major importers of Indian organic coconut as reflected by the probability of retention at 26.95. The most unstable market among the importing countries was United Kingdom, Singapore and Thailand with zero per cent retention.

Table No 3: Actual and estimated quantity of organic coconut exports from Tamil Nadu (2013-14 to 2017-18)

Year	United kingdom	Singapore	Malaysia	United states	Dubai	Thailand	Total
2013-2014	12.92	14.64	15.49	16.48	17.29	23.18	100
2014-2015	12.96	15.04	15.05	15.59	17.33	24.03	100
2015-2016	13.30	14.85	14.96	15.72	17.55	23.62	100
2016-2017	13.14	14.78	15.14	15.94	17.45	23.55	100
2017-2018	13.15	14.86	15.08	15.79	17.44	23.68	100

Inference: The actual and estimated quantity of organic coconut isincreasing for all the six organic coconut importing countries from the year 2013 -14 to 2017 – 18.

Table No 4: Projected export share of Tamil Nadu's organic coconut to major importing countries from 2013-14 to 2017-18 (percentage)

Year	United kingdom	Sing	Malays	United states	Dubai	Thailand
2013-2014	12.92	14.64	15.49	16.48	17.27	23.173
2014-2015	12.96	15.04	15.05	15.59	17.31	24.02
2015-2016	13.30	14.85	14.96	15.70	17.55	23.60
2016-2017	13.14	14.789	15.12	15.94	17.45	23.55
2017-2018	13.12	14.86	15.08	15.79	17.44	23.68

Inference

It was found that the projected export share of Indian organic coconut to United Kingdom has increased from 12.92 per cent to 13.12 per cent. It is also observed that the projected shares were found to be constant for Singapore, Malaysia, Dubai, and Thailand. With respect to United States, the projected share has decreased from 16.48 per cent to 15.79 per cent.

Table No 5: Constrains faced by organic coconut farmers

Sl.No	Constrains	Average score	Rank
1	Procedure for getting organic certification is too long	74.4	2
2	Absence of subsidy	8.26	5
3	High cost of organic certification labels	49.6	3
4	less cost for the produce	33.06	4
5	price fixing mechanism for organic produce is not good	82.66	1

Inference

It was found that theprice fixing mechanism for organic produce is not good was the major constrains faced by the farmers followed by Procedure for getting organic certification is too long, followed by High cost of organic certification labels.

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ASSOCIATION OF GENETIC MARKERS WITH PHENOTYPIC TRAITS BY USING SSR MARKERS IN FINGER MILLET [*Eleusine coracana* (L.) Gaertn]

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Abstract

Finger millet [*Eleusine coracana* (L.) Gaertn] is an important crop used for food, fodder and industrial purposes. With the objective of increasing the utilization of finger millet germplasm in crop improvement, a composite collection consisting of 1000 accessions was developed, evaluated in three environments for 15 agronomic traits and profiled using 20 SSR markers. In this study has reported the marker-trait associations by using Simple Sequence Repeats markers.Allelic data on 959 accessions and 20 markers based on quality index was used for further statistical analysis. A total of 231 (121 common and 110 rare)alleles were detected in the composite collection. Significant variation of all the agronomic traits was observed. Marker UGEP8 in LG3 and UGEP56 in LG9 showed strong association with days to 50 % flowering in composite collection in over all the tree environments. Several other markers were associated with the traits but were not consistent across environments.

Introduction

Finger millet (Eleusine coracana L. Gaertn) is an important crop in several countries of Asia and Africa used for food, fodder, and industrial purpose.Finger millet is a highly self-fertilized allotetraploid (2n = 4x = 36) derived from the wild tetraploid progenitor E. coracana subsp. africana. The A genome donor is believed to be E. indica. Both E. floccifolia or E. tristachya have been considered as potential B genome donors to E. corancan based on rDNA restriction pattern (Hilu *et al*., 1992) and genomic in situ hybridization (Bisht and Mukai, 2001).

In finger millet the diversity has been studied using morphological characters like growth habit, leaf architecture or floral morphology (Rachie and Peter., 1997). At molecular level, DNA markers such as RFLP (Muza *et al*., 1997), RAPD (Das *et al*., 2007), SSRs (Dida *et al*.2007) have been used to determine genetic diversity. Comparative analysis of finger millet genetic map with rice genetic map was a novel attempt that reported high level of conserved co-linearity between the two genomes (Srinivasachary *et al*., 2007). Low molecular variation was reported in the cultivated finger millet in the past as the results were based on limited number of germplasm and markers. With the discovery of large numbers of genomic SSR markers (Dida *et al*., 2007), it is now possible to conduct extensive molecular diversity and QTL analysis in finger millet.Population structure using 79 finger millet accessions and 45 SSR markers have been reported (Dida *et al*., 2008). The present study aimed to assess the genotypic diversity, to dissect the population structure of global composite collectionand to find marker- trait associations in global finger millet composite collection.

Materials and Methods

All the 1000 accessions of the finger millet composite collection including four internal checks (VR708, VL149, PR202 and RAU8) were grown in the field. The DNA was extracted from single seedling of each accession by high throughput 96- well plate mini preparation method.From the preliminary screening of 31 SSR markers (Dida *et al*., 2007) on an eight diverse finger millet genotypes (IE4709, IE6082, IE2921, IE5177, IE4057, IE4443, IE2564 and IE3025), 20 polymorphic SSR markers were selected to genotype the composite collection. Of these, 19 SSRs belong to dinucleotide repeats and one to trinucleotide repeats. The 20 SSR markers used for genotyping were mapped on nine chromosomes.

Polymerase chain reaction (PCR)

The PCR reactions were conducted in 96-well and 384-well micro-titer plates in a GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA, USA) Thermal Cycler. The PCR reactions were performed in 5 µl volume in 384-well PCR plates. The reaction mixtures contained 10 pmol of primer, 25 mM $MgCl_2$, 2mM dNTP, 0.3 unit of Taq polymerase and 1x PCR buffer (Applied Biosystems, Foster City, CA, USA). The touch down PCR protocol was used for the following reaction of following: three-minute denaturation cycle, followed by first five cycles of 94^{o}C for 20 seconds, 60 ^{o}C for 20 seconds and 72 ^{o}C for 30 seconds, then by 30 cycles of 94 ^{o}C for 20 seconds. After completion of 30 cycles, a final extension of 20 min at 72 ^{o}C to ensure amplification to equal lengths of both DNA strands. The amplified PCR products were tested on 1.2 per cent agarose gel to check for the amplification of the products.

Genotyping

The PCR products were size-separated by capillary electrophoresis using an ABI 3700 DNA analyzer (Applied Biosystems, Foster City, CA, USA). The PCR products of 4 primer pairs labeled with different dyes (FAM, VIC, NED and PET) could be pooled (post-PCR), because of the different signal spectra of the fluorophores used. The products of the same fluorophore-labeled primers were also pooled, when they had non-overlapping

amplicons in terms of size. The pooled PCR products were mixed with 0.25 µl of the GeneScan 500™ LIZ® internal size standard and 7 µl of Hi-Di™ Formamide (Applied Biosystems, Foster City, CA, USA). The final volume was made up to 12 µl with sterile double-distilled water. This mixture was denatured for 5 minutes at 95°C and cooled immediately on ice.

Fragment size fractionation

After denaturation, the plate with samples was placed into the sequencer machine (ABI Prism 3700 DNA analyzer). The capillary run was performed using the "GeneScan2_POP6 Default" run module and "G5" filter-set. The analysis module used was "GS500 analysis". The fragments were separated in a 50 cm capillary array using POP6 (Performance Optimized Polymer) as the separation matrix.

Data processing

After the capillary runs were over, the raw data were processed with Genescan 3.1 software (Applied Biosystems) to size the peak patterns in relation to the internal size standard GeneScan 500™ LIZ®. The principle behind this is that standards are run in the same lane or capillary injection as the samples, which contain fragments of unknown sizes labeled with different fluorophores. Genescan® analysis software automatically calculates the size of the unknown DNA sample fragments by generating a calibration sizing curve based upon the migration times of the known fragments in the standard. The unknown fragments are mapped onto the curve and the sample data is converted from migration times to fragment size. Genotyper 3.7 (Applied Biosystems) was used for allele calling. The peaks were displayed with base pair values and height (amplitude) in a chromatogram and the allelic data were exported in to Excel spread sheet for further analysis.

Association mapping

Phenotyping

Phenotyping of composite collection along with four check cultivars (VR708, VL149, PR 202 and RAU 8) was carried out in three environments, viz., 2005-'06 post rainy at Tamil Nadu Agricultural University, Coimbatore (E1), 2006 rainy (E2) and 2007 rainy (E3) at ICRISAT, Patancheru. This experiment was conducted in augmented design with one of the four control cultivars repeated after every nine entries in all the environments. Data on 15 quantitative traits [days to 50% flowering (DF), plant height (PH), number of basal tillers (BTN), culm branching (CB), flag leaf blade length (FLBL) and width (FLBW), flag leaf sheath length (FLSL), peduncle length (PL), panicle exertion (PE), ear head length (EHL) and width (EHW), length and width of longest finger (LLF and WLF), number of fingers per ear head (NF) and plot yield (PY)] were recorded following finger millet descriptors. Mean, range and broad sense heritability were calculated for all traits to study the variability present in the germplasm material.

Association of markers with traits

All association tests were run with the mixed linear model (MLM) method in TASSEL 1.9.4 (http://www.maizegenetics.net/), a recently developed unified mixed-model method simultaneously taking into account multiple levels of both gross level population structure (Q) and finer scale relative kinship (K). The population structure matrix (Q) was identified by running STRUCTURE at K = 4. Only markers with an allele frequency of 5% or greater were included in the association analysis.

Results and Discussion

Substantial variation was observed for all traits and high heritability showed greater importance of the traits in revealing marker trait associations. The marker trait association of composite collection data was validated with reference set data. It was observed that the marker-trait association varied with the environments and population used.In the present study, association analysis resulted inconsistent association between the traits and markers for most of the traits mainly due to limited number of random and non trait specific markers. However, in the present study, QTL for days to 50 per cent flowering had consistent association with UGEP8 in LG3 (E2, E3 and pooled for both composite collection and reference set) and UGEP56 in LG9 (E2 and E3 in composite collection and E1 in reference set). It indicated relatively tight linkage between the trait and marker. Also the association varied in different sample size consisting of composite collection with 959 accessions and reference set with 300 accessions. It has been suggested that large numbers of molecular markers are needed to better cover the entire nuclear genome for such association studies (Jensen 1989). However, in our study only 20 markers were used. The marker UGEP3 on LG3 was associated with seven traits (PH, DF, BTN, CB, EHL, FLBL, FLBW) in composite collection and (BTN, FLBL, PL, EHW, PY, FL, PH) in reference set. Also majority of the markers were found to be associated with more than one trait, such an association may arise due to pleotropic effect of the linked QTL on different traits (Culp *et al*., 1979).

Conclusion

The global finger millet composite collection showed rich allelic diversity (231 alleles, 11.6 alleles per locus, 121 common alleles and 110 rare alleles at 1%). The markers UGEP8 and UGEP56 were consistently associated with days

to 50 per cent flowering indicating relative strong association between marker and traits. Extensive study of these markers in mapping population would be helpful for confirmation of QTL.

Table 1. Association of 20 SSR loci with agronomic traits in finger millet composite collection in three environments and pooled.

Traits	Environment	Marker	Linkage Group	Position (cM)	P
Days to 50% flowering	E1				
		UGEP11	5A	63.5	0.034
	E2	UGEP56	9A	7.4	0.028
		UGEP8	3B	65.2	0.001
	E3	UGEP56	9A	7.4	0.025
		UGEP8	3B	65.2	0.016
	Pooled	UGEP8	3B	65.2	0.048
Plant height	E1	UGEP3	3A & 3B	75.8 & 64	0.013
		UGEP65	8A	31.6	0.044
	E2	UGEP68	9B	0	0.046
		UGEP104	3B	124.7	0.042
Basal tiller numer	E2	UGEP3	3A & 3B	75.8 & 64	0.039
		UGEP8	3B	65.2	0.003
Clum branching	E2	UGEP8	3B	65.2	0.047
		UGEP26	5B	121.1	0.017
Flag leaf blade length	E2	UGEP90	6B	23.3	0.035
	pooled	UGEP26	5B	121.1	0.038
Flag leaf blade width	E2	UGEP56	9A	7.4	0.019
		UGEP65	8A	31.6	0.001
	pooled	UGEP3	3A & 3B	75.8 & 64	0.028
Flag leaf sheath length	E2	UGEP26	5B	121.1	0.016
		UGEP18	1B	70.3	0.001
Peduncle length	E1	UGEP18	1B	70.3	0.024
	E2	UGEP65	8A	31.6	0.028
	Pooled	UGEP11	5A	63.5	0.02
Ear head length	E1	UGEP56	9A	7.4	0.001
	E2	UGEP8	3B	65.2	0.001
	E3	UGEP107	1A	9.5	0.001
	pooled	UGEP26	5B	121.1	0.032
Length of longest finger	E2	UGEP68	9B	0	0.054
	E3	UGEP3	3A & 3B	75.8 & 64	0.001
Plot yield	E3	UGEP104	3B	124.7	0.03

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ON FARM TESTING OF ROOT ROT DISEASE MANAGEMENT TECHNIQUE IN GROUNDNUT IN PUDUKKOTTAI DISTRICT OF TAMIL NADU

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Abstract

Groundnut (Arachis hypogaea L.) is an important oil seed crop, grown throughout the tropics. Even though the groundnut crop suffers from several diseases, fungal and viral pathogens are major constraints to its production. Among the fungal diseases, root rot caused by Macrophominha pahseolina (Tassi) Goid., a warm dry weather pathogen, is responsible for economic losses to an extent of 80%. The present work was aimed to develop an integrated management strategy involving biocontrol agents and fungicide to combat dry root rot in groundnut. Two technologies were tested for the management of root rot disease in comparison with farmer practice of no seed treatment (TO1). The Technology options were seed treatment with carbendazim (2g/kg of seeds) (TO2) and Seed treatment with Propiconazole (2ml/kg of seed) + soil application of Bacillus subtilis (2.5kg/ha) + Drenching with 0.01% carbendazim. TO 3 recorded lower root rot disease incidence (3.2), maximum yield of 18.75 q/ha and BCR of 2.23 followed by TO 2 recorded root rot disease incidence (8.3 PDI), yield (17.20 q/ha) and BCR of 2.12. The farmers showed interest in adopting this technology, and highly satisfied because of higher yield through efficient management of root rot disease in groundnut.

Key words: Groundnut, Macrophominaphaseolina, carbendazim, Propiconazole, Bacillus subtilis

Introduction

Groundnut is an important oilseed crop in Pudukkottai district and it has been grown both in Kharif and Rabi season. It is a major edible oilseed crop and its uses are as edible oil, seeds, vines and dry fodder as an excellent nutrient for cattle and Rhizobium bacterial root nodule provide nitrogen status of the soil. The growth and yield of groundnut is affected by a number of diseases, among which, root rot caused by Macrophominaphaseolina (Tassi) Goid., causes severe yield loss especially under dry conditions.Extensive use of chemicals for the control of soil borne plant pathogen has several disadvantages viz., pesticide residues in soil and crop produce, ground water pollution, emergence of resistant races and killing of nontarget beneficial microbes, etc. Biological agents provide an alternative to chemicals in the management of soil borne diseases as they are eco-friendly in nature.

Materials and Methods

On farm trials were conducted at ten farmers' field in the Thiruvarangulam block and Aranthangi blocks of Pudukkottai district to assess the root rot disease management technique in rainfed groundnut. Initially the selected farmers were sensitized on the importance of biocontrol agents and time of application viz., seed treatment and soil application. The trials were taken up in ten farmers' field each in an area of about 0.40 ha. Each treatment, seven replications were maintained in each location. Each replication had plot size of 5x 6 feet. Besides, the following technologies viz., TO1: Seeds are sown directly without any seed treatment, TO2: Seed treatment with carbendazim @ 2g/kg of seed, and TO3: Seed treatment with Propiconazole@ 2ml/kg of seed + soil application of Bacillus subtilis 2.5kg/ha + Drenching with 0.01% carbendazimwere also demonstrated in the trial.

Result and discussion

Groundnut disease management techniques were tested by Krishi Vigyan Kendra, Tamil Nadu Agricultural University, Vamban, Pudukkottai district in the farmer's fields. Two technologies were tested for the management of root rot disease in comparison with farmer practice of no seed treatment (TO1). The Technology options were seed treatment with carbendazim (2g/kg of seeds) (TO2) and Seed treatment with Propiconazole (2ml/kg of seed) + soil application of Bacillus subtilis(2.5kg/ha) + Drenching with 0.01% carbendazim.

TO 3 recorded lower root rot disease incidence (3.2), maximum yield of 18.75 q/ha and BCR of 2.23 followed by TO 2 recorded root rot disease incidence (8.3 PDI), yield (17.20 q/ha) and BCR of 2.12. When no treatment was imposed (TO1), higher root rot disease incidence (12.6), lower yield (15.20 q/ha) and BCR of 1.95 were recorded. The farmers showed interest in adopting this technology, and highly satisfied because of higher yield through efficient management of root rot disease in groundnut. Meena *et al*. (2001) reported the reduction of groundnut root rot significantly in field experiments by seed treatment with powder formulation ofP. fluorescens which was also on par with carbendazimdrenching and suggested that more than one mechanismmight be responsible for suppression of the pathogen.Seed inoculation with plant growth promoting bacteria have been reported to result in higher pod yield in the field trials in addition to suppression of soil borne fungal pathogens (Dey *et al*., 2004). Antagonistic ability of several PGPR on M. phaseolinaand their growth promotion ability were demonstrated byDeshwal*et al*. (2003), Arora *et al*. (2001) and Dey *et al*., (2004) with variation in their performance. This may be due to the

difference in soil conditions, initial inoculum and other parameters. In spite of these differences, the biocontrol agents performed consistently in reducing disease incidence and increasing yield.

Table 1. Assessment of root rot disease incidence in groundnut

Sl.No	Technology option	Disease incidence (%))	Yield (q/ha)	Net profit (Rs.)	B: C ratio
1	Seeds are sown directly without any seed treatment,	12.6	15.70	29675	1.95
2	Seed treatment with carbendazim @ 2g/kg of seed, and	8.3	17.2	36300	2.12
3	Seed treatment with Propiconazole@ 2ml/kg of seed + soil application of Bacillus subtilis 2.5kg/ha + Drenching with 0.01% carbendazim	3.2	18.75	41375	2.23
	CD (p=0.05%)	0.441	0.812	1684.84	0.099
	SEd	0.202	0.373	773.27	0.045

*values are mean of ten locations. Each location, seven replications were maintained for each variety.

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DEVELOPMENT OF PRE BREEDING MATERIALS IN BLACKGRAM VARIETIES ALTERNATIVE TO ADT 5

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Abstract

In an attempt to develop the pre breeding materials in Black gram varieties Alternative to ADT 5 we made inter varietal hybridization between 8 Blackgram varieties (ADT3, ADT 5, VBN 4, ADT 6, VBN 5, VBN 6, VBN 7, VBN 8) and the restructuring of ADT 5 Blackgram variety. In the Hybridization programme, 15 F_1 Crosses were derived from 3 Lines and 5 Testers through Line X Tester Mating System. The F_1's are raised in the field and are in the vegetative stage. In the Restructuring of ADT 5 Blackgram variety we selected 121 plants based on short stature and multi pod characters and recorded the yield attributing characters. The next Generation of the selected plants were raised in the Field and checked for purity and yield attributing characters.

Key words: Blackgram, Pre breeding materials, Alternative ADT 5

Introduction

Blackgram (Vigna mungo) is an important short-duration pulse crop grown in many parts of India. Blackgram grain contains 24% protein, 60% carbohydrates, 1.3% fat and is the richest source of phosphoric acid among pulses (5-6% richer than others). It is used as a nutritive fodder specially for milch cat tle. It is also used as green manuring crop. Being deep rooted crop, helps in binding soil particles and thus prevents soil erosion. It fixes atmospheric N (42 kg/ha/year) to the soil through symbiosis and improves fertility of soil.

India is the largest producer as well as consumer of black gram. Nearly 70% of worlds blackgram production comes from India. It produces about 1.5 to 1.9 million tons of urad annually from about 3.5 million hectares of area, with an average productivity of 500kg per hectare. Blackgram accounts for about 13 % total pulses area and 10 % total pulses production in India. In India about 2-3 million tonnnes of pulses are imported annually to meet the domestic consumption requirement. Thus, there is need to increase production and productivity of pulses in the country by producing high yielding and widely adopted varieties through various breeding methods.

Materials and methods

The present investigation titled "Development of Pre breeding materials in Blackgram varieties alternative to ADT 5" was conducted in the Agricultural College and Research Institute, Eachangkottai. The "Hybridization Programme" was carried out at A Block and "Restructuring of ADT 5 Blackgram variety" was carried out at D Block.

Hybridization Programme:

Experimental materials:

The experimental material consists of 3 Lines (ADT3, ADT 5, ADT 6)and 5 Testers (VBN 4, VBN 5, VBN 6, VBN 7, VBN 8). ADT3, ADT 5, ADT 6 varieties were collected from TamilNadu Rice Research Institute, Aduthurai and VBN 4, VBN 5, VBN 6, VBN 7, VBN 8 varieties were collected from National Pulses Research Centre, Vamban.

Parents used in the Investigation Programme:

Parents	Characters
ADT 3	Suitable for rice fallow
ADT 5	Suitable for rice fallow. Resistant to major diseases like YMV, root rot and leaf crinkle and to stem fly.
ADT 6	High yield; moderately resistant to YMV, leaf curl virus and powdery mildew; suitable for rice fallow
VBN (Bg) 4	Resistant to Yellow Mosaic Virus
VBN (Bg) 5	High yield and resistant to yellow mosaic virus disease in all seasons
VBN 6	High yield and resistant to yellow mosaic virus disease in all seasons
VBN(Bg) 7	High yield and resistant to yellow mosaic virus disease, powdery mildew and leaf crinkle disease
VBN 8	High yield and resistant to yellow mosaic virus disease and moderately resistant to powdery mildew

Methods

Fifteen crosses were made with 3 Lines (ADT3, ADT 5, ADT 6) and 5 Testers (VBN 4, VBN 5, VBN 6, VBN 7, VBN 8) in a Line X Tester Mating system. The F_1 Hybrid seeds along with Parental lines were raised in Randomised Block Design. The experiment consisted of 15 crosses (ADT3 X VBN 4, ADT3 X VBN 5, ADT3 X

VBN 6, ADT3 X VBN 7, ADT3 X VBN 8, ADT 6 X VBN 4, ADT 6 X VBN 5, ADT 6 X VBN 6, ADT 6 X VBN 7, ADT 6 X VBN 8, ADT 5 X VBN 4, ADT 5 X VBN 5, ADT 5 X VBN 6, ADT 5 X VBN 7, ADT 5 X VBN 8).
Restructuring of ADT 5 Blackgram variety:

Experimental Materials

The Experimental materials consisted of 121 Lines selected from ADT 5 Blackgram variety raised in 1.5 Acres in the A Block at Agricultural College and Research Institute, Eachangkottai.

Method

The Plants were selected based for short statured and multi pod cluster charactes. The 121 Lines were raised in Plant to Row method in the D Block at Agricultural College and Research Institute, Eachangkottai.The plants were observed for the characters viz.,days to 50% flowering, days to maturity, plant height (cm), no. of clusters per plant, no. of pods per plant, no. of seeds per pod, 100-seed weight (g)and seed yield per plant (g):

Results

Early Flowering

The line 82 was found to be early flowering followed by the line 24. These lines are of great value in developing early maturing variety and could be used in the further varietal development process.

Uniformity:

The line 90 has uniformity in growth followed by the lines 92 and 23. These lines are of great value in developing uniform maturing variety.

Plant height

To meet out the requirement of Blackgram ,various methods to be involved for the production of high yielding with yield attributing characters. For this study we taken aaduthurai varieties as lines which are high yielder and suits for all tracts in Tamilnaduand Vambanvarieties are having YMWV resistance which are used as testers.for this study .Weused LXT method for Hybridization programme ,. In this case we attempted crossing between various crosses and was observed High crossing percentage in ADT3 X VBN 8 the cross which was 12.1%, followed by the cross ADT 5 X VBN 6 and ADT 5 X VBN 4 having crossing percentage 12% and 10.5 % respectively . These crosses are of great value in future for varietal improvement.

In Restucturing of ADT 5 Blackgram variety we selected 121 plants from ADT blackgram variety field , based on the morphological characters and high number of pods which are responsible for high yielding. Among 121 plants maximumno.of pods observed from 8th plant which had 45pods followed by 42 and 40 from 109 and 20th plant .Hundred seed weight for improving yield per plant was recorded. Maximum seed weight from 55th plant having 5.91 gms ,followed by 5.48 gms and 5.4 gms. Contrast to 100 seed weight , yield per plant will vary from the above plants from the selected plants. Maximum seed weight /plant obtained was 15.93 g/plant(68th plant) followed by 14.67g(31st plant) and 12.69 g (47th plant).

Also we concentrated in uniformity and early flowering plants for synchoronized flowering and quick maturity to reduce the duration .The Early flowering plants observed on 27 and 28 days which are used for developing early maturity variety in further generation.

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Restructuring of ADT 5 Blackgram variety:

Plant no.	*Plant height (cm)*	*No. of pods*	*100 Seed weight*	*Total Seed weight*
1	33.0	42	4.07	11.22
2	23.0	25	4.10	4.53
3	28.0	45	3.80	3.78
4	25.5	35	4.25	9.31
5	35.3	42	4.60	9.14
6	20.0	37	3.89	9.16
7	19.0	21	3.43	3.65
8	28.6	45	5.16	5.17
9	15.0	21	4.73	5.56
10	17.0	22	4.07	5.34
11	21.0	35	3.72	7.32
12	26.0	35	4.01	8.88
13	17.9	20	4.11	4.96
14	23.0	25	4.10	5.76
15	26.4	26	3.73	7.45
16	33.9	20	3.72	4.41
17	16.0	23	3.20	3.62
18	17.0	30	4.40	8.20
19	24.0	27	4.30	7.27
20	27.0	40	4.52	11.10
21	16.5	28	4.66	7.09
22	24.0	25	3.58	5.38
23	20.5	18	3.74	4.36
24	18.4	21	3.90	4.89
25	19.0	17	4.15	3.87
26	25.0	32	4.54	7.49
27	32.0	26	4.86	4.86
28	19.5	19	3.66	4.22
29	19.0	26	4.04	5.94
30	17.0	29	3.78	3.99
31	34.0	47	4.75	14.67
32	27.5	18	5.09	6.15
33	19.5	18	4.18	4.20
34	28.0	36	4.78	8.28

35	20.0	20	4.80	4.76
36	19.0	13	3.36	3.31
37	23.0	23	5.00	9.04
38	16.0	15	4.85	3.87
39	28.0	21	5.23	5.24
40	22.0	17	4.32	4.96
41	22.5	23	4.71	6.06
42	22.0	23	4.40	5.31
43	23.9	21	5.48	7.32
44	20.0	27	4.10	4.13
45	37.2	16	4.73	4.72
46	32.0	37	5.13	10.05
47	33.0	38	4.12	12.69
48	29.4	30	4.57	4.36
49	27.0	30	4.95	9.99
50	29.7	25	5.00	8.08
51	21.5	35	4.56	6.76
52	25.0	25	5.13	7.51
53	23.0	33	5.14	7.79
54	23.5	23	4.46	5.95
55	34.3	29	5.91	8.55
56	16.0	12	4.33	3.01
57	24.0	36	5.09	5.16
58	30.4	32	4.00	8.85
59	31.3	22	3.83	3.86
60	33.6	31	4.60	7.80
61	18.0	19	2.95	2.88
62	17.5	14	4.61	2.64
63	33.3	36	5.02	6.18
64	19.0	21	4.33	5.14
65	34.5	24	5.00	8.73
66	27.0	27	5.77	6.61
67	23.0	27	4.52	5.25
68	30.7	43	5.10	15.93
69	31.0	27	5.35	7.82
70	29.0	25	4.72	6.80
71	32.0	27	5.40	9.65
72	35.0	28	5.32	7.23
73	21.7	23	4.98	7.55
74	21.9	19	4.62	4.63
75	23.0	20	4.35	10.47
76	27.0	38	4.60	10.82
77	26.0	28	4.82	7.83

78	17.0	22	4.19	5.32
79	28.0	40	4.39	10.98
80	23.0	21	5.18	8.55
81	22.0	22	2.62	1.27
82	23.5	22	3.49	5.28
83	35.6	25	5.10	7.26
84	25.0	27	4.37	7.99
85	30.0	23	4.96	7.28
86	35.0	32	4.89	4.89
87	31.6	20	4.55	5.55
88	29.0	36	4.65	8.45
89	17.0	15	4.59	5.48
90	19.0	13	4.12	4.12
91	25.0	21	4.34	6.14
92	29.6	30	4.13	7.94
93	21.5	26	4.57	6.54
94	22.0	29	4.79	9.09
95	25.1	25	4.34	6.79
96	26.0	35	4.67	9.95
97	-	-	4.73	8.00
98	-	-	5.20	3.06
99	29.0	39	4.01	9.61
100	24.0	24	4.51	7.02
101	24.0	27	4.56	6.41
102	21.0	-	4.61	5.60
103	16.0	26	3.98	6.32
104	24.0	29	4.71	8.75
105	33.0	25	5.10	4.02
106	25.0	28	4.61	6.67
107	24.0	17	3.55	3.54
108	30.0	29	4.33	6.40
109	29.5	42	4.99	9.72
110	25.0	27	4.78	7.36
111	40.2	41	4.46	9.34
112	37.8	29	5.30	8.87
113	40.0	31	4.42	7.81
114	28.0	15	4.22	5.03
115	22.0	40	4.29	7.20
116	20.1	26	3.65	5.02
117	22.0	29	4.44	8.23
118	17.0	15	4.00	3.49
119	21.0	25	3.64	5.54
120	30.0	30	5.10	7.72
121	22.0	20	5.15	5.14

Days to 50% Flowering:

Line	Days to 50% Flowering DAS										
1	40	22	30	43	39	68	35	91	30	116	
2	39	23	30	44	37	69	39	92	28	117	
3	-	24	26	45	34	70	39	93	30	118	39
4	-	25	35	46	37	71	39	94	34	119	
5	-	26	31	47	32	72	37	95	31	120	39
6	38	27	32	48	35	73	38	96	35	121	39
7	42	28	31	49	35	74	35	97	34	-	-
8	30	29	36	50	38	75	36	98	33	-	-
9		30	20	51	33	76	39	99	31	-	-
10	29	31	34	52	37	77	39	100	32	-	-
11	31	32	40	53	38	78	37	101	37	-	-
12	34	33		54	36	79	39	102	37	-	-
13	31	34	35	55	39	80	40	103	36	-	-
14	33	35	34	56	37	81		104	35	-	-
15	36	36	33	57	30	82	38	105	39	-	-
16	34	37	40	58		83	34	106	39	-	-
17	32	38	34	59	34	84	39	107	38	-	-
18	34	39	31	60	35	85	33	110	39	-	-
19	30	40	30	61	35	86	38	111	36	-	-
20	40	41	32	62	27	87	40	112	37	-	-
21	34	42	37	63		88	37	113	36	-	-
64	34	108	35	66	37	89	34	114	37	-	-
65	33	109	39	67	30	90	32	115	37	-	-

FLOWERING PHENOLOGY AND POLLINATOR INTERACTIONS OF GMELINA ARBOREA IN TAMIL NADU

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Abstract

Gmelina arborea is a fast-growing, multipurpose tree species. The study on flowering phenology and pollinator interaction was conducted in 10 year old Gmelina plantation at Anavayal, Pudkkottai district. Tamil Nadu,India. The trees started blooming from the fourth week of February and reached a peak in March. The anthesis started late-night (23.30 hrs to 04.00 hrs) and the peak time of flowering was recorded between 02.00 hrs and 03.00 hrs (54%). Flower opening starts from the base of the inflorescence to the tip. The flower and short-stalked and 4-5cm long. The stigma receptivity began at the time of anther dehiscence and remained up to 14. 00 hrs in plantation and peak stigma receptivity was recorded between 09.00-10.30 hrs. A flower produced 15-35µl of nectar with 20-40 percent sugar concentration. The pollinator activity was recorded over 24 hours and subsequently, the observations were confined between 05.0 am to 02.00 pm for 15 days in each season. Birds, bees, butterflies, Xylocopa, ants and beetles, and small insects were common visitors of G. arborea flowers. The squirrel and parrot are the real menaces for low fruit set.

Key Words: Gmelina arborea, Flower, Stigma receptivity and Pollinators

Introduction

The interaction of flowers and pollinators is an important ecological process. It is a critical mechanism for the occurrence of cross-sexual reproduction. (Ashman *et al*., 2004). A pollinator's effectiveness can be divided into a 'quantity' and a 'quality' component, which are its visitation rate and the amount of pollen transferred per visit, respectively. The 'quantity' and 'quality' of pollination services provided by different pollinator types frequently differ (Schemske and Horvitz, 1984; Fishbein and Venable, 1996). Many plants can reproduce vegetatively, but cross-pollination provides the genetic mixing required for long-term population stability and adaptation. This generation of new genetic traits is critical in a plant's ongoing battle against rapidly evolving competitors, pests, and diseases. Indeed, recent research indicates that the evolution of self-pollination strategies is frequently an evolutionary dead-end that leads to extinction (Pattemore, 2017).

G. arborea Roxb. is an indigenous fast-growing, multi-utility tree species that belongs to the family of Lamiaceae. It is a medium-sized to large tree, native to India. It is an important plantation species in many tropical areas around the world. The wood is used for construction, furniture, carriages, sports items, musical instruments and artificial limbs. It is also used as raw material for cellulose (Foelkel *et al*., 1978), firewood, pole wood (Moya, 2004a), particleboard (Chew and Ong, 1989), veneer (Sicad, 1987) as well as the structural purpose (González *et al*., 2004). The study of floral biology and pollination ecology of the species is mandatory for control pollination and production hybrid. Considering the importance of multipurpose utility very little work was carried out on phenology and reproductive biology. The present investigation aimed to study flowering phenology and pollinator interactions in the Gmelina arborea

Materials and Methods

Study Site

The experiment was conducted in the ten year old G. arborea plantation established in Anavayal Pudukkottai district, Tamil Nadu, India in two consecutive years 2019,2020 from December to April. The geographic position of the study area is N 10°20'9.40" E latitude 79° 5'57.70" longitude and elevation 49 MSL. The temperature varies from 23 - 35° C and receives an annual rainfall of 750-850 mm. The Gmelina plantation is established in sandy loam soil with good drainage.

Flowering phenology and morphology

A flowering phenology study was conducted in Ten year old Gmelina plantation. The floral phenophases including time of anthesis, anther dehiscence and duration of the stigma receptivity were recorded. The inflorescence length was measured after full blooming. The total numbers of flowers per inflorescence were counted in 50 randomly selected inflorescences and the average was taken. 50 mature buds were tagged on 10 different trees followed for anthesis, anther dehiscence and stigma receptivity were recorded as per the method suggested by Dafni (1992). The data on flower morphology was recorded using Leica microscope attached image analyzer (Leica Q Win). The numbers of fruits per inflorescence were counted in 50 randomly selected inflorescences.

Study of pollinators

The pollinator activity was recorded for 24 hours and subsequently, the observations were confined between 05.0 am to 02.00 pm for a period of 15 days in each season. The floral visitors were collected using standard entomology procedure (Daffni, 1992) and transported to a laboratory for identification.

Results and discussion

The flowers are produced in paniculate cymes, which arise at the terminal and lateral shoots. The flowers are short-stalked, large, bisexual, odorless and zygomorphic. The calyx is tubular and has five-lobed at the tip. The corolla is brownish-yellow has a short tube with the upper lip formed by two petals and the lower lip by one broad central and two lateral petals. Both calyx and corolla are densely hairy, giving the leathery appearance of flowers. The stamens are four and occur in two pairs, inserted near the base of the corolla tube. Raju and Rao, (2006) reported G. arborea flowers are produced in paniculate cymes, the flowers are large, short-stalked, bisexual, odourless and zygomorphic. The flowers open during 0800-1000 h. the anther are dithecous and dehisce by longitudinal slits. Flowers are large bright in colour that produced about 15-35µl of nectar in natural population and plantation.

Table 1. Flower Morphology and Phenology of Gmelina arborea flowers

Floral characters	Observations
Type of inflorescence	Terminal cyme
No. of flower/ inflorescence	12-17
Flower type	Large, bisexual, pubescent
Anthescing	Night
Peak time of flowering	02 00-03 00 Hrs
Symmetry	Zygomorphic
No. of stamens	4 stamens in two pairs, epipetalous,
Calyx	Tubate, five lobed at tip
Corolla	Brownish yellow colour, short stalk, upper lip by two lateral petals and lower lip by one broad central and two lateral petals
No. of anther flower	4
Anther dehiscence	08.00am-09.30am
Mode of anther dehiscence	Longitudinal slits
Average no. of pollen/anther	6000-7000
Average size of pollen length	25-35µm
Mean No of pollen grains/ flower	24000-28000
Pollen release time	Mid-morning
Pollen viability	4-5 hrs
Mean No of ovule/flower	4
Type of ovary	2 carpels, each carpel has 2 cell
Nature of the style and stigma	Style long, end with short stigma with unequal lobes.
Pollen-ovule ratio	7000:01:00

The anterior pair is longer, while the posterior pair is short. The anther is dithecous and dehisces in longitudinal slits. The ovary has two carpels, each with two cells. Each cell has one ovule. The style is long and terminated with a short stigma having two unequal lobes. The stigma lies between the anthers of the long and short pair of stamens. Floral characteristics of trees from natural population and plantation were almost similar, except for a minor variation observed in the time of anthesis, number of pollen/anther and pollen ovule ratio (Table 1). In both plantations and natural populations, trees started blooming from the fourth week of February and reached a peak in March.

Stigma receptivity and anther dehiscence

The mature flower buds started late-night (23.30 hrs to 04.00 hrs) and the peak time of flowering was recorded between 02.00 hrs and 03.00 hrs (54%) (Table 2). The stigma receptivity began at the time of anther dehiscence and remained up to 14. 00 hrs and peak stigma receptivity were recorded between 09.00-10.30 hrs. A flower produced 15-35µl of nectar with 20-40 percent sugar concentration. Nagarajan *et al*. (2010) reported that mangrove species of Bruguiera flowers are large, long-lived and produce 40-60µl of nectar/flower per day.

Table-3. Progression of anthesis, anther dehiscence and stigma receptivity after flower opening in plantation at Pudukkottai

Time (Hrs)	No of flower opened(100Nos. x 5 Replication)	Flowers Opened (%)	No. of flowers with anther dehisced(100Nos.x 5 Replication)	Flowers with anther dehisced (%)	No. of flowers with stigma receptive(100Nos.x 5 Replication)	Flowers with stigma receptive (%)
00-01	25	5	0	0	0	0
01-02	60	12	0	0	0	0
02-03	270	54	0	0	0	0
03-04	75	22	0	0	0	0
04-05	45	9	0	0	0	0
05-06	25	5	0	0	0	0
06-07	0	0	0	0	0	0
07-08	0	0	75	15	0	0
08-09	0	0	100	20	80	16
09-10	0	0	225	45	240	48
10-11	0	0	75	15	210	42
11-12	0	0	0	0	0	12

Observations on flower visitors

Pollination syndromes are suites of flower characteristics like morphology, colour, nectar and odor that supposedly attract particular pollinators to specific flowers (Ollerton, 1998). In the present study, flower visitors included bees, butterflies, birds, squirrels and insects. Vectors visited flowers at sunrise depending on nectar availability. Bolstad and Bawa (1982) concluded that the native bees in G. arborea plantations in Costa Rica were effective pollinators and this seems to have been confirmed by subsequent observations in the Ston Forestal seed orchard. In most countries, the pollination vectors for G. arborea are insects in the Hymenoptera. However, little census information on what insects are visiting flowers, their foraging range and their effectiveness in pollination is available (Dvorak, 2004).

Six insect species belonging to the insect order; Hymenoptera and Lepidoptera were collected from the inflorescences during the study period. The foraging activities of major flower visitors concerning pollination were observed during day time. Flower visitors' visitation was just before too soon after sunrise. The major pollinators were honey bees like Apis dorsata, Apis mellifera and Apis cerana and Apis Ceraae indica. All the species showed intense foraging activity from 06.00 hrs to 12.00 hrs. (Table 3). Honey bees forage for both nectar and pollen and butterflies for nectar alone after anthesis. They were mostly attracted by colour of the flower and its fragrance. All the insects were pollen carriers and their frequent interplant movement facilitated cross-pollination.

The carpenter bee Xylocopa sp. visited flowers, between 07.00-12.30 hrs foraged nectar and collected pollens. The pollen-collecting bees usually landed on the lower lip or upper lip and gathered pollen from anthers. Sometimes they landed on the anther lobes and foraged for the pollen. The bees directly landed on the coherent anther lobe and

affected the pollination. Bird visitors were Pycnonotus sp. Leptocomazeylonica, Nectariniaasiatica and Lorikeets, which foraged from early morning to afternoon. All the birds and squirrels perched on shoot or inflorescence resulting in the removal of flowers. All passerine birds were found to collect nectar from flowers.

Table 3. List of flower visitors and foraging nature in G. arborea

S.No	Species	Common Name	Order	Family	Foraging nature
1	ApisdorsataFab.	Rock bee	Hymenoptera	Apidae	P
2	Apis mellifera L.	European honey bee	Hymenoptera	Apidae	N and P
3	Apiscerana indica Fab	Indian honey bee	Hymenoptera	Apidae	N and P
4	Trigona iridipennisSmith	Dammer bee	Hymenoptera	Apidae	P
5	Xylocapasp.	Carpenter Bee	Hymenoptera	Apidae	N and P
6	Pachliopta hector Fab.	Crimson rose	Lepidoptera	Papilionidae	N and P
7	Euploea core Cramer	Common crow	Lepidoptera	Danaidae	N
8	CinnyriszeylonicaLatham	Purple-rumped sunbird	Passeriformes	Nactariniidae	N
9	Cinnyris asiatica Latham	Purple- sunbird	Passeriformes	Nactariniidae	N
10	PycnonotusluteolusLesson	White-browed bulbul	Passeriformes	Pycnonotidae	N
11	LoriculusvernalisSparrman	Vernal hanging parrot	Psittaciformes	Psittacidae	N
12	Funambulus palmarum	Indian palm squirrel	Rodentia	Sciuridae	

Conclusion

G. arborea peak flowering was observed between 04.00-05.00 hrs and peak stigma receptivity was recorded between 09 00-11 00 hrs. The flower produced 15-35µl of nectar with 20-40% sugar concentration. The pollen grains per flower varied from 24000-28000 and the pollen ovule ratio was approximately 7000:1. The average size of pollen length is 25-35µm. It was observed that some species of bees, butterflies, birds, ants, beetles and some small insects were common visitors of flowers. The major pollinators were honey bees (Apis dorsata, Apis mellifera and Apis cerana), pollen bees, butterflies, insects and birds. All the birds, lorikeets and squirrels landed on shoot or inflorescence resulting in the removal of flowers. All bees, butterflies and passerine birds were found to collect nectar from flowers. The information on flower morphology and pollinator interaction will help the design of seed orchards and tree Tree improvement through hybridization.

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INTEGRATED BEST MANAGEMENT PRACTICES FOR RESOURCE CONSERVATION IN SUGARCANE (*SACCHARUM OFFICIANARUM*)

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Abstract

The results of an on farm trial to evaluate suitable resource conservation and best management practices in sugarcane through combined mechanized cultivation was conducted in farmer's field at Ottai village in Vanur taluk of Villupuram Dist. The Sugarcane variety CoC 25 was chosen as test cultivar CoC 25 and Co Si 8 at Cuddalore and Sirugamani centres respectively. The aim of the experiment was to evolve best management practices for resource conservation in sugarcane through combined mechanized cultivation. Two treatments such as T_1 (Crop geometry: Adoption of 150 cm inter row spacing, Sett treatment with bio-inoculants (G.diazotrophicus and AM fungi), Irrigation: sub surface drip fertigation, In-situ trash decomposition after cane harvest with TNAU Bio-mineralizer @ 2kg/tonne of trash and Mechanisation with power weeder (30 &60 DAP), Earthing up (90 DAP), Detrashing (120&210 DAP) and T_2 (Control: Spacing 90 cm, manual cutting and planting, sett treatment with fungicide, surface flood irrigation, manual weeding, trash burning, no detrashing and earthing up, manual harvesting) were adopted. Parameters such as soil physico-chemical properties, microbial population, weed flora, water use efficiency, labour saving, growth and yield of sugarcane were assessed. Mechanization of sugarcane cultivation enhances the population of soil microbes and also the monetary returns in terms of net income (1,99,048 Rs./ha) and BC ratio (2.17) through increased cane yield altogether with reduction in cost of cultivation compared to manual cultivation of sugarcane. Consolidated results obtained from the non replicated trials raised at farmer's field at Ottai village of Vanur taluk of Vilupuram District in Cuddalore centre and at Mannukulam village of Thanjavur District in Sirugamani centre revealed that the soil texture were sandy clay loam and clay loam in respective experimental sites. At both the locations soil is neutral in pH with low in available 'N', high in available 'P' and medium in available 'K'. Regardless of the experimental sites, the mechanized cultivation of sugarcane registered higher mean values of millable canes (1,19,500/ha), cane length (210.5 cm), girth (8.05cm), single cane weight (1.72 kg), population of soil microbes (Actinomycetes, Bacteria and Fungi), cane yield (132.5 t/ha), net income (Rs.210136/ha) and BC ratio (2.24) altogether with reduced cost of cultivation compared to manual cultivation of sugarcane. Adoption of Mechanization in sugarcane cultivation enhances the values of varied yield parameters, cane yield and economic returns which also minimized the cost of cultivation of sugarcane. The impact of complete mechanization practice on physico-chemical soil properties, microbial population, weed flora, water use efficiency, labour saving, growth and yield of sugarcane could be ascertained.

Introduction

Sugarcane farm mechanization is very advantageous and minimize labour demand and facilitate faster operations. It aims to improve the productivity of land labour by improving the protection and comfort of farm labour, and to protect the environment by enabling specific operations and raising the potential revenue. Efficient machinery helps in timely farm operation, input use efficiency, increasing productivity by about 30 percent. Development and introduction of high capacity, precise, reliable and energy efficient equipment and their judicious use can bring in precision and timeliness in field operations.

Materials and Methods

A non-replicated field trial was planted at farmer's field at Ottai village in Vanur taluk of Villupuram Dist. The Sugarcane variety CoC 25 and Co Si 8 were selected at Cuddalore and Sirugamani centres respectively. The soil of the experimental field was sandy clay loam in texture with neutral in soil pH in both the centres. The soil was low in available 'N', high in available 'P' and medium in available 'K'. The data on varied crop bio-metrics, population of soil microbes, juice quality and economic out turn were recorded.

Results and discussion

The results are presented in Tables 1,2,3&4. Mechanization of sugarcane cultivation enhances the population of soil microbes and also the monetary returns in terms of net income (Rs.1,99,048/- per ha) and BC ratio (2.17) through increased cane yield altogether with reduction in cost of cultivation compared to manual cultivation of sugarcane.

Table 1. Effect of mechanization in sugarcane on growth yield attributes and cane yield (Sirugamani centre).

Treat ments	Population of Tillers (000/ha) 90 DAP)	Millable canes (000/ha)	Yield attributes Cane length (cm)	Cane girth (cm)	Single cane weight (kg)	Cane yield (t/ha)	CCS (%)	Sugar yield (t/ha)
T_1	168.4	117.8	203	7.9	1.61	128.9	11.2	14.4
T_2	155.0	108.3	178	7.0	1.25	116.5	10.8	12.6

Higher population of tillers (1,68,400/ha), millable canes (1,17,800/ha), cane length (203cm), cane girth (7.9cm), single cane wt (1.61 kg), cane yield (128.9 t/ha) and sugar yield (14.4 t/ha) were obtained with the adoption of mechanization in sugarcane cultivation compared to conventional manual cultivation of sugarcane.

Table 2. Effect of mechanization on soil microbial population and monetary returns (Sirugamani centre)

Treat ments	Soil microbes population			Gross income (Rs./ha)	Cost of cultivation (Rs./ha)	Net income (Rs./ha)	BC ratio
	Actinomycetes (10^2 cfu/g soil)	Bacteria (10^6cfu/g soil)	Fungi (10^4cfu/g soil)				
T_1	53	10	12	368654	169606	199048	2.17
T_2	40	5	7	333190	178206	154984	1.86

Table 3. Comparison of mechanization with manual cultivation of sugarcane on crop indices, cane and sugar yield (Cuddalore centre)

Treat ments	Germination (%) 35 DAP	Population (000/ha) Tillers (000/ha) 90 DAP	Eco.Shoots (000/ha) 210 DAP	Milable Canes (000/ha)	Yield Parameters Cane Length (cm)	Cane girth (cm)	Individual wt (Kg)	Cane Yield (t/ha)	CCS (%)	Sugar Yield (t/ha)
T_1	24.5	182.5	136.8	121.5	218	8.2	1.82	136.0	11.5	15.6
T_2	86.7	174.2	129.3	112.6	197	7.7	1.37	120.5	11.0	13.2

The practice of mechanization in sugarcane resulted with the higher population of tillers (1,82,500 Nos./ha), economic shoots (1,36,800/ha), millable canes (1,21,500/ha) compared to manual cultivation of sugarcane. The higher values of yield parameters and yield viz., cane length (218 cm), cane girth (8.2 cm), individual cane weight (1.82 kg), cane yield (136.0 t/ha), and sugar yield (15.6 t/ha) were also resulted with mechanized cane cultivation.

Table 4. Comparison of mechanization with manual cultivation of sugarcane on population of soil microbes and economics (Cuddalore centre).

Treat ments	Population of soil microbes			Gross income (Rs./ha)	Cost of (Rs./ha)	Net income (Rs./ha)	BC ratio
	Actinomycetes (10^2 cfu/g soil)	Bacteria (10^6cfu/g soil)	Fungi (10^4cfu/g soil)				
T_1	58	12	14	388960	167735	221225	2.31
T_2	43	7	9	344630	177835	166795	1.93

The population of soil microbes viz, Actinomycetes, Bacteria and Fungi were higher with mechanized cultivation of sugarcane. In addition to reduced cost of cultivation, through enhancing the cane yield the practice of mechanization enhanced the net income to Rs. 2,21,225/- per ha) and the BC ratio (2.31).

Consolidated results obtained from the non-replicated trials raised at farmer's field at Ottai village of Vanur taluk of Vilupuram District in Cuddalore centre and at Mannukulam village of Thanjavur District in Sirugamani centre revealed that the soil texture were sandy clay loam and clay loam in respective experimental sites. At both the locations soil is neutral in pH with low in available 'N', high in available 'P' and medium in available 'K'. Regardless of the experimental sites, the mechanized cultivation of sugarcane registered higher mean values of millable canes (1,19,500/ha), cane length (210.5 cm), girth (8.05cm), single cane weight (1.72 kg), population of soil microbes (Actinomycetes, Bacteria and Fungi), cane yield (132.5 t/ha), net income (Rs.2,10,136/- per ha) and BC ratio (2.24) altogether with reduced cost of cultivation compared to manual cultivation of sugarcane.

Conclusion

Adoption of Mechanization in sugarcane cultivation enhances the values of varied yield parameters, cane yield and economic returns which also minimized the cost of cultivation of sugarcane.

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EVALUATION OF THE SELECTED GROUNDNUT (*Arachis hypogaea* L.) LINES FOR YIELD AND HAULMS

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Abstract

Groundnut varieties that combine high haulm yield with high pod yield are very desirable for farmer. Variance components (genetic, phenotypic, and error), broad sense heritability, genotypic coefficient of variation, phenotypic coefficient of variation, genetic advance, and genetic advance as a percentage of the mean are estimated in this study. Out of these 47 groundnut genotypes, MLT-SBER-19-20-04 is the superior genotype studied and it is the best choice for dual purpose utilization of farm cultivation.

Key words: Groundnut, Variance components, superior genotype, dual purpose utilization

Introduction

Groundnut, or peanut (Arachis hypogaea L.) is a species in the legume family Fabaceae. The crop is the second most important cultivated food legume and the fourth largest edible oilseed crop in the world (Shilman *et al*. 2011). Groundnut is now grown worldwide in the tropical and temperate zones primarily as an oil seed crop. India ranks second in the field area and third in production of groundnut in the world. Groundnut is an important oilseed crop grown in the arid and semi-arid tropics of India under rainfed condition.

Groundnut grows well in sandy loam or sandy clay loam soil with a pH of 6.5 -7.0.Optimum soil temperature for good germination of groundnut is 30 °C. Groundnut takes 90-130 days to mature and it requires a minimum rainfall of 500 mm and a maximum rainfall of 1250 mm. The rainfall should be distributed well during flowering and pegging of the crop. The groundnut cannot withstand frost, long and severe drought or water stagnation. Groundnut varieties are classified under bunch type, semi-spreading, spreading type. The application of gypsum 400 kg/ha at the pegging stage will enhance the pod formation. Whalen and chang (2001) reported that application of phosphorus fertilizer in combination with farmyard manure enhances the effectiveness of P fertilizers resulting in higher groundnut yields. Reddy (1991) stated the increase groundnut shelling percentage, 100 kernel weight, numbers of pods and pod yield per plant following application of FYM. The benefits of FYM addition in groundnut growth and yield has also been attributed to a range of factors including improvement in soil physical, microbial as well as nutrient availability such as N, P, K and Ca, and were more pronounced where combined with inorganic fertilisers (Deshmukh *et al*., 2005).

Methods and materials

The study was based on the 47 genotypes of groundnut which were obtained from Regional Research Station, Virudhachalam. The 47 genotypes of groundnut were selected based on mean pod and haulm yield as well as the days to physiological maturity. The field experiment was carried out in 'A' block research plot at Agricultural College and Research Institute, Eachangkottai, Thanjavur. The 47 genotypes were raised in the field of an area 0.32 acre . 1.6 ton FYM was applied initially after ploughing. The plot size is 4 x 3 m formed depending upon the availability of water, slope of the land and type of soil. The each genotypes were sown in line (i.e. Line sowing- 7 Lines per plot). The seeds were dibbled in the plots at a spacing of 30 cm between rows and 10 cm between plants. Irrigation was regulated based on physiological growth phases such as pegging, flowering and pod development for irrigation and adequate soil moisture is maintained.

Results and Discussion

The agronomic performances of the genotypes used in this study were given in Table 1. This is an indication that the traits were less influenced by the environment (Hamidou *et al*., 2012). Consequently, broad sense heritability was high in these traits. On the other hand, genetic variance of days to 50% flowering and shelling percentage were low compared to their error variances and therefore moderate broad sense heritability was recorded for these traits. The high broad sense heritability observed is an indication that direct selection for number of pods per plant and kernel yield can be done effectively (Ntare and Williams, 1998). Genotypic coefficient of variation (GCV) was highest for number of pods per plant and lowest in shelling percentage. The same trend was observed for phenotypic coefficient of variation with pod yield being the highest while shelling percentage showed the least PCV. Genetic advance was the highest in pod number per plant and the least in days to 50% flowering. However, it was haulm yield and number of pods per plant recorded the highest genetic advance as a percentage of the mean.

Number of days to 50% flowering ranged from 56 to 62 with a mean of 57. Average number of pods per plant was 30 with some genotypes having as low as 20 pods per plant and others as high as 45 pods per plant. The highest mean pod yield recorded was 3.17 t/ha with a mean of 1.21 t/ha . The highest mean kernel yield recorded

was 1.90 t/ha with an average of 0.78 t/ha. Average haulm yield recorded was 4.3 t/ha with the highest being 7.82 t/ha and the lowest being 2.71 t/ha. Shelling percentage ranged from 58 to 70%.

Table 1. Variance components, heritability, coefficient of variation, and genetic advance of 47 genotypes of groundnut

TRAITS	σ²g	σ²p	σ²e	H^2	GCV %	PCV %	GA	GAM %
Days to 50% flowering	2.75	4.06	1.31	0.68	9.71	14.33	2.82	9.96
No. of. Pods/plant	7.94	8.74	0.8	0.91	26.45	29.45	5.61	18.9
Pod yield (t/ha)	0.52	0.84	0.32	0.62	48	77.56	1.17	67.01
Kernel yield (t/ha)	0.75	1.03	0.38	0.72	26.2	31.8	1.53	57.8
Shelling percentage	6.38	8.13	1.75	0.78	3.98	4.68	5.43	8.34
Haulm yield (t/ha)	2.78	4.33	1.54	0.64	21.5	24.3	3.62	89.16

σ²g: genotypic variance, σ²p: phenotypic variance, σ²e: error variance, H^2: broad sense heritability, GCV: genotypic coefficient of variations, PCV: phenotypic coefficient of variation, GA: genetic advance, and GAM: genetic advance as a percentage of mean

Number of Days to Flowering: There was a significant ($p < 0.05$) difference among the genotypes for days to 50% flowering. Flowering time in groundnut is an indicative of the maturity period of genotype (Upadhyaya.et.al, 2006). The late maturing groundnut genotypes generally took more days (>30 days after planting) to reach 50% flowering. Some genotypes like MLT-SBER-19-20-01,MLT-SBER-19-20-02,MLT-SBER-19-20-03,MLT-SBER-19-20-04 and MLT-SBER-19-20-05 reached 50% flowering after 60 days (Table 1).

Haulm Yield: One major importance of groundnut is the use of the haulms for livestock feeding (Martey *et al*., 2015). Groundnut haulms after harvest have a high economic value as they are sold to livestock farmers. The haulms also contain high amounts of nitrogen which has the potential to improve soil fertility when incorporated into the soil (Ahiabor et.al., 2011). Therefore, groundnut varieties that combine high haulm yield with high pod yield are very desirable for farmer. Average haulm yield differed significantly ($p < 0.05$) among the genotypes and ranged from 2.71 to 7.82 t/ha (see Table 3). Genotypes MLT-SBER-19-20-04, MLT-SBER-19-20-02, MLT-SBER-19-20-05, MLT-SBER-19-20-03, VG 19543 and VG 19576 recorded haulm yield above 5 t/ha . These genotypes exhibited tolerance to leaf spots infection (data not shown) and therefore maintained most of their foliage at the time of harvest. Contrary to this, VG 19535 which is very susceptible to foliar diseases (Padi, 2008), shed most of its leaves by the time of harvest and therefore it is not surprising that it recorded the least haulm yield below 3 t/ha at the time of harvest.

Conclusion

Based on the agronomic performances of the genotypes used in this study, MLT-SBER-19-20-04 cultivar has high haulm yield and pod yield which will be suitable for dual purpose. Hence MLT-SBER-19-20-04 is the superior genotype out of these 47 genotypes studied and it is the best choice for dual purpose utilization of groundnut farm cultivation.

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AGRICULTURE SELF-RELIANCE IN INDIA: POSSIBILITIES AND OPPORTUNITIES

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Abstract

The Indian rural agrarian economy achieved the nations' food security from 50 million tonnes production in 1951 to 292 million tonnes food grains production in 2019. In horticulture, livestock and fisheries production also increased several folds. In this context, it is right time to address the nation's nutrition security, reduction of oilseeds imports, promotion of exportable commodities and value addition based products from the perishable horticultural and animal husbandry products. It also supports to convert the subsistent farmers in to successful farm entrepreneurs in farm and agri-business sectors with quality conscious international branding of local farm products to be sold globally through integrating farmers, rural infrastructure, logistics, warehousing, value addition, quality certification works, through farmers' centric institutions such as cooperatives, Farmers' Producer Organizations (FPOs), Self Help Groups (SHGs), Not for profit organizations (NGOs) and other private owned firms in rural area to make "rurban" village clusters to access all urban amenities and employment opportunities in a decentralized potential "agrarian - Industrial – services sector" settlement patterns with the global connectivity to support globalized, potential market oriented,information linkage based socio- economic transformation process. Moreover, the agricultural sector has the potential opportunities in coming future through capitalizing the creative and viable international economic opportunities through achieving self-reliance and self-sustainability to upgrade our nations' status from the developing category in to the developed upper middle income status.

Key Words: Agriculture, self-reliance, economy, agri-business

Introduction

Globally in 1700s; India had the strongest international economy of (24%) followed by China (23%). Later on, the colonization led Industrialization supported European economies in 1850s followed by the privatized corporate United States' economies in 1900s. The industrialized innovative economies enriched the global service sectors and its' contributions highlighted the global economy in the past century. Moreover, in 1990s, the global economic structural transformation process pressurized the globalization led economic policies promoted enormous development opportunities in the developing economics such as China and India. At the same time, these two highest populated countries' human resources capitalized various knowledge oriented, skilled based global human resource potentials in the economic transformation process. It has supported well in the economy of China and India with the share value of (15.5%) and (3.3%) respectively in the global GDP nominal value of 87.75 trillion US dollars in 2019.

In this context, it is timeto focus on sustainable development process to reach people from various social sections as well as through promoting economic development initiatives measures to retain their larger stake economy in one side and addressing sustainable development goals in another side for equitable and sustainable development of the country. Here, the agricultural sector plays very important role in the sustainable development process. It is also supportive for effective utilization of natural resources and its' conservation and management. Under this context, in India, it is urgent need to focus agricultural sector to regularize and mainstream the economic system services to capitalize the foreign direct investments for agri-preneurship and entrepreneurship development process, export promotion, sustainable industrial corridors, semi urban and rurban development process, need based education, health, food processing, etc. for creative and speedy growth oriented self-reliant economic development process.

Methodology

The study was conducted with the support of various secondary sources based reviews and the present-day need based policy documents; So, it acts as a conceptual article for the benefit of the academicians.

Discussions

Agriculture for self-reliant economy – Importance

India can be an economic superpower with the support of self-reliant in agriculture sector.Moreover, the villages are the foundation of civilization in India. "The soul of India lives in its villages", In India, around 833.1 million people (68.84%) of people live in 640,867 different villages with diversified culture, values and social cohesiveness.The rural agrarian economy achieved the nations' food security from 50 million tonnes production in 1951 to 292 million tonnes food grains production in 2019. Further, in horticulture, livestock and fisheries production also increased several folds. But, the horticultural, livestock and fisheries perishables products value addition and processing,

addressing the nation's nutrition security, reduction of oilseeds imports, promotion of exports markets based on the International markets, addressing affordable and accessible quality food and perishable horticultural and animal husbandry products to marginal sections of people are the challenges to trigger the countries' economic development in multi-dimensional ways. To access the opportunities, it is right time to convert the subsistent farmers in to successful farm entrepreneurs in farm and agri-business sectors with production and value addition of farm products with quality conscious international branding of local farm products to be sold globally through integrating farmers, rural infrastructure, logistics, warehousing, value addition, quality certification works, etc through farmers' cooperatives, Farmers' Producer Organizations, Self Help Groups, Not for profit organizations (NGOs) and other private owned firms. It can also provide a platform to micro food enterprises (MFEs), agricultural allied activities of animal husbandry, sericulture, mushroom culture, bio fertilizer and vermiculture, bee-keeping, herbal cultivation, in-land fisheries, integrated farming system practices and other commercial allied agricultural operations.

It should be possible to revaluate the existing agricultural and allied sectoral infrastructures, human resources potentials, national and international consumers preferences, public sectors policy priorities, private sectors' investment priorities, training and other facilitation potentials, information and communication technologies utilization opportunities, regional opportunities and natural resources potentiality particularly land and water for making effective farm based alternative livelihood and other commercial agro business and agro export opportunities to make potential market efficiency with easy marketing channels can provide livelihood, ownership and employment opportunities in rural area to make "rurban" village clusters to access all urban amenities and employment opportunities in a decentralized potential "agrarian - Industrial – services sector" transformational settlement patterns with the global connectivity through globalized, information linked, potential market oriented socio- economic Transformation process.

Possibilities and Opportunities in Agriculture sector for self-reliant economy

The probablepossibilitiessuch as accessing 2 million hectares of suitable lands for oil palm cultivation to yield 8 mt of palm oil to reduce the oil imports and save our foreign exchange reserves, standardisation and promotion of indigenous technology knowledge (ITK) in agriculture,connecting strongest social capitalist self-help groups (SHGs) and FPOs based organisations to boom the green economy through linking through corporate social responsibility (CSR) funds and corporate connectivity for facilitation and effective business promotion in a globalized manner to enhance the globalized agricultural production, secondary agricultural operations and human resources based Knowledge and skilling related to sustain effective agricultural economic opportunities with efficient production, value addition, marketing and supply chain management may provide a scope of private participation in numerous sectors. Effective utilisation of Mahatma Gandhi National Rural Employment Guarantee Act (MGNREGA) can also create adequate work days for creation of effective rural infrastructures, Addressing long-term commodity wise plans in local, regional and international markets with respective quality standards about the commodities' comparative advantages, available market, production, productivity, consumption, subsidies and incentives may facilitate to fix a long term vision to address the self-reliance in Agriculture. It also supports to prioritize other sectors financial allocation to speed up the Indian economy through addressing the nations' self-reliant economic demand and contribution in International economic avenues.

Further, the new agricultural export policy aimed at doubling farm exports by 2022. However, the Indian farm sector needs to adapt its production and value addition system in to the requirements of the world. India's farm exports commodities of marine products, rice, spices, buffalo meat, sugar, tea and coffee, fresh fruits and vegetables and cotton. But, still, India has not attained its' potential export opportunities particularly in the developed nations' context, due to quality standard norms, consumer's commodity demand and their preferences have been ignored. Hence, it is right time to offer incentives for promotion of high-value agri and allied sectors produce to address the doubling farmer's income in 2025. The recent infusion of Rs 1 lakh crores to strengthen agricultural infrastructure development works through modernizing processing, value addition and better marketing platforms,reforms to enhance land leasing, private investment, agricultural Research &Development areas,improvement in irrigation facilities, easy loan and crop insurance scheme, access to scientific support, and better prices for their produceefforts may transform the agriculture sector to local to regional, regional to national, national to international market accessibilities to cater to the needs of the global market.

Important programmes and activities to support the Agriculture sector for its' self-reliance

The natural resources conservation based climate smart agricultural practices, reduction of agricultural inputs utilization patterns through implementation of neem coated Urea technologies, encouraging farmers to shift from diesel pump set to solar pumps, promotion of conservation, Integrated farming technologies, Agri-Horti-Forestry based farming system practices and organic farming technologies, block chain for climate intelligence, forecasting solutions, machine learning to identify crop stages, soil health monitoring, artificial intelligence, plant image recognition reduces crop and natural resources wastages. Further, the Agricultural Infrastructure Fund of INR

1 lakh crores also supports to set up cold chain and post-harvest management infrastructure for adoption of innovative solutions for cold chain storage and supply chain management for pre- and post-harvest crops.Further, the biggest agriculture reforms though the electronic national agricultural market (Enam) based transparent and easily accessible marketing platforms also enhances the market share to the farmers on their commodity.

The transformed socio-entrepreneurial agricultural environment has tremendous potential to adopt various innovations and widespread initiation and risk-taking atmosphere for establishment of agri-tech start-ups, micro and small and medium enterprises entrepreneurial ecosystem of the country to enhance rural livelihoods and reduce costs associated with climatic uncertainties and traditional farming practices. It also helps to improve the economy recovery, strengthen global standard supply chains across industries and improve the domestic and export production potentials. It also promotes enlarged and relatively inelastic demand and supply systems to boost up crop management and climate resilience, etc to improve the financial efficiency of rural banks by vibrant cash flow to support infrastructure and other sectoral development process. In this way the transformed agrarian economy supports industrial (GDP share of 26%) and service sector (GDP share of 60% in 2015) through digital and smart economic pathways to utilize the full potentialopportunities of the decentralized agricultural based eco-friendlyrural village settlement pattern for effective global economic, socialand environmental benefits. It also avoids migration and population pressure related issues to existing megacities and other urban settlements. Because, even now also sixty percent of Indian population livesin rural area depends on agriculture.

Agriculture led Rural Transformational Process

The agriculture led socio- economic transformation process address the rural problems such as the lack of gainful employment, food insecurity, migration to urban areas, illiteracy, poorinfrastructural housing and poor health. It also supports "social entrepreneurship", road connection from various towns and cities, favourable infrastructural climate conditions and the drinking water sources, alternate livelihood opportunities, promotion ofoptimum utilization of available resources of land, water and gainful family farming to support the rural cluster based settlement for sustainable development to reduce the rapid centralized urban human settlements and other natural resources degradation issues. Moreover, the "Rurban" based village cluster settlement supports decentralized industrial developments, controls overcrowding, sustainable housing, promotion of employment opportunities, need based transport, Water, Sewage and solid waste disposal in an eco-friendly manner. At the same time,in another side, India can be utilizingits full potential opportunities of the decentralized agricultural based eco-friendly rural village settlement pattern for effective sustainable development for local, regional, national and international economic development process.

Agriculture led"Rurban settlements" for sustainable development process

The rurban settlements'support to make desirable standard of living with sustainable natural resource conservation and utilization patterns particularly in the potential southern and western regions of the country in first phase. Under this context, government of India initially planned to develop rural clusters with economic activities, developing skills, local entrepreneurship and providing infrastructure amenities through the scheme of Shyama Prasad MukherjiRurban mission (SPMRM) which aims to develop 300 rural clusters in all states and union territories with fourteen suggested components of continuous piped water supply, agro processing, agri services, storage and warehouses, sanitation, solid and liquid water supply, management, health care connectivity, skill development, public transport, Liquid Petroleum Gas connections and digital connection (Shyama Prasad MukherjiRurban mission 2015).

Further, the "rurban settlement" patterns supportssocial entrepreneurial perspective of hub and spoke model of agrarian based industries and business supported the village's development in a multiple way. Moreover, the social entrepreneurship based development supported by the village natural capital of farm lands, water sources, flora and fauna to sustain the agricultural economy. Later, agricultural economy based activities lead the secondary and tertiary sector development through the industrialization of cotton spinning mills, sugar, rice, value added processing and other agro industries with national and international connectivity. The well-connected by road to all the important cities act as a hub for the large-scale trading of agro and value added commodities to support the farm based business activities in the rural cluster in a rapid way. Like the way, the small town act as a hub and the decentralized village cluster settlements with in the radius of 100 square kilometres act as a spokes for the Hub and spoke model of sustainable development process. The village cluster also supports education, health, other services sectors establishments and it also supports the foreign capital investment patterns. In this way, over the period of 50 years long term periods the rurban villages acts as a sustainable and developed region in health, water, sanitation and education, communication facilities, entertainments, increased individual social responsibility and, Industrial and business activities.

The technological penetration leads the village community development in the industrial and service sector opportunities, improved access to health with the government hospitals and urban linked private hospitals supported

better health access to the villagers. The increased employment opportunities in abroad and employment opportunities in small and medium enterprises also indirectly helps the rurban based village development process. The enhanced education reduces the negative impact of the castism and worst taboos which is prevailing major development issues in the present context. Also, the sustainable development reduces poverty through financial, environmental (living conditions), and social development (including equality of income) perspectives.

Economic Reforms to harness the Global economic opportunities

In India, due to the lesser value of contribution to International economy, unaccounted, unaudited and unorganized economic activities which is not valued in the mainstream economics and more dependency of international economy for oil imports and other industrial products. Hence, to overcome these challenges to become an India's economic contribution to lead the upper middle income country status. It is right time to adopt the digital services, Goods and Service Tax and other tax reforms, merger of financial institutions, Adhar based direct cash transfers and direct benefit money transfer schemes, liberalization of foreign direct investment opportunities, etc for supporting the regulating the mainstream economic services for effective Gross Domestic Product (GDP) measurement process harness the economic development benefits. Further, the developed upper middle income status maintenance and sustainability is feasible through capitalizing the creative and viable international economic opportunities through achieving self-reliance and self-sustainability in agriculture and make for world and skilling India to making India as a self-reliant 5-6 trillion dollars' economy in 2025 and 20-25 trillion dollars' economy in 2050.

The programme on "Aatmnirbhar Bharat Abhiyan" and National Infrastructure Pipeline (NIP) with the investments over INR 100 lakh crore to build world-class infrastructural facilities. Infrastructure ranging from physical structures to digital highways attract innovations in lay outing and construction of roads and railways, green buildings, smart energy meters, waste and water treatment, Internet of technologies, telecommunications, sensors, security solutions for accessing global linkages through airports, consignment tracking for shipments, Artificial Inteligence for work-flow management, and drone surveillance. It may mitigate the nation's infrastructure deficiencies and transform the economy in a self-reliant in a competitive and comparative advantage opportunities in the globalized market. The self-reliant sustainable economic developments are resilience to future geo-economic shocks and to be self-sufficient, global manufacturing hub with enhanced sustainable development Indicators.

Conclusion

In the context of making India to be a potential contributor of global economy, major emphasis should be given to agricultural based decentralized rural economy to harness the globalization opportunities with Information and communication technologies led innovations to boost up the local agrarian economy to creative global economy. Though, our present India's GDP (PPP) Valued $10.51 trillion as compare to the GDP Nominal value of $2.94 trillion. This variation should be narrow down to make a developing economy in to getting the status of decent middle income country. Because as compare the middle income countries GDP nominal and GDP PPP of Mexico Nominal GDP valued $1.22 trillion against the Mexico GDP (PPP)value of $2.57 trillion, South Korea Nominal GDP valued $1.63 trillion against South Korea GDP (PPP) of $2.14 trillion, Brazil Nominal GDP valued $1.85 trillion against Brazil GDP (PPP) of $3.37 trillion and At last, China Nominal GDP valued $14.14 trillion against China GDP (PPP) of $27.31 trillion revealed that, the middle income countries' GDP nominal and GDP (PPP) variation is less as compare to the India's GDP nominal and GDP (PPP) values.Hence, the public, private stake holders, people and other development stake holder's role is very important to promote the Agriculture led rural cluster based developments for Self-reliance to become a global growth engine to feed the world under the next generations emerging global issues and challenges!

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PLANT -POLLINATOR INTERACTION IN INDIGENOUS SPECIES MITRAGYNA PARVIFOLIA (ROXB.) KORTH

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Abstract

Mitragyna parvifolia (Roxb.) Korth is one among the fast-growing with medium to large deciduous tree species belonging to the family Rubiaceae which has multiple utility. It is commonly known as Kadam. Butterflies have been identified as one of the sources potential pollinators.. Wide range of insects, including bees, wasps, and butterflies, were attracted to the Mitragyna flowers. The insects in the Family: Nymphalidae and Family: Pieridae have been identified as the most likely pollinators of the Mitragyna parvifolia trees. Butterflies are the most frequent visitors in M. Parvifolia tree. A total of 16 species of butterflies, among that Common emigrant, tawny coaster, white four rings, plain tiger and blue tiger were recorded as frequent visitors of M.parvifolia. Other insect groups such as bees, wasps and moths were also observed the flower visiting in the M.parvifolia tree in the months of may – June.

Introduction

The genus Mitragyna consist of 11 species, mainly distributed in tropical and arid/ semi-arid parts of Africa, India, China, Bangladesh, Myanmar, Sri Lanka, Pakistan and South-East Asia, Andaman and Nicobar Island (Govaerts *et al*., 2015). Out of 11 species, seven species are distributed in India, and Miragyna parvifolia is one among the tree species. The species exhibits many therapeutic properties and therefore used in the local / traditional medicine for many ailments (Pandey *et al*., 2006). Due to over exploitation and habitat destruction are the major constraints in conserving the wild stocks of this species which resulted as endangered tree species in Rajasthan (Panwar and Tarafdar 2006; Rai and Lalramnghinglova 2011). Conservation of M. parvifolia germplasm in situ and ex situ is an important pre-requisite to prevent loss of genetic diversity. M. parvifolia grow up to 1300 m, msl elevation, and widely grows in 20-35°C of temperature with mean rainfall between 1500 and 2500 mm and wide range of fertile soil optimum soil pH is 5.5-6.5. M. parvifolia wood quality is equal to that of teak, so it is commercially important as timber value for making furniture, cooperages in paper industry, widely used in agricultural appliances, and construction materials. The species is well recognized for its pharmaceutical medicinal properties of the alkaloids in it (indole and oxindole) (Brown *et al*., 2017). In the Chenchus, Yerukalas, Yanadis and Sugalis of Gundur District, Andhra Pradesh, tribals used fresh leaves sap of M. parvifolia to treat jaundice (Rao and Pullaiah, 2001). Valaiyans tribe, population of Sirumalai hills, Madurai district, Western Ghats, Tamil Nadu utilized stem bark of M. parvifolia for rheumatic pain. The fruit juice augments the quantities of breast milk in lactating mothers and also work as lactodepurant agent. Butterflies are one of the large taxonomically grouped insects (Lepidoptera: Rhopalocera) with 19,238 species in world wide and 1,501 species in India (Evans, 1932). Butterflies are important components of biodiversity and play a vital role in the ecosystem between flora and fauna. Most of butterflies are diurnal with attracting scaly wings called jewels. Butterflies are good indicators of environmental changes in the ambient features of any ecosystem. These insects are natural resources as they help in pollination, natural propagation and they feed on specific host plant foliage, nectar and pollen as their food. Hence the present study was carried out to explore the role of pollinators in a selected native tree species Miragyna parvifolia.

Materials and Methods:

The study was conducted in the selected (4 CPTs) M. Parvifolia twenty years old trees situated at Forest Campus, (Latitude 11°1′3.2" longitude 76°56′57" altitude 442 m) R. S. Puram, Coimbatore which is situate in the Coimbatore district of Tamil Nadu. The floral phenophase of anthesis, total number of flowers per inflorescence were counted 100 randomly selected inflorescence. The pollinator activity was recorded over the period of 24 hours and subsequently the observations were confined between 06.00 am to 06.00 pm for a period of 15 days. The floral visitors were collected using standard entomological procedure (Daffni, 1992) and butterflies were identified using field guides (Kehimkar, 2008; and Wynter-Blyth, 1957). Photographs were taken using (Nikon D3400) for species identification. Butterflies are the most frequent pollinators of M. parvifolia.

Results

The study was mainly focused on floral visitor and pollinators visit on the indegenous tree of M. parvifolia. The number of flowers visited per unit of time and the amount of time spent at each bloom are indicators of insect movement, which, in turn, is an indicator of the insects mobility. This demonstrates the efficiency with which the floral resource can be utilized. The length of time spent by each species of butterfly varies, as does the time spent on different plants by the same species. When a lot of nectar accumulates, the butterfly

requires more time to extract the nectar, and fewer flowers are visited. To sum up, there is a scarcity of data on butterfly flower visits and pollination. Accordingly, our preliminary observation was anthesis 6.30 pm to 12.30 am, peak time anthesis is 7 pm to 8 pm and an Inflorescence bear up to 110 – 175 flowers. The butterflies were started M. parvifolia flower visit from 07.00 am to 06.00 pm. A total of 16 species of butterflies belonging to four families, followed Papilionidae one species, Pieridae four species, Nymphalidae nine species and Lycaenidae with two species were recorded as frequent flower visitors of M. parvifolia. Other insect groups such as bees, wasps and moths were also observed the flower visiting in the M. parvifolia tree. Among the sixteen butterflies, species of Acraea terpsicore, Junonia lemonias, Ypthima ceylonica, Azanus ubaldus, Tirumala limniace, Hypolimnas bolina, Danaus chrysippus, and Catopsilia pomona were most frequently visited. The highest peak time of butterfly visitation is observed between 07.30 am to 09.00 am, then continuously reduced the number of flower visit. The butterfly flower handling time varied between 0.2 to 6.23 minutes. Honey bees visited at 6.00 am, peak time of visit is 7.00 am, No. of Visit is 270 times and the honey bees flower handling time varied between 0.15 to 0.25 minutes. Beetles visited at 6.40 am, peak time of visit is 7.20 am, No. of Visit is 26 times and the beetle's flower handling time varied between 01.20 to 6.63 minutes. Wasps visited at 7.10 am, peak time of visit is 8.00 am, No. of Visit is 30 times and the wasp's flower handling time varied between 0.17 to 0.30 minutes (Table-1). And finally moths visited during night time. For a proper appraisal of butterflies as users of floral nectar and pollen vectors, detailed studies in this intriguing and complex field of inquiry across several geographical regions are required.

Table – 1: Recorded Flower visitors in Mitragyna parvifolia inflorescences.

S.No	Common Name	Species	Time of Visit	Peak time of visit	No. of visit	Flower handling time (minutes)
Family: Papilionidae						
1	Common Rose	Pachliopta aristolochiae Fabricius, 1775	7.35 am	07.40 am	12	0.5 - 0.10
Family: Pieridae						
2	Pioneer	Belenois aurota (Fabricius, 1793)	07.12 am	08.45 am	59	0.4 – 1.0
3	Crimson-tip	Colotis danae (Fabricius, 1775)	07.00 am	07.30 am	21	0.2 – 0.38
4	Common Emigrant	Catopsilia Pomona (Fabricius, 1775)	08.00 am	09.00 am	142	0.5 – 0.30
5	Common Gull	Cepora nerissa (Fabricius, 1775)	08.08 am	08.40 am	76	0.8 – 1.0
Family: Nymphalidae						
6	Tawny Coaster	Acraea terpsicore Linnaeus, 1758	07.15 am	08.00 am	205	0.10 - 3.30
7	Yellow Pansy	Junonia hierta Fabricius, 1798	08.17 am	8.34 am	54	0.25 - 2.12
8	Lemon Pansy	Junonia lemonias Linnaeus, 1758	07.08 am	07.37 am	167	0.15 - 6.23
9	White Four-ring	Ypthima ceylonica Hewitson, [1865]	07.08 am	07.50 am	155	0.30 – 6.0
10	Blue Tiger	Tirumala limniace (Cramer, [1775])	07.35 am	08.00 am	125	0.6 – 0.54
11	Danaid Eggfly	Hypolimnas bolina (Linnaeus, 1758)	08.12 am	08.45 am	102	0.5 – 0.36
12	Double branded Crow	Euploea Sylvester (Fabricius, 1793)	07.40 am	08.06 am	45	0.14 – 1.15
13	Plain Tiger	Danaus chrysippus (Linnaeus, 1758)	07.55 am	08.14 am	120	0.10 - 0.53
14	Stripped Tiger	Danaus genutia (Cramer, [1779])	07.13 am	07.56 am	116	0.5 – 0.13
Family: Lycaenidae						
15	Bright Babul-Blue	Azanus ubaldus (Stoll, [1782])	8.40 am	8.45 am	117	0.20 – 1.26
16	Common Shot Silverline	Spindasis ictis Hewitson, 1865	08.00 am	08.25 am	36	0.20 - 0.45
17	Honey bee	Apis sps	06.00am	07.00am	270	0.15-0.25
18	Beetles		06.40am	07.20am	26	01.20-6.63
19	Wasp		07.10am	08.00am	30	0.17-0.30
20	Moth		During night time			

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BEHAVIOR OF CHLORPYRIFOS IN SOIL ECOSYSTEM

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Abstract

Chlorpyriphos is a non-systemic organophosphate insecticide, acting as a cholinesterase inhibitor with contact, stomach, and respiratory action ,The findings of the experiments are summarized here under.There were no drastic change was found in the initial and final characteristics of the Irugur sandy loam and clay loam soils by the application of Chlorpyriphos such as nitrogen, phosphorous and minor change in the pH, EC and there was no difference found in the organic carbon.Thechlorpyriphos LC_{50} and LC_{95} were calculated for sandy loam soil and clay loam soil by using the probit analysis based on earthworm mortality. Sandy loam has LC_{50} value of 10.88 $mgkg^{-1}$and LC_{95} of 106.71 $mgkg^{-1}$. Clay loam has LC_{50} value of 22.72 $mgkg^{-1}$and LC95 of 155.66 $mgkg^{-1}$ There were significant difference in the initial and final weight of earthworms by the application of Chlorpyriphos.

After spiking the chlorpyriphos in the sandy loam and clay loam soil the degradation of chlorpyriphos was occurred, and it was analysed by using GC at different interval resulted in the decrease residue with increase in dissipation percentage.The calculated half lives from regression equation were 37.62 days in sandy loam soil and 12.54 in the clay loam soil respectively

Introduction

India is now the second largest manufacturer of pesticides in Asia after China and ranks twelfth globally. The total production of pesticide in the world was 186830 million tones (India Stat, 2017).The production of Chlorpyriphos in India was 9880 million tones in 2015 and it was 9540 million tones in 2014. Total pesticide consumption in Tamil Nadu is 2000 metric tones of which Chlorpyriphos contributes 9.54 metric tones (Indiastat, 2017).

Majority of the farmers are unaware of pesticide types, level of poisoning, safety precautions and potential hazards on health and environment. Pesticide users such as farmers in developing nations like India are at a much higher risk of pesticide exposure due to lack of adequate safety measures and awareness.

Pesticides are considered hazardous chemicals since there is no strict compliance of pesticide regulation particularly in developing countries, pesticides remains a health risk. Pesticides have chemical classes such as organophosphates (OPs), organochlorines, synthetic pyrethroids, carbamates, in which organophosphates are said to have high Lethal Dose-50 (LD_{50}) (Arora *et al*., 2011).

The degradation study of Chlorpyriphos in different soil types of Tamil Nadu is very less especially in the Irugur soil series. The ecotoxicological study related to Chlorpyriphos is also limited in terrestrial and aquatic ecosystems. Considering the ecotoxicological importance of Chlorpyriphos and the environmental problems caused by it, this study was undertaken for proper investigation of the behavior of Chlorpyriphos and its bioavailability in the terrestrial and aquatic ecosystem.

Objectives

The major objective of this investigation is:

17. To study the behavior of Chlorpyriphos in different soil types of Irugur soil series

Methods

Location

The laboratory studies were conducted at the Department of Environmental Sciences, Tamil Nadu Agricultural University, Coimbatore. Soil sampling of Irugur series was done at Vellamadai village, Coimbatore district, Tamil Nadu.

Collection

The soil samples of Irugur series were identified and collected from the Vellamadai village. The target pesticide was not applied during the past 5 years or more in any of the chosen sites. The surface debris were removed, and the samples were collected from the top 0-15 cm and 15-30 cm.Before characterization, soil samples were passed through a 2mm sieve and stored. The processed soil samples were stored in polythene bags and then analyzed for some important physical and chemical characteristics. The soil samples were also used in the laboratory incubation experiment. Analysis of soil

Mechanical properties

The International Pipette method suggested by Piper (1966) was followed to determine the mechanical composition of the experimental soils.

Chemical properties

The soils were air dried and sieved to pass through a 2mm sieve and stored in polythene bags. pH and EC at 1: 2.5 soil and water ratio

The pH of soil was measured in water (1: 2.5) after half an hour equilibration with a glass electrode pH meter. The electrical conductivity of the supernatant suspension was measured using a conductivity bridge (Jackson, 1973). Chlorpyriphos in soil. Experiments were conducted to study the persistence and dissipation of chlorpyriphos , residues on sandy loam and clay loam soil under laboratory conditions. Blank samples were collected from non treated plot for past 10 years and the experiments were conducted by application of pesticide and residue analyzed using Gas Chromatography (GC).

Sample preparation

Degradation kinetics of Chlorpyriphos was quantified using laboratory incubation experiments. Soils were prepared according to the procedures by Racke*et al*. (1994). A 400 g of moist soil was weighed into a 1 L amber glass flask with caps (Fig 4.4). Twenty mL of 100 mg L^{-1}Chlorpyriphos solutions (in acetone) were added to one quarter of the soils, left for half an hour to allow the acetone to evaporate and then mixed with the rest of the soils. The flasks were then shaken on a horizontal shaker at 200 rpm for half an hour to ensure Chlorpyriphos was mixed homogenously with the soil. The flasks were stored at room temperature.

Soils were incubated in darkness and 10 g of soils were retrieved for analysis from each flask on day 0, 3, 5, 7, 14, 21 and 28 days. The weight of the incubation flasks was recorded to permit periodic addition of water so that constant moisture contents of soils could be maintained. All experiments were carried out in triplicates. All the collected samples were transported to the laboratory and processed immediately. All the processed samples were stored at -4˚ C before analysis

GC Analysis

Chlorpyriphos residues were analysed by adopting QuEChERS method (Paramasivam and Banerjee, 2012; Sinha *et al*., 2012). The residues of chlorpyriphos were estimated by GC coupled with Flame Photometric Detector (GC FPD).

Results

Chlorpyriphos dissipation study: The Chlorpyriphos dissipation study was conducted by spiking a known concentration (5ppm) in the sandy loam and clay loam soil to determine the half life, resulted in higher dissipation % in clay loam soil. The residue remined after incubation was showing 0.58 ppm with a dissipation % of 72.76 in clay loam soil where as 32.49% dissipation had occurred in the sandy loam soil resulted in half life of 37.62 days whereas half life of clay loam soil was obtained as 12.54 days.

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IMPACT OF ARBUSCULAR MYCORRHIZAL FUNGAL (GLOMUS SP.)INOCULATION ON ELICITING ANTIOXIDANT DEFENSE RESPONSE IN BLACKGRAM AGAINST *Spodoptera litura* INFESTATION

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Abstract

Arbuscular mycorrhizal fungi (AMF) augments plant defense to various biotic stress, besides to improving nutrient uptake, mainly phosphate.As a major defense response, production of antioxidants is accelerated in plants under stress. These metabolites productionintensified further when AMF colonized plants are subjected tostress. With this background, experiments were conducted to explore the impacts of Glomusintraradices on the stimulation of antioxidants defense in blackgram against Spodopteralitura. G. intraradicesinoculation stronglyaugmentedthe antioxidants defense metabolites, superoxide dismutase,catalase,peroxidase, phenylalanine ammonium lyase, carotenoids and tannins up on S. litura infestation in blackgram than AMF un-inoculated plants infested with S. litura.Theseoutcomes revealed that tolerance against S. litura in blackgram could be primed by mycorrhizalinoculation.Hence, AMF couldbe recommended as a bio-protectant against S. litura in blackgram.

Keywords: arbuscular mycorrhizal fungi, Spodoptera litura, blackgram, plant defense, antioxidants

Introduction

Arbuscular mycorrhizal fungi (AMF) is a unique soil fungi belongs to the phylum Glomeromycota which forms symbiotic association with almost 80% of land plants including agriculturally important crops. These fungi benefits to improve plant growth and health via various mechanisms viz., improve the uptake of water and immobile inorganic phosphate from soil, increase the leaf area, chlorophyll content, and photosynthetic rate, as well as regulating endogenous phytohormones production. Furthermore, it also improves plant defense against various biotic stresses. In the current scenario, climate fluctuation lead to an increase of pest incidence in crops, which in turn affects crop production. To overcome this stress, plants have evolved with diverse defense systems, includes the production of antioxidants metabolite, which protects the plants from negative effects of oxidative stress created by herbivorous insect. Interestingly, AMF symbiosis assists to augment antioxidants metabolite production in plants and thus potentially fortify both direct and indirect defense against the herbivorous insect (Mohamed *et al*., 2021 and Selvaraj *et al*., 2021).

Objective

To elucidate antioxidants defence metabolitesin blackgram colonized with AM fungi against herbivorous insect

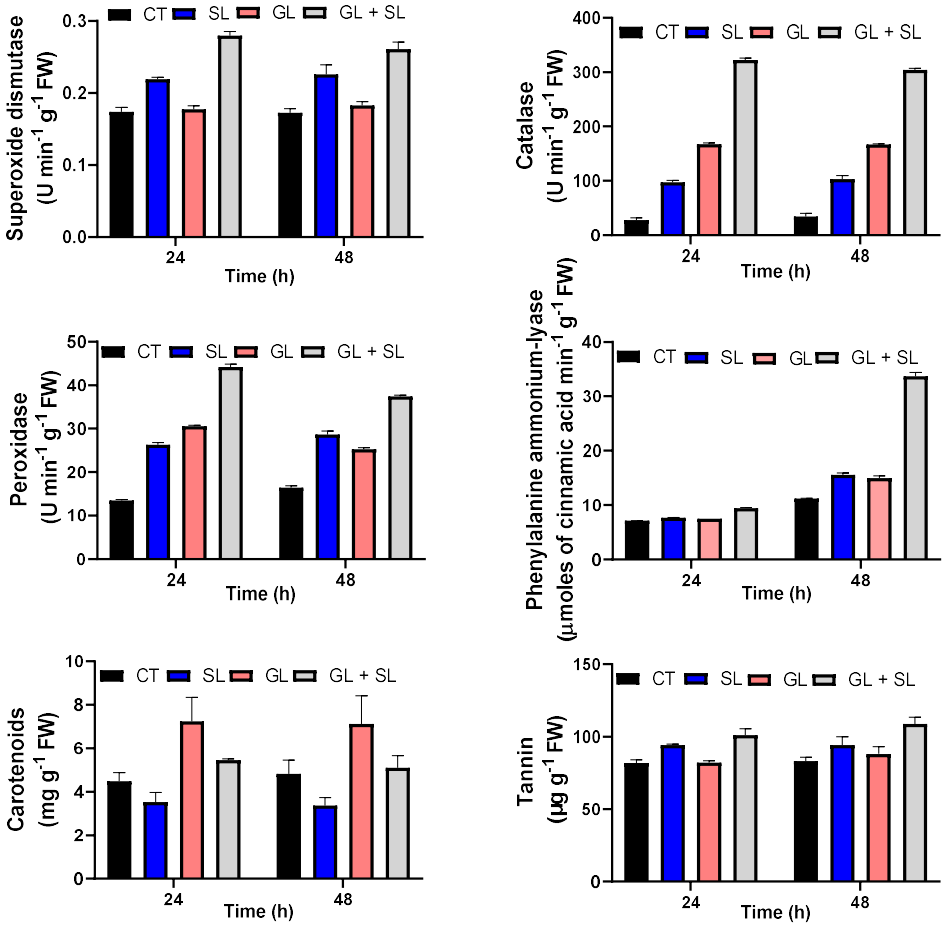
Materials and Methods

A pot culture experiment was conducted in the Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore by following completely randomized design (CRD) with four treatments. Blackgram seeds were surface sterilized with 10 % sodium hypochlorite for 10 min and rinsed thoroughly in distilled water for five times. Then, the surface sterilized seeds were treated with AMF spore (Glomus intraradices) at 5 spore's $seed^{-1}$. The seeds were sown in pots (17cm x15cm x15cm) filled with autoclaved red soil and sand (2:1) mixture. The plants were irrigated once in 2 days with 50-100 ml tap water and once in 4 days with 100 ml of Hoagland's nutrient solution. The herbivory treatment was initiated by releasing the three numbers of 3rd instar S. litura larvae on 40 days old blackgram seedling. The treatments include (CT) - Control without G. intraradices and S. litura, (SL) - S. litura, (AMF) - G. intraradices,and (AMF+ SL) - G. intraradices + S. litura. Leaf samples were collected after 24 h of *S. litura* larvae release and analyzed antioxidants metabolite (Hajiboland *et al*., 2020).

Results

In the current study, antioxidants metabolite of blackgram plants colonized with G. intraradices was influenced tremendously upon exposure to S. litura at different time intervals (24 and 48 h), which signifying the importance of G. intraradices in priming the defense against S. litura. Antioxidants metabolites, like superoxide dismutase (0.279 & 0.261U min^{-1} g^{-1} FW)), catalase (322.07 & 304.18U min^{-1} g^{-1} FW), peroxidase (44.19 & 37.40U min^{-1} g^{-1} FW), phenylalanine ammonium lyase (9.47 & 33.69µmoles of cinnamic acid min^{-1} g^{-1} FW) and tannins (101.16 & 108.87µg g^{-1} FW), except carotenoids, were significantly augmented in G. intraradices inoculated plants after 24 and 48 h of S. liturainfestation respectively (Figure 1). Mycorrhizal colonization was examined in all the treatments. Only AMF inoculated blackgram plants showed the presence of mycorrhizal colonization and it registered maximum of 78.33 %.

Figure 1. Production of leaf antioxidant metabolites of blackgram plants inoculated with AMF and infested by S. litura



Conclusion

The current investigation reveals that blackgram plants colonized with AMF not only improved plant growth by enhancing nutrient availability, but also aided in sustaining plant health during Spodoptera litura infestation. AMF colonization has improved the tolerance against S. litura infestation by modulating the production of defense antioxidant enzymes and metabolites. These results clearly indicated that AM fungi may be recommended as bioprotective agent during cultivation of field crops.

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STUDIES ON THE SURVIVAL OF BACTERIAL INOCULANTS IN RAINFED SORGHUM IN BLACK COTTON SOIL

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Abstract

Sorghum popularly known as Jowar is the most important food and fodder crop of dry land agriculture. The influence of biofertilizers like Azospirillum, phosphobacteria, Azophos (a 50:50 combination of Azospirillum and phosphobacteria) mycorrhiza individually and in combination was investigated in pre-monsoon sowing of APK 1 sorghum in a randomized replicated field trial in the Regional Research Station at Aruppukottai. The adherence of the inoculated bacteria was seen in all treatments. The results showed the feasibility of biofertilizer treatment in pre-monsoon sowing as the bacterial inoculants survived on seeds up to 21 days even though they were sown in dry soil and remain there until receipt of showers. However the inoculates were higher in Azophos + Mycorrhiza and Azospirillum. The appearance of the soil and hotness prevailing in Southern Tamil Nadu during summer gives an impression that this soil might not have any microbiological activity particularly in summer. Hence, the present study was focused on the survival of bacterial inoculants in rainfed sorghum.

Introduction

Sorghum crop is grown under both rainfed as well as irrigated condition. In rainfed crop two different practices of sowing viz., pre-monsoon sowing in a dry soil 2-3 weeks well ahead of monsoon showers and sowing at optimum soil moisture after monsoon rains are practiced. In the former practice adopted in southern districts of Tamil Nadu the seeds remain buried in dry soil in hot weather until soaking rain drenches the soil and seed. Indian farmers are highly dependent on rainfall and all activities of agriculture starting from sowing to post harvest are affected either directly or indirectly by weather especially by rainfall. The sorghum grain is used primarily as human food in various forms such as roti/bhakri (unleavened bread) or is cooked like rice or made into porridge with pulverized sorghum grown in Southern India. The sorghum grain and fodder is used for production of ethanol, starch and paper (Doifode, 2021).

Materials and Methods

The pre-monsoon sowing practiced in certain tract of Southern districts was simulated in pots with soil brought from RRS, Aruppukottai. In pre-monsoon sowing seeds are sown in soil anticipating rain in 2-3 weeks, the seeds are subjected to stress there in hot sun. Seeds are sown in earthen pots of 27 cm in diameter and left buried in the soil for three weeks. The soil was kept dry without irrigation and the soil temperature was recorded. The survival of inoculants on the seeds after sowing and prior to germination was investigated.

Results and Discussion

The results on the survival of bacterial inoculant on sorghum seeds in pre-monsoon sowing and left in the soil up to 3 weeks and the soil temperature recorded are presented in Table 1 and 2 respectively. Both Azospirillum and Phosphobacteria survived on seeds when the seeds were sown in a dry soil and left for 21 days without water simulating the condition in pre-monsoon sowing. Not only they survived but also exhibited an increase at certain sampling periods. The Azospirillum survived on seeds up to 21 days. The Azophos + Mycorrhiza treated seeds showed a higher population on 7 DAS, 14 DAS and 21 DAS when compared to other treatments. Control seeds also recorded a population of 0.13 to 0.17 x 10^4 cfu g^{-1} population which was negligible. The results indicated the survival of Azospirillum on seeds when they are sown and left in the field under dry condition.

Azophos treated seeds showed an increased number of phosphobacterial population, which recorded 41 x 10^3 cfu g^{-1} of seed immediately after seed treatment. Its population declined subsequently. The same trend was observed when phosphobacteria alone was seed treated. The biofertilizer treated seeds showed a higher population of phosphobacteria when compared to control. Soil temperature recorded during the period of experiment in soil ranged from 31-42ºC. The results clearly showed that the inoculated phosphobacteria survived on seed despite the dryness and high soil temperature. Besides the survival of inoculated organisms it is likely that soil adhering the seeds when removed for testing also might have contributed to the presence of Azospirillum and phosphobacteria as these were also observed in uninocualted seeds. It is not only the bacterial inoculants adhered to seed when inoculated but also survived when the treated seeds were sown in a dry soil subjected to hot dry condition until the showers drench the soil enabling the seeds to germinate. In the present study under a simulated dry condition in pots containing the black cotton soil the sorghum seeds treated with biofertilizer established survival of inoculated

bacteria for 3 weeks. The soil microbial profile is altered by season, soil organic matter (Mellado-Vazquez *et al*., 2016), plant root exudates, soil carbon dynamics (Chen *et al*., 2016), soil type, soil fertility, soil pH, soil moisture and soil temperature.

Table 1. Survival of biofertilizers on sorghum sown in soil (pot culture)*

Sl. No.	Treatments	Azospirillum (x 10^4)				Phosphobacteria (x10^3)			
		0 DAS	7 DAS	14 DAS	21 DAS	0 DAS	7 DAS	14 DAS	21 DAS
	Azospirillum 3 pkt / ha seed rate	0.47	0.17	0.13	0.20	-	-	-	-
	Phosphobacteria	-	-	-	-	12	6	3	3
	Azophos	0.45	0.44	0.36	0.13	41	29	26	14
	Azophos + Mycorrhiza	0.47	0.41	0.38	0.33	20	12	8	9
	Phosphobacteria + Mycorrhiza	-	-	-	-	20	9	8	12
	Control (Uninoculated)	0.17	0.17	0.13	0.13	12	3	2	3
	Azospirillum culture	0.14**	-	-	-	-	-	-	-
	Azophos culture	0.38**	-	-	-	5**	-	-	-
	Phosphobacteria culture	-	-	-	-	4**	-	-	-

** cfu 10^7 on wet weight basis
*Moisture content of soil 8.4 at 0 DAS, 5.1 at 7 DAS

Table 2. Soil temperature recorded in pots during the period of experiment*

DAS	Temperature (ºC)	DAS	Temperature (ºC)
0	38	11	40
1	39	12	39
2	38	13	40
3	39	14	34
4	40	15	36
5	38	16	31
6	40	17	39
7	40	18	40
8	39	19	42
9	35	20	40
10	39	21	39

*Moisture content 8.4 at 0 DAS; 5.1 % at 7 DAS

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BIO-PRIMING AND INTE*GRATED MANAGEMENT OF SESAME DISEASES

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Abstract

Sesame production is declining day by day in the traditional sesame growing areas due to severe biotic stresses such as root rot and powdery mildew may cause heavy yield losses in sesame, if management is not proper. Hence, possible attempts were made to assess the effect of IDM modules with chemicals and biocontrol agents on disease incidence and yield of sesame. The field trials were conducted at Regional Research Station, Vridhachalam during Kharif seasons of 2020 with three IDM modules: M_1-Biointensive: ST with *Trichoderma asperellum* @ 10 g/kg, furrow application of enriched *Trichoderma* (2.5 kg *Trichoderma* sp. + 100 kg Vermicompost) @ 250 kg/ha, spray of *Pseudomonas fluorescens* @ 10 g/l at 30-35 DAS, wettable sulphur @ 2 g/l at 50-60 DAS; M_2-Chemical: ST with carbendazim @ 2 g/kg, spray of combi product (Tebuconazole 50% + Trifloxysrobin 25%) @ 0.5 g/l at 30-35 DAS and second spray at 50-60 DAS; M_3-Adoptive: ST with *T. asperellum* @ 10 g/kg, furrow application of enriched *Trichoderma* (2.5 kg *Trichoderma* sp. + 100 kg Vermicompost) @ 250 kg/ha and spray of combi product (Tebuconazole 50% + Trifloxysrobin 25%) @ 0.5 g/l at 30-35 DAS and second spray at 50-60 DAS; M_4-Untreated check: Control in Randomized block design with five replications using the susceptible variety VRI-1. The results of the field experiment revealed that Module - M_3 - ST with *T. asperellum* @ 10 g/kg, furrow application of enriched *Trichoderma* (2.5 kg *Trichoderma* sp. + 100 kg Vermicompost) @ 250 kg/ha and spray of combi product (Tebuconazole 50% + Trifloxysrobin 25%) @ 0.5 g/l at 30-35 DAS and second spray at 50-60 DAS was effective in managing the major diseases of sesame which recorded minimum root rot disease incidence (5.04 %) and powdery mildew (6.96 PDI) with higher yield of 644.0 kg/ha.

Key words: Sesame, Biocontrol, fungicide, Management ,root rot, powdery mildew

Introduction

Area and production of sesame is declining day by day in the traditional sesame growing areas due to severe biotic stresses such as Root rot and Powdery mildew may cause heavy yield losses in sesame, if management is not proper. Due to existing health risk and pollution hazards by use of chemical fungicides in plant disease management, it is the need of the hour to minimize pesticide usage. Hence, possible attempts were made to assess the effect of IDM modules with chemicals and biocontrol agents on disease incidence and yield of sesame.

Materials and Methods

The field trials were conducted at Regional Research Station, Vridhachalam during Kharif seasons of 2020 with three IDM modules: M_1-Biointensive: ST with Trichoderma asperellum @ 10 g/kg, furrow application of enriched Trichoderma (2.5 kg Trichoderma sp. + 100 kg Vermicompost) @ 250 kg/ha, spray of Pseudomonas fluorescens @ 10 g/l at 30-35 DAS, wettable sulphur @ 2 g/l at 50-60 DAS; M_2-Chemical: ST with carbendazim @ 2 g/kg, spray of combi product (Tebuconazole 50% + Trifloxysrobin 25%) @ 0.5 g/l at 30-35 DAS and second spray at 50-60 DAS; M_3-Adoptive: ST with T. asperellum @ 10 g/kg, furrow application of enriched Trichoderma (2.5 kg Trichoderma sp. + 100 kg Vermicompost) @ 250 kg/ha and spray of combi product (Tebuconazole 50% + Trifloxysrobin 25%) @ 0.5 g/l at 30-35 DAS and second spray at 50-60 DAS; M_4-Untreated check: Control in Randomized block design with five replications using the susceptible variety VRI-1.

Results and Discussion

The results of the field experiment revealed that Module - M_3 - ST with T. asperellum @ 10 g/kg, furrow application of enriched Trichoderma (2.5 kg Trichoderma sp. + 100 kg Vermicompost) @ 250 kg/ha and spray of combi product (Tebuconazole 50% + Trifloxysrobin 25%) @ 0.5 g/l at 30-35 DAS and second spray at 50-60 DAS was effective in managing the major diseases of sesame which recorded minimum root rot disease incidence (5.04 %) and powdery mildew (6.96 PDI) with higher yield of 644.0 kg/ha. This was followed by Module II (M_2) comprising of ST with carbendazim @ 2 g/kg, spray of combi product (Tebuconazole 50% + Trifloxysrobin 25%) @ 0.5 g/l at 30-35 DAS and second spray at 50-60 DAS which recorded root rot disease incidence (10.64 %) and powdery mildew (12.34 PDI) with yield of 616 kg/ha. Control recorded the maximum root rot disease incidence (24.08%) and powdery mildew (22.64 PDI) with minimum yield of 473 kg/ha. The BCR varied significantly among the treatments. Higher BCR was recorded in M3- Adoptive treatment (2.24) whereas the check recorded the least (1.68). Papavizas and Lumsden (1980) opined that changes in soil reaction due to increased activity of introduced Trichoderma species might be one among the reason s for the increased seedling growth beside production regulating substances by the antagonists. Rettinassababady *et al*., (2000) found that significant reduction in black

gram powdery mildew incidence due to foliar spray of neem oil (3 %) might be due to the presence of sulphur containing compounds.

Table .1 Bio-priming and Integrated management of major diseases of sesame

S. No	Modules	Root rot (%)	Powdery mildew (PDI)	Yield (kg/ha)	B:C ratio
1.	M_1-Biointensive	12.70(13.46)	13.34(17.63)	537	1.87
2.	M_2-Chemical	10.64(9.70)	12.34(16.55)	616	2.16
3.	M_3-Adoptive	5.04(6.62)	6.96(7.08)	644	2.24
4.	M_4-Untreated check	24.08(25.12)	22.64(24.81)	473	1.68
C.D (0.05)		4.13	5.11	25.67	
C.V		8.08	11.32	3.28	
S.Em		2.20	1.58	15.77	

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EFFICIENCY INDICES OF WEED MANAGEMENT IN COTTON

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Abstract

Weeds are considered as a major biotic constraint for high production. The weeds which germinate before or simultaneously with the crop are frequently capable of forming a leaf canopy over cotton. To manage the weed effectively the present study was taken during winter seasons of 2008-09 and 2009-10 at Tamil Nadu Agricultural University, Coimbatore. Field trials were laid out in randomized block design with treatments replicated thrice. The results showed that weed control efficiency (WCE) was maximum under pre-emergence application of pendimethalin (38.7%) at 4.0 kg ha^{-1} at 25 and 45 DAS. Pre-emergence application of pendimethalin registered higher WCE ranging between 93.45 and 65.8 per cent. The weed free check with maximum yield was taken as the base to work out the weed index that gives the magnitude of yield reduction due to weed competition in other treatments. The highest yield reduction of 51.7 and 103 per cent occurred under unweeded control during winter 2008-09 and winter 2009-10, respectively.

Keywords: Cotton, Pendimethalin, Weed control efficiency, Weed Index, SDR

Introduction

Cotton the "white gold or the king of fibres" is one of the most important commercial crops in India. Cotton is known for the fibre and oil from seed, which plays a prominent role in the national and international economy. In India, cotton cultivation provides livelihood for over 4 million farming families. It produces only 3.95 million bales of lint every year with a productivity of 567 kg ha^{-1} (Anonymous, 2008). The key role that cotton plays in our country can be gauged from the fact that nearly 15 million farmers spread out in more than 10 states are dependent on cotton cultivation (Prasad and Prasad, 2009). Cotton must be kept weed free for a period after emergence in order to avoid crop loss (Coble and Byrd, 1992). Pendimethalin is now commercially available as 30% EC in market and with increase in active ingredient percentage, it is necessary to evaluate its effect on weeds and crops.

Materials and method

Field experiments were laid out in Field No. 73 and 36C during winter seasons of 2008-09 and 2009-10, respectively in Eastern Block farm of Tamil Nadu Agricultural University, Coimbatore. The farm is situated at 11° North latitude and 77° East longitude at an altitude of 426.72 m above Mean Sea Level. The soils of the experimental sites were sandy clay loam in texture with low in available nitrogen, medium in available phosphorus and high in available potassium. Cotton (Gossypium hirsutum L.) variety MCU 13 was raised during winter season of 2008-09 and Bunny Bt during 2009-10.

Field trials were laid out in randomized block design with treatments replicated thrice. In the present study, various weed management practices viz., pre-emergence pendimethalin 38.7% EC at 1.5, 2.0, 2.5 and 4.0 kg ha^{-1} + HW, pendimethalin 30% EC at 1.0 kg ha^{-1} + HW, EPOE trifloxysulfuron at 10g ha^{-1} + HW, PE pendimethalin 30% EC at 1.0 kg ha^{-1} + PWW, pendimethalin 30% EC at 1.0 kg ha^{-1} + CRM + HW, PWW on 25 and 45 DAS, hand weeding twice, weed free and unweeded checks were included.

The following efficiency indices were calculated

Relative density

The relative density (RD) of weeds was worked out using the following formula

$$RD\,\% = \frac{\text{No. of weeds of individual species}}{\text{Total no. of weeds}} \times 100$$

Relative dry weight

The relative dry weight (RD_{wt}) of individual weed species was worked out using the following formula and expressed as per cent.

$$RD_{wt} = \frac{\text{Dry weight of weeds of individual species (gm}^{-2})}{\text{Total dry weight of weeds (g m}^{-2})}$$

Weed control efficiency

Weed control efficiency (WCE) was calculated as per the procedure given by Mani *et al*. (1973).

$$WCE\ \% = \frac{WD_c - WD_t}{WD_c} \times 100$$

Where,

WCE - weed control efficiency (per cent)

WD_c - weed biomass (g m^{-2}) in control plot.

WD_t - weed biomass (g m^{-2}) in treated plot.

Summed dominance ratio

The summed dominance ratio for individual weed species was worked out using the following formula

$$SDR = \frac{RD + RD_{wt}}{2}$$

RD = Relative density (no. m^{-2})

RD_{Wt} = Relative dry weight (g m^{-2})

Weed index

Weed index (WI) was calculated as per the method suggested by Gill and Vijaya Kumar (1969).

$$WI = \frac{X - Y}{X} \times 100$$

Where, X = yield (kg ha^{-1}) from minimum weed competition plot

Y = yield (kg ha^{-1}) from the treatment plot for which WI is to be worked out.

RESULTS AND DISCUSSION

Relative density

During winter 2008-09 season cotton crop, broad leaved weeds were dominant followed by grasses and sedges at early stages of crop growth, subsequently the grassy weeds dominated dominated the weed flora followed by broad leaved weeds and sedges.

At 25 DAS, the relative density of grasses was relatively lesser with application pendimethalin (38.7%) at 2.0 kg ha^{-1}, whereas, the density of broad leaved weeds and sedges were lesser with trifloxysulfuron at 10 g ha^{-1}, while, the grassy weed density was more in this treatment. During 2009-10 crop season, broad leaved weeds were dominant followed by grasses and sedges at the early stages of crop growth and later on the grasses become the dominant weed flora followed by broad leaved weeds and sedges. The relative density of grasses was lesser with pendimethalin (38.7%) at 2.0 kg ha^{-1} whereas, the density of broad leaved weeds and sedges were lesser with trifloxysulfuron at 10 g ha^{-1} while, the grass weed density was more in this treatment at 25 DAS. Srinivasan *et al*. (1992) reported application of Thiobencarb (1 kg/ha) + 2,4- DEE (0.5 kg/ha) in rice of rice-mung cropping system controlled weeds effectively than Anilophos (0.3 kg/ha) + 2,4-DEE (0.5 kg/ha)as it indicated by lower relative density and relative dry weight.

Relative dry weight

During winter 2008-09, at 25 DAS the relative dry weight of grassy weeds was higher followed by broad leaved weeds in unweeded control treatment. The relative dry weight of grassy weeds was lesser with pendimethalin (38.7%) at 2.0 kg ha^{-1}. During 2009-10 at 25 DAS, the relative dry weight of grassy weeds was higher followed by broad leaved weeds in unweeded plot. The relative dry weight of grassy weeds was lesser with pendimethalin (38.7%) at 2.0 kg ha^{-1}.

Summed dominance ratio (SDR)

The summed dominance ratio (SDR) of weeds gives a clear picture of the dominance of the weed in the respective treatment and effectiveness of the weed control treatments. In the first crop, it was found that lesser values of SDR for grassy weeds were observed under pendimethalin (38.7%) at 2.0 kg ha^{-1} and pendimethalin (38.7%) at 2.5 kg ha^{-1} at 25 DAS. Similar results was also obtained by Mian *et al*. (2007) who computed SDR was the highest in grasses indicating principal dominancy as compared to other species. At 45 DAS lesser SDR values of broad leaved weeds and sedges were observed with trifloxysulfuron at 10 g ha^{-1} and higher values of SDR for broad leaved weeds were observed with pendimethalin applied treatments.

During 2009-10, lesser values of SDR for grassy weeds were observed pendimethalin (38.7%) at 2.0 kg ha^{-1} + HW and pendimethalin (38.7%) at 2.5 kg ha^{-1} at 25 DAS. At 45 DAS lesser SDR values of broad leaved weeds and sedges were observed with trifloxysulfuron 10 g ha^{-1} followed by HW and higher values of SDR for broad leaved weeds were observed with pendimethalin applied treatments. The reduction of summed dominance ratio of

Echinochloa spp. and Cyperus difformis in herbicide treated plots indicated their effectiveness in weed control (Ramanjaneyulu *et al*., 2006).

Effect of herbicides on weed control efficiency

Weed control efficiency (WCE) showed the maximum value under pre-emergence application of pendimethalin (38.7%) at 4.0 kg ha^{-1} at 25 and 45 DAS. Pre-emergence application of pendimethalin registered higher WCE ranging between 93.45 and 65.8 per cent. The results of the present study indicated that application of pendimethalin (38.7%) at different doses followed by hand weeding produced higher WCE throughout the crop period which was comparable with the conventional weeding at 45 DAS. The integrated weed management practice gave the broad spectrum weed control as a result of longer persistency in the soil profile. Manual weeding is usually rendered difficult especially during the monsoon seasons due to intermittent rains and consequently the moisture content of the soil would be too high for mechanical manipulation. Hence, application of pendimethalin (38.7%) at 2.0 to 4.0 kg ha^{-1} followed by hand weeding is a quite suitable option to overcome the weed problem in cotton. Similar finding was reported by Balasubramanian (1992) who found that the weed control efficiency was comparatively higher with the application of pendimethalin at 1.0 kg ha^{-1} as compared with 0.5 and 0.75 kg ha^{-1}.

Lower dose of pendimethalin 38.7% at 1.5 kg ha^{-1} and pendimethalin 30% at 1.0 kg ha^{-1} followed by hand weeding, power weeder weeding and crop residue mulch application resulted in higher WCE. However, it was not persistent throughout the cropping period. This might be possibly due to lower dose of herbicide which was not sufficient to break the metabolism of the weeds. Though it suppressed the weeds for shorter period, it took longer time to control the weeds as against higher doses.

Effect of treatments on seed cotton yield

Pendimethalin (38.7%) at 2.0 kg ha^{-1} + hand weeding recorded higher seed cotton yield of 58 and 32 per cent during winter 2008-09 and 2009-10 seasons, respectively over unweeded control. The next best treatment was the pendimethalin (38.7%) at 2.5 kg ha^{-1} + hand weeding. Application of pendimethalin at 1.0 kg ha^{-1} in combination with inter culturing plus hand weeding gave 199.4 per cent increase in seed cotton yield over untreated check was reported by Ali *et al*. (2005). Gnanavel and Babu, (2008) also reported maximum seed cotton yield with pendimethalin and fluchloralin combination coupled with hand weeding as compared with control. Hand weeding twice recorded lower seed cotton yield during winter season due to poor control of grasses and broad leaved weeds. Cotton being a wide spaced and slow growing crop is sensitive to weed competition at early stages of growth than at later stages. Due to heavy infestation of weeds under unweeded check, there was 32 to 58 per cent reduction in seed cotton yield. Weeds compete with crop for light, nutrients and water. Hence, the crop under unweeded control may not be able to obtain the above growth factors in optimum quantity resulting in reduced leaf area, dry matter production and number of leaves. This would have reflected in poor yield under unweeded control. Presence of weeds throughout the growing season caused poor crop growth and caused yield reduction in unweeded check (Bhoi *et al*., 2007).

Conclusion

The success of the weed control operations is dependent on the time of weed seedling emergence, weed species and stage of crop growth. Timely applications of effective herbicide are able to reduce losses when there is an occurrence of targeted weeds, optimize herbicides efficacy against weeds and also minimize production cost or protect crops against injury.

Table 1. Weed control efficiency (WCE) as influenced by weed management practices in cotton

Treatments	Weed control efficiency (%)					
	Winter 2008-09			Winter 2009-10		
	DAS			DAS		
	25	45	60	25	45	60
T_1 - Pendi 38.7% at1.5 kg/ha + HW	77.82	45.46	65.13	80.71	48.70	71.29
T_2 - Pendi 38.7% at 2.0 kg/ha + HW	86.12	62.48	74.94	87.93	63.46	79.20
T_3 - Pendi 38.7% at 2.5 kg/ha + HW	86.23	65.80	75.29	88.02	65.96	78.48
T_4 - Pendi 38.7% at 4.0 kg/ha + HW	86.40	52.47	75.09	88.17	52.24	79.22
T_5 - Pendi 30% at 1.0 kg/ha + HW	76.18	41.42	63.53	79.29	45.18	69.65
T_6 - EPOE Trifloxy at 10g/ha + HW	76.26	52.58	70.20	79.35	54.19	68.46
T_7 - Pendi 30% at 1.0 kg/ha + PWW	75.61	29.44	58.46	75.31	34.74	65.62
T_8 - Pendi 30% at 1.0 kg/ha + CRM + HW	76.36	63.29	74.74	79.44	61.14	65.59
T_9 – PWW on 25 and 45 DAS	6.85	18.10	35.49	7.85	33.69	32.11
T_{10} - HW on 25 and 45 DAS	12.10	48.37	74.10	13.65	61.28	79.96
T_{11} - Weed free check	98.18	97.50	97.46	98.42	98.41	97.58
T_{12} - Unweeded check	0.00	0.00	0.00	0.00	0.00	0.00

Data not statistically analysed

Table 2. Weed index as influenced by weed management practices in cotton

Treatments	Weed Index (%)	
	Winter 2008-09	Winter 2009-10
T_1 - Pendi 38.7% at 1.5 kg ha^{-1} + HW	23.90	49.50
T_2 - Pendi 38.7% at 2.0 kg ha^{-1} + HW	8.15	12.50
T_3 - Pendi 38.7% at 2.5 kg ha^{-1} + HW	16.19	30.8
T_4 - Pendi 38.7% at 4.0 kg ha^{-1} + HW	27.53	56.51
T_5 - Pendi 30% at 1.0 kg ha^{-1} + HW	17.24	36.62
T_6 - EPOE Trifloxy at 10g/ha + HW	17.84	38.80
T_7 - Pendi 30% at 1.0 kg/ha + PWW	26.10	52.31
T_8 - Pendi 30% at 1.0 kg/ha + CRM +HW	26.65	50.67
T_9 – PWW on 25 and 45 DAS	28.14	64.42
T_{10} - HW on 25 and 45 DAS	10.63	14.51
T_{11} - Weed free check	0.00	0.00
T_{12} - Unweeded check	51.7	103.3

Data not statistically analysed

Fig 1. Effect of weed management methods seed cotton yield in cotton

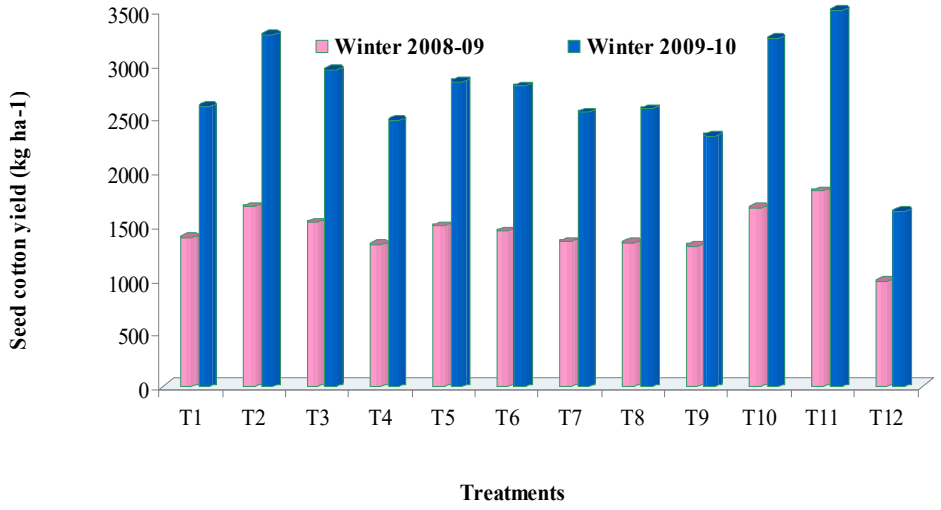

Table 3. Relative density (per cent) of weeds as influenced by weed management practices in cotton

Treatments	Relative density (per cent)						Relative dry weight (per cent)					
	Winter 2008-09			Winter 2008-09			Winter 2008-09			Winter 2008-09		
	Grasses	Sedge	BLW	Grasses	Sedge	BLW	Grasses	Sedge	BLW	Grasses	Sedge	BLW
T_1 - Pendi. (38.7%) at 1.5 kg ha^{-1} + HW	35.25	16.69	48.05	17.74	22.91	59.34	33.6	13.3	53.1	19.00	26.35	54.65
T_2 - Pendi. (38.7%) at 2.0 kg ha^{-1} + HW	29.29	17.00	53.71	17.82	23.14	59.04	32.2	12.5	55.3	20.28	26.17	53.54
T_3 - Pendi. (38.7%) at 2.5 kg ha^{-1} + HW	28.57	16.28	55.15	18.10	22.43	59.47	34.2	13.7	52.2	21.62	26.51	51.87
T_4 - Pendi. (38.7%) at 4.0 kg ha^{-1} + HW	30.91	16.63	52.45	20.38	20.97	58.65	31.3	20.5	48.2	21.28	25.59	53.13
T_5 - Pendi. (30%) at 1.0 kg ha^{-1} + HW	33.89	16.81	49.30	19.17	23.87	56.97	34.2	14.1	51.6	18.61	26.59	54.80
T_6 - EPOE Trifloxy. 10 g ha^{-1} + HW	64.71	10.11	25.19	38.92	18.23	42.85	58.9	6.1	35.0	90.74	5.40	3.86
T_7 - Pendi. (30%) at 1 kg ha^{-1} + PWW	43.37	14.39	42.24	20.20	22.96	56.84	35.0	12.9	52.1	19.27	26.72	54.01
T_8 - Pendi. (30%) at 1 kg ha^{-1} + CRM + HW	30.27	17.87	51.86	16.19	23.87	59.95	36.7	7.2	56.2	20.01	26.23	53.76
T_9 - PWW on 25 and 45 DAS	42.62	12.23	45.15	24.48	19.04	56.48	51.0	8.7	40.3	46.33	10.39	43.28
T_{10} - HW on 25 and 45 DAS	33.35	16.30	50.35	18.31	22.84	58.85	52.6	2.5	44.9	44.38	10.22	45.41
T_{11} - Weed free check	32.76	18.10	49.14	19.18	23.30	57.53	49.1	11.0	39.9	39.58	26.39	34.04
T_{12} - Unweeded control	45.07	13.54	41.39	32.18	19.15	48.68	49.1	9.9	41.1	46.45	10.35	43.21

Data not statistically analysed

Table 4. Summed dominance ratio of weeds as influenced by weed management practices in cotton

Treatments	25 DAS						45 DAS					
	Winter 2008-09			Winter 2009-10			Winter 2008-09			Winter 2009-10		
	Grasses	Sedge	BLW	Grasses	Sedge	BLW	Grasses	Sedge	BLW	Grasses	Sedge	BLW
T_1 - Pendi. (38.7%) at 1.5 kg ha^{-1} + HW	36.02	18.75	45.23	24.36	24.09	51.56	54.28	9.17	36.55	52.27	7.22	40.52
T_2 - Pendi. (38.7%) at 2.0 kg ha^{-1} + HW	36.57	17.46	45.97	22.82	23.75	53.43	54.52	7.53	37.95	53.56	6.05	40.39
T_3 - Pendi. (38.7%) at 2.5 kg ha^{-1} + HW	36.01	19.21	44.78	23.61	23.59	52.80	53.43	7.80	38.77	53.28	6.05	40.67
T_4 - Pendi. (38.7%) at 4.0 kg ha^{-1} + HW	35.01	21.86	43.14	23.56	23.40	53.04	55.49	9.42	35.09	52.62	5.83	41.54
T_5 - Pendi. (30%) at 1.0 kg ha^{-1} + HW	36.43	19.10	44.47	24.16	24.50	51.34	54.73	9.64	35.63	52.17	7.30	40.53
T_6 - EPOE Trifloxy. 10 g ha^{-1} + HW	67.63	7.26	25.11	80.79	6.58	12.64	74.40	4.54	21.06	76.37	3.47	20.17
T_7 - Pendi. (30%) at 1 kg ha^{-1} + PWW	36.29	18.04	45.68	23.82	24.74	51.43	51.52	9.58	38.90	50.10	8.18	41.72
T_8 - Pendi. (30%) at 1 kg ha^{-1} + CRM + HW	37.41	15.03	47.56	24.07	24.06	51.86	49.60	12.23	38.17	51.70	8.85	39.45
T_9 - PWW on 25 and 45 DAS	46.61	13.48	39.92	43.04	13.52	43.43	48.80	9.83	41.36	50.68	8.41	40.90
T_{10} - HW on 25 and 45 DAS	47.86	9.58	42.57	40.17	13.74	46.09	51.21	12.08	36.71	52.15	8.30	39.55
T_{11} - Weed free check	43.53	15.83	40.65	36.96	21.74	41.30	45.98	13.31	40.71	41.81	15.69	42.50
T_{12} - Unweeded control	45.53	13.38	41.09	43.03	13.30	43.67	45.69	10.05	44.26	44.86	10.81	44.33

Data not statistically analysed

WATER HARVESTING THROUGH FARM POND AND SUPPLEMENTAL DRIP IRRIGATION FOR RAINFED COTTON

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Abstract

Field experiments were conducted at Cotton Research Station, Veppanthattai during 2014-2015 and 2015 – 2016 to carry out the studies on tillage with crop residues and supplemental irrigation through drip system on rainfed Bt cotton. The experiments were laid out in a strip plot design with three replication. The main plot treatments were conventional tillage, minimum tillage without crop residue, minimum tillage with crop residue @ 2.5 t ha^{-1} and minimum tillage with crop residue @ 5 t ha^{-1} .Conventional tillage comprised of one disc ploughing, four cultivator ploughing and two harrowing (0.3-0.4 m deep). Minimum tillage included only one ploughing (cultivator) and two harrowing (0.1-0.12 m deep). The sub plot comprised of irrigation treatments viz., supplemental drip irrigation through drip system one, two and three times and without irrigation (control), during the cropping period. Under paired row planting system (120 + 30 x 60 cm spacing), one 12 mm lateral was laid out between two rows of Bt cotton. The supplemental irrigations were given to the crops at the time of moisture stress period determined based on the visual symptoms of wilting. Recommended dose of fertilizers viz.,120:60:60 kg NPK ha^{-1} was applied to Bt cotton. The results revealed that minimum tillage (BBF) with crop residue application @ 5 t ha^{-1} (M_4) recorded higher No. of sympodial branches $plant^{-1}$, No. of bolls $plant^{-1}$ and seed cotton yield .

Key words : Cotton, supplemental drip irrigation, yield , minimum tillage

Introduction

In India, out of 142 million hectare of arable lands, 60 per cent (85.2 million ha) is rainfed area (Wani *et al*., 2012). The cultivable area in Tamil Nadu is around 7 million hectares and 55 per cent of which is under rainfed condition (TNTDPC, 2011). Under changing climate due to heavy precipitation the runoff is expected to be more and hence effective harvesting of this runoff water is the need of the hour, which can be efficiently utilized for irrigating the crops at critical stages. Though there are experiments with surface and sprinkler irrigations using farm pond harvested water, no such work has been done to use this harvested water for supplemental irrigation through drip irrigation systems for better utilization scarce rainwater for crop production under rainfed conditions.

Conservation tillage is often associated with a varying degree of tillage reduction, which may leave some crop residues on the soil surface. Three key principles have been identified such as minimal soil disturbance, permanent residue cover and planned crop rotations, which are considered essential to its success (Hobbs *et al*., 2008). Conservation tillage also reduces soil disturbance, maintain soil organic matter and enhance the soil quality (Zentner *et al*., 2004). Supplemental irrigation is a key strategy to unlock rainfed yield potentials. The objective of supplemental irrigation is not to provide stress-free condition through the crop growth for maximum yields, but to provide just enough water to tide over moisture scarcity at critical growth stages to produce optimal yields per unit of water (Sharma and Smakhtin, 2004). Drip irrigation has been practiced for its effectiveness in reducing soil surface evaporation, increasing the crop yield and water use efficiency. There is paucity of information on conservation tillage, crop residues along with supplemental drip irrigation on different crops in rainfed situation. Hence, an attempt has been made to study the impact of tillage, crop residue and supplemental irrigation through drip system on Bt cotton.

Materials and methods

Field experiments were conducted during kharif season of 2014 and 2015 at central block of Cotton Research Station, Veppantattai. The soil of the experimental farm was clay loam in texture with low in nitrogen , medium in phosphorus and potassium . The experiments were laid out in strip plot design with three replications. The main plot treatments were conventional tillage, minimum tillage without crop residue, minimum tillage with crop residue @ 2.5 t ha^{-1} and minimum tillage with crop residue @ 5 t ha^{-1}. The conventional tillage comprised of one disc ploughing, four cultivator ploughing and two harrowing (0.3-0.4 m deep). The field operations for minimum tillage comprised one ploughing (cultivator) and two harrowing (0.1- 0.12 m deep). Broad bed furrows were formed (BBF) in each plot. The BBF had 8.0 m long, 120 cm wide and 30 cm furrow and 15 cm height. The size of each plot was 48 square meter.

A buffer zone of 2.0 m spacing was provided between plots.

Irrigation water was pumped out using 5.0 HP oil engine from the farm pond and conveyed to the main line of 63 mm OD (outer diameter), PVC (Poly vinyl chloride) pipes after filtering through filter. From the main, sub mains of 40 mm OD PVC pipes were drawn. From the sub main, laterals of 12 mm linear low density polyethylene (LLDPE)

pipes were installed at an interval of 1.20 m. Each lateral was provided with individual tap control for imposing respective irrigation schedules. Along the laterals, inline drippers with a discharge capacity of 4 lph were spaced at 0.6 m. Single lateral was used for a paired row. Sub mains and laterals were closed at the end with end cap. After installation, trial run was conducted to assess mean dripper discharge and uniformity co-efficient. Under paired row planting system (120 + 30 x 60 cm spacing) one 12 mm lateral was laid out between two rows of Bt cotton. The supplemental irrigations were given to the cotton at the time of moisture stress period determined based on the visual symptoms of wilting. Recommended dose of fertilizers viz.,120:60:60 kg NPK ha^{-1} were applied to Bt cotton.

Results and discussion

Effect of treatment on yield attributes and yield of Bt cotton

The effect of tillage practice with crop residue and supplemental irrigation practices had significant influence on yield attributes like No. of sympodial branches $plant^{-1}$, No. of bolls $plant^{1}$ and seed cotton yield of Bt cotton during the both the years (Table 1). Minimum tillage (BBF) with crop residue application @ 5 t ha^{-1} (M_4) recorded higher yield attributes like, No. of sympodial branches $plant^{-1}$, No. of bolls $plant^{-1}$ and seed cotton yield. Bauer *et al*. (2005) reported that conservation tillage produced higher seed cotton yield as compared to conventional tillage.

Supplemental irrigation through drip system had significant influence on yield attributes like No. of sympodial branches $plant^{-1}$, No. of bolls $plant^{-1}$, boll weight and seed cotton yield of Bt cotton during both the years. Among the sub plots supplemental irrigation through drip system three times recorded higher No. of sympodial branches $plant^{-1}$, No. of bolls $plant^{-1}$ boll weight and seed cotton yield of Bt cotton during 2013 and 2014. Patil *et al*. (2004) reported that 10 per cent increased seed cotton yield (1329 kg ha^{-1}) under drip irrigation over surface irrigation (1210 kg ha^{-1}) at Dharward. Sampathkumar *et al*. (2006) found that higher seed cotton yield from drip irrigation where the yields obtained were 38 per cent higher than the furrow irrigation. Two supplemental irrigations from farm pond to cotton + blackgram recorded higher cotton equivalent yield of 0.940 t ha^{-1} compared to without supplemental irrigation (0.78 t ha^{-1}) at Kovilpatti, Tamil Nadu (AICRPDA, 2003). Xing *et al*. (2011) reported that using supplemental irrigation at jointing stage kernel weight per ear is more than 1.82 g mm^{-1} at filling stage. So, supplemental irrigation in critical period of maize growth is an effective way to water saving and increasing yield in semi arid region.

Effect of treatment on yield attributes, yield and economics of Bt cotton

The effect of tillage practice with crop residue and supplemental irrigation practices had significant influence (Table 2). Minimum tillage (BBF) with crop residue application @ 5 t ha^{-1} (M_4) registered higher gross income (Rs. 1,05,600 and 1,08,400 /ha), net return (Rs. 74,900 and 77,700 /ha) and cost benefit ratio (3.4 and 3.6) (Table 4) during the year 2012 and 2013 respectively. This was followed by minimum tillage (BBF) with crop residue application @ 2.5 t ha^{-1} (M_3) and it was comparable to the conventional tillage method of treatment (M_1). Similarly in the sub plot treatment supplemental drip irrigation given three times (S_3) during the crop growing period registered higher gross income (Rs. 1,07,200 and 1,12,000 /ha), net return (Rs. 78,000 and 82,200 /ha) and cost benefit ratio (3.6 and 3.8) during the year 2012 and 2013 respectively. This was followed by supplemental drip irrigation given two times (S_2) during the crop growing period.

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Table 1. Effect of different conservation, crop residue application and supplemental irrigation on yield attributes and yield of cotton.

Treatments	No. of sympodial branches/plant		No. of Bolls/plant		Boll weight (g)		Seed Cotton Yield (kg/ha)	
	2012	2013	2012	2013	2012	2013	2012	2013
M_1	18.1	18.3	24.6	25.3	5.4	5.5	2180	2200
M_2	18.5	18.6	25.3	26.3	5.5	5.7	2325	2360
M_3	19.0	18.9	28.6	29.2	5.6	5.8	2436	2480
M_4	19.5	19.6	29.5	30.4	5.6	5.8	2640	2710
S.Ed	0.4	0.3	0.4	0.5	0.04	0.05	34	38
CD (P=0.05)	0.9	0.7	0.8	1.0	NS	NS	73	82
S_1	19.5	19.8	26.5	27.2	5.3	5.4	2280	2310
S_2	20.1	20.3	28.0	29.1	5.5	5.6	2470	2560
S_3	21.0	21.6	29.3	30.2	5.7	5.7	2680	2800
S_4	18.1	18.4	24.0	26.2	5.0	5.0	2164	2180
S.Ed	0.7	0.8	0.7	0.8	0.06	0.07	27	30
CD (P=0.05)	1.5	1.7	1.6	1.7	NS	NS	59	65
M at S S.Ed.	0.4	0.7	0.8	0.9	0.03	1.04	126	130
CD (P=0.05)	NS	NS	NS	NS	NS	NS	NS	NS
S at M S.Ed.	0.6	0.8	0.6	0.7	0.05	0.06	138	145
CD (P=0.05)	NS	NS	NS	NS	NS	NS	NS	NS

Table 2. Effect of tillage and weed management practices on economics of cotton

Treatments	Gross Return (Rs/ha)		Net return (Rs/ha)		B.C ratio	
	2012	2013	2012	2013	2012	2013
M_1	87,200	88,000	58,364	59,164	3.0	3.1
M_2	93,000	94,400	63,163	64,563	3.1	3.2
M_3	97,440	99,200	67,180	68,940	3.2	3.3
M_4	1,05,600	1,08,400	74,900	77,700	3.4	3.5
S_1	91,200	92,400	63,020	64,220	3.2	3.3
S_2	98,800	1,02,400	70,100	73,700	3.4	3.6
S_3	1,07,200	1,12,000	78,000	82,800	3.6	3.8
S_4	86,560	87,200	58,610	59,250	3.1	3.1

BIOMASS POTENTIAL AND QUALITY OF PERSIAN CLOVER (T*RIFOLIUM RESUPINATUM* L.) AS AFFECTED BY ORGANIC & CHEMICAL FERTILIZERS

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Abstract

An experimental was conducted with factors included nitrogen fertilizer with three levels (20, 40 and 60 kg ha $^{-1}$ as urea), phosphorus with three levels (30, 60 and 90 as diammonium phosphate) and vermicompost with two levels (0 (control) and 5 tonnes ha -1).The data related to green fodder yield revels that 40 kg N ha -1 and 60 kg P 2 O 5 ha -1 along with 5 tonnesvermicompost ha -1 showed highest green fodder yield in order of 345.63 & 346.21 during respective years of experimentation with CP (21.12%); DMD (66.38%) and CF (24.17%). The result displayed that inorganic fertilizer along with vermicompost offers better growth medium for higher green fodder production.

Key words: Persian clover, biomass, fertilizers, vermicompost, quality

Introduction

Persian clover (Trifolium resupinatumL.) is native forage (Fabaceae) of Turkey, Iraq, Afghanistan and Iran (Taylor, 1985). It is an annual, prostrate or semi-erect branched legume, up to 40-100 cm high. It forms dense swards and has a rosette growth habit under grazing. Clover plants require large amounts of mineral nutrients such as N, P and K for their growth and development (Fraser*et al*., 2009). Nutrient availability is one the most important factors during plant development. The use of biological fertilizers is a critical component to crop production in sustainable farming systems. Sustainable agriculture, especially organic agriculture, is a low input system that implies the efficient use of biological resources. In such a system, fertilizing with organic fertilizers such as vermicompost is considered as major contribution to sustainable crop production (Alam, 2007). However, there are scientific evidences supporting the idea that the application rate of chemical fertilizers could be reduced (to achieve optimum yield levels) if they were applied along with organic fertilizers. Vermicompost is a nutritive 'organic fertilizer' rich in NPK (nitrogen 2-3%, phosphorus 1.55-2.25% and potassium 1.85-2.25%), micronutrients, beneficial soil microbes like 'nitrogen-fixing bacteria' and 'mycorrhizal fungi' and are scientifically proving as 'miracle growth promoters & protectors (Sinha,, *et al*. 2009).

Materials and Methods

The experiment was conducted at the Researach farm- Agronomy ,FoAWaduraof Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, India during, 2017-18 and 2018-19. The area is designated as temperate zone. The experimental design was split plot design with three replications. Experimental factors included nitrogen fertilizer with three levels (20, 40 and 60 kg ha^{-1} as urea), phosphorus with three levels (30, 60 and 90 as diammonium phosphate) and vermicompost with two levels (0 (control) and 5 tonnes ha^{-1}).Persian clover being multicut annul is capable of producing high quantities of herbage the first cut of the crop was carried out on 5th May and 26th April during both the respective years, and after each cut crop was supplied with light irrigation for quick regeneration. Parameters such as plant height, number of branches per plant & green fodder yield were calculated.

Results and Discussion

Nitrogen application showed a significant variation in plant height of Persian clover during both years of experimentation. A perusal of data indicated that the plant height significantly increased with nitrogen application at 40 kg ha^{-1}, however, beyond this dose i.e. 60 kg ha^{-1} no significant increase in the plant height over 40 kg ha^{-1} was noticed (Table 1). The data also revealed that increasing the rates of phosphorus from 30 to 90 kg ha^{-1} enhanced the plant height. Significantly highest plant height (100.62 cm in 2013-14 and100.89 cm in 2014-15) was recorded with 90kg P_2O_5 ha^{-1} however, beyond 60 kg P_2O_5 ha^{-1} there was no significant effect in plant height.Highest plant height was found in plots supplies with 5 t vermicompost ha^{-1}as 100.56 and 103.05 during both years respectively. The data also revealed that there was increase in number of lateral braches, however statistically the performance of treatments in terms of number of lateral branches was non-significant during both the years (Table 1). The data related to green fodder yield revels that 40 kg N ha^{-1} and 60 kg P_2O_5 ha^{-1} along with 5 tonnesvermicompost ha^{-1}

showed highest green fodder yield in order of 345.63 & 346.21 during respective years of experimentation. Some quality parameters such as: crude protein (CP); dry matter digestible (DMD) and crude fiber (CF) were also assessed during the study. V_5 (5 tones vermicompost ha^{-1} with reduced application of chemical fertilizers N_{40}& P_{60}) treatment as compared to V_0 (no application of vermicompost with highest chemical fertilizers) significantly increased quality parameters such as: CP (21.12%); DMD (66.38%) and CF (24.17%). The result displayed that inorganic fertilizer along with vermicompost offers better growth medium for higher green fodder production

Conclusion

It can be considered from the results in terms of clover that fertilizers alone were not effective for higher biomass yield. It appears that application of vermicompost had positive effect on vegetative growth and green fodder yield. Regarding the combined application of nitrogen phosphorus and vermicompost, it can be concluded that vermicompost (5 ton ha^{-1}) + nitrogen (40kg ha^{-1}) + phosphorus(60kg ha^{-1}) is more favorable for vegetative growth, green fodder yield and quality parameters such as; crude protein (CP); dry matter digestible (DMD) and crude fiber (CF) in Persian clover. The result displayed that inorganic fertilizer along with vermicompost offers better growth medium for higher green fodder production.

T**able1. Efeect of Plant height and number oflateral branches for green foffer yield**

Treatments	**Plant height (cm)**		**Number of lateral (branches/plant)**		**Green fodder yield (quintals/ha)**	
	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15
N_{20}	91.47	92.13	3.56	3.39	302.01	306.33
N_{40}	100.45	101.09	3.57	3.62	315.21	318.52
N_{60}	100.78	102.13	3.60	3.85	320.43	322.19
SE(m) ±	0.84	0.85	0.16	0.17	1.84	1.86
CD (5%)	2.46	2.74	NS	NS	5.40	5.53
P_{30}	94.33	94.45	3.58	3.68	308.06	311.14
P_{60}	97.75	98.41	3.72	3.88	314.14	319.61
P_{90}	100.62	100.89	3.43	3.92	315.18	323.16
SE(m) ±	0.84	0.85	0.16	0.16	1.84	1.86
CD (5%)	2.46	2.48	NS	NS	5.40	5.54
V_0	94.58	95.91	3.48	3.13	297.04	298.10
V_5	100.56	103.05	3.67	3.91	328.05	333.26
SE(m) ±	0.68	0.69	0.13	0.15	1.50	1.56
CD (5%)	2.01	2.22	NS	NS	4.38	4.67

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GENOTYPIC ANALYSIS OF MOISTURE DEFICIT EFFECTS ON BIOCHEMICAL ALTERATIONS IN *PASPALUM SCORBICULATUM L.*"

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Abstract

Drought is the most important abiotic factor considered as one of the crop performances limiting factors and a threat for successful crop production. Drought is also commonly expressed as a shortage or absence of rainfall causing a loss in rain-fed agriculture. Millets might provide alternative climate-smart crops, as their adaptations to challenging environment are better than the current major crops of the world.The present investigation was conducted in the Centre of Excellence in millets, Athiyandal, Tiruvannamalai district. It was done in Summer, 2018 and 2019.Randomized block design was used to conduct this experiment. It has four replications. A study was conducted to determine the biochemical and yield responses to water stress in varagu. Terminal stress was imposed at Flower initiation periodof crop growth. The plants submitted to stress suffered a decrease in the amount of Chlorophyll and yield content. To evaluate and compare water stress effects on Chlorophyll content, Chlorophyll Stability Index and Relative Water Content of varagu. The aim of this study was to evaluate and compare water stress effects on chlorophyll content, relative water content, chlorophyll stability index and yield of varagu genotypes, as well as reveal which genotypes better adopts to water stress conditions using these parameters. From this assessment the most reliable parameter for drought tolerant, it is evident that the Chlorophyll stability index (90 %) and Relative water content (88 %) is high in TNPsc 176 varagu genotype than the other genotypes.

Key words: Chlorophyll content, Relative Water Content, Chlorophyll Stability Index, yield and Water stress.

Introduction

Millets are resilient to the extreme climatic and soil conditions. The similarities of millets are that they are all grown under extreme environmental conditions, especially those of inadequate moisture and poor soil fertility which are poorly suited to the major crops of the world. The ability for water or plant water use efficiency is greater, will be more resistant to drought and adaptation of some plants, such as plants CAM and C4 is that their metabolic pathway, allow them to exploitation of the dry environments, as well as the mechanisms of adaptation of plants that are activated in response to water stress (Kafi and Mahdavi Damghani, 1999). Millets possess a C4 photosynthesis system; hence, they prevent photorespiration and, as a consequence, efficiently utilize the scarce moisture present in the semi-arid regions. Since C4 plants are able to close their stomata for long periods, they can significantly reduce moisture loss through the leaves.

Chlorophyll is one of the major chloroplast components for photosynthesis (Rahdari *et al*, 2012). The decrease in chlorophyll content under drought stress has been considered a typical symptom of pigment photo oxidation and chlorophyll degradation (Anjum *et al*, 2011). Decreased of chlorophyll content during drought stress depending on the duration and severity of drought level (Zhang and Kirkham, 1996). A decrease of total chlorophyll content with drought stress implies a lowered capacity for light harvesting. Since the production of reactive oxygen species is mainly driven by excess energy absorption in the photosynthetic apparatus, this might be avoided by degrading the absorbing pigments (Mafakheri *et al*, 2010). In relation to drought effect on chlorophyll a and b in leaf, we can express that drought is due to chloroplastic proteins hydrolysis, decreasing of leaf pigments and chlorophyll destruction as a primary stagein degradation of proteins (Synerri *et al*., 1993)

At the onset of water stress, inhibition of cell growth, leading to a reduction in leaf development. Lower leaf surface causes less water uptake from the soil and transpiration is reduced. Plenty of water to form effective for a longer period, the soil is kept. Restrictions on the leaf surface could be the first line of defense against water (Kafi and Mahdavi Damghani, 1999). Drought avoidance is the ability of plants to maintain relatively high tissue water potential despite a shortage of soil moisture, whereas drought tolerance is the ability to withstand water deficit with low tissue water potential.

A study by Winkel *et al*. in Niger where the annual rainfall is around 200 mm investigated the impact of water deficit at three stages of pearl millet development. The three stages were prior to flowering, at flowering and at the end of flowering. According to the findings of the work, the grain yield of pearl millet was severely reduced when moisture was limited, Abiotic and Biotic Stress in Plants - Recent Advances and Future Perspectivesand at the

flowering stage but not at the end of flowering. On the other hand, in pearl millet, terminal drought in which irrigation was terminated from the flowering until crop maturity, was severe, as it resulted in 60% yield loss(Bidinger and Mahalakshmi,1987). The mid-season stress, which occurred from one month before flower initiation to full flowering, resulted in only 7% yield loss. An artificially created water stress environment will be used to provide the opportunity in selecting superior genotype out of a large population.

Tolerance to abiotic stresses is very complex, due to intricate of interaction between stress factors and various molecular, biochemical and physiological phenomenon affecting plant growth and development (Razmjoo *et al*., 2008). High yield potential under drought stress is the target of crop breeding. In many cases, high yield potential can contribute to yield in moderate stress environment (Blum, 1996). In plants, a better understanding of the morpho-anatomical and physiological basis of changes in water stress resistance could be used to select or create new varieties of crops to obtain a better productivity under water stress conditions (Martinez *et al*., 2007).

Millets play a significant role in the livelihood of the population of developing world especially due to their enormous contribution to the food security of all countries. However, these crops have not been sufficiently studied. Breeding for drought tolerance is the major objective of many crop-breeding programmes due to the widespread prevalence of the moisture-deficit problem in global agriculture. A number of crops with drought tolerance have been developed. There are two options for the managment of crops in water-limiting environments: the genetic and agronomic (Saxena and John, 2002). The genetic approach requires robust and reproducible screening methods for the identification of traits of drought tolerance in germplasm and breeding materials, and incorporation of the same into high-yielding varieties using conventional and biotechnological tools.

Materials and Methods

A field experiment was conducted at Centre of Excellence in millets, Athiyandal, Tiruvannamalai district. It was done in Summer, 2018 and 2019.Randomized block design was used to conduct this experiment. It has four replications. Five varieties of Paspalum scorbiculatum L., CO 3, TNAU 86, TNPsc 176, TNPsc 301 and TNPsc 313 were used in this study. Terminal stress was imposed at Flower initiation period of crop growth. Duration of the crop was 120 days. Varagusown with a spacing of 45 cm x 10 cm and raised with recommended package of practices.

Chlorophyll content

Contents of fractions of 'a', 'b' and total chlorophyll were estimated in a fully expanded young leaf at the specified time intervals and expressed in mg g^{-1} fresh weight (Yoshida *et al*., 1971).

Relative Water Content (RWC)

Leaf samples were taken from the youngest fully expanded leaves for recording relative water content. Relative water content was estimated by Weatherly (1962) method and expressed in percentage.

Chlorophyll stability index (CSI)

Chlorophyll stability index was calculated by the method described by Murthy and Majumdhar (1962).

Results and Discussion

Maintenance of adequate soil moisture is essential for successful crop production. Drought endurance recovery needs the special characters to minimize yield loss and reduced water has for irrigated crops. The aim of small millet breeding programme is to produce cultivars suitable for dry land production system that have high yield potential and enhance water use efficiency. Varagu is relatively drought-tolerant, but severe water stress at flower initiation period can slow plant development, cause reduced leaf area, leaf size, chlorophyll content, and thus reduce the varagu yield. This is of agricultural importance since the incidence to stress is unpredictable and plants may be exposed to drought stress at any time during their life cycle under field conditions.

In the present study, there was significant reduction in growth expressed in plant height irrespective of genotypes, treatments and their interaction. Drought stress imposed at pre flowering stage as more pronounced effect on plant height than at any other stages. The plant height recorded at pre flowering stage was reduced in the moisture stress-imposed treatment from 96 to 85 cm the reduced plant stature under moisture deficit stress are similar to that reported by Gerik*et al*. (1996) and Ball *et al*. (1994). The genotype TNPsc 176 has the highest value (85.7 cm) than other genotypes at grain filling stage. Root elongation during drought may help plants get deeper water, thus avoiding water deficits near the soil surface. Elongation also could reduce the water lost by drainage when precipitation allows recovery after the drought (Ludlow and Muchow, 1990). If, however, water is unavailable deeper in the soil profile, longer roots may reduce shoot dry weight and harvest index by allowing the preferential partitioning of photosynthate to roots at the expense of shoots. The genotype TNPsc 176 has increased root length (22.3) significantly at grain filling stage than other genotypes. Data on leaf area revealed significant differences between the genotypes. The genotype TNPsc 176 maintains a higher mean leaf area during grain filling stage than others even under the stress condition (453.8). The genotype CO 3 has the lowest value 397.3 than other genotypes at all stages irrespective of the treatment effects.

Hussain *et al*. (2009) reported decline in LAI of sunflower exposed to drought at budding and flowering stages.

Drought also suppresses leaf expansion and tillering (Kramer and Boyer 1995), and reduces leaf area due to early senescence (Nooden 1988). All these factors contribute to reduced dry matter accumulation and grain yield under drought. In pearl millet (Pennisetum glaucum L. Leeke), drought at flowering increased the rate of ear abortion due to a decline in assimilate supply to developing ears (Yadav *et al*. 2004). In drought-stressed maize, kernel set was lost leading to low grain yield (Schussler and Westgate 1995). Likewise, water deficit at anthesis increased pod abortion which reduced yield in soybean (Liu *et al*. 2003). The role of photosynthetic pigments such as chlorophyll (chl) contents, carotenoids, and xanthophylls are also vital in carbon fixation, as they are involved in capturing solar radiation to drive the photosynthetic mechanism. Drought stress severely decreased chl a and chl b contents in marigold (Asrar and Elhindi 2011).

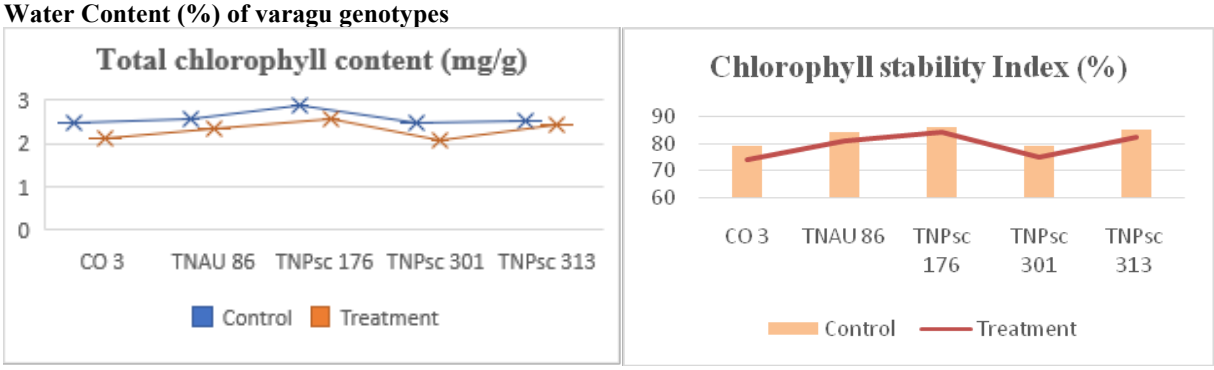
Drought lowered RWC in tomato and caper bush (Capparis spinosa L.) (Ozkur *et al*. 2009). In sunflower, RWC, leaf water potential and osmotic potential were affected by drought (Tezara *et al*. 2002). However, different genotypes behaved differently; drought-tolerant genotypes maintained higher leaf water potential for longer and wilted later than sensitive genotypes upon exposure to drought (Ouvrard *et al*. 1996). Breeding approach is often used to explore genetic variability for drought tolerance among crop genotypes for desired agronomic traits to then breed genotypes better able to perform in drought-prone areas (Ashraf 2010).

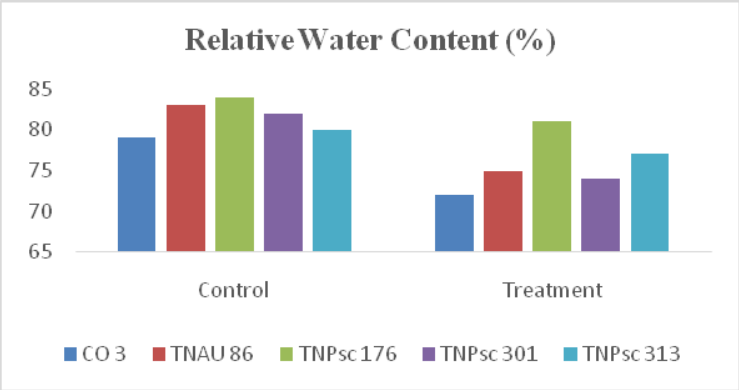
Water stress inhibits cell enlargement and reduce plant growth by affecting various physiological and biochemical processes, such as leaf area, chlorophyll content and photosynthesis.The genotype TNPsc176 maintained a higher total chlorophyll content in all stages than others even under the stress conditions (2.572). The total chlorophyll values were significantly lower in the case of TNPsc 301(2.074). The chlorophyll stability index is an indicative of the maintenance of photosynthetic pigments under drought situation. Among the genotypes the TNPsc 176 and TNPsc 313 maintained a good mean values (84% and 82%).

Table. 1. Effect of drought on plant height (cm) , root length (cm), leaf area(cm^2 $plant^{-1}$) and Total Chlorophyll content (mg/g) of varagu genotypes

Genotypes	**Plant height (cm)**		Root Length (cm)		Leaf Area (cm^2 $plant^{-1}$)		Crop Growth Rate (g m^{-2} day^{-1})		Total chlorophyll content (mg/g)	
	C	T	C	T	C	T	C	T	C	T
CO 3	88.5	81.3	15.3	16.7	419.1	397.3	0.512	0.452	2.489	2.127
TNAU 86	95.0	74.3	14.7	17.3	435.0	412.4	0.608	0.538	2.587	2.329
TNPsc 176	96.8	85.7	15.0	22.3	481.1	453.8	0.676	0.564	2.867	2.572
TNPsc 301	87.3	76.0	16.7	17.6	450.3	421.3	0.527	0.433	2.467	2.094
TNPsc 313	88.5	79.3	16.9	20.1	478.3	424.5	0.592	0.496	2.537	2.457
SEd	1.09	1.46	0.22	0.23	2.06	2.15	0.05	0.04	0.02	0.03
CD (P=0.05)	3.35	4.52	0.69	0.72	6.35	6.63	0.10	0.08	0.07	0.09

Fig. 1 Effect of drought on Total Chlorophyll content (mg/g), Chlorophyll Stability Index (%) and Relative Water Content (%) of varagu genotypes





Using plant breeding is a good approach for improving drought tolerance, also, produce appropriate droughttolerant genotypes could be another technique to manage drought stress and improve plant response to stresses.Drought stress is one of the most serious threats to world food security. There are various negative effects on plant growth and total yield occurs under drought conditions, therefore, plants have different responses for adapted and survive with drought conditions such as morphological, biochemical, physiological responses, and a molecular mechanism. Plants acclimatize with drought stress through use various strategies which include drought escape, drought avoidance and drought tolerance. Plant breeders using Plant Physiology and classical breeding techniques for improving plant drought tolerance, could improve plant tolerant for drought stress. Millets might provide alternative climate-smart crops, as their adaptations to challenging environment are better than the current major crops of the world.

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AUGMENTATION OF GREEN GRAM PRODUCTIVITY THROUGH MULTI NUTRIENT FOLIAR FERTILIZATION

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Greengram (Vignaradiata L.) is one of the important pulse crops in India. It is a protein rich staple food which contains about 25 per cent protein, almost three times that of cereals. Soil application of nutrients is often not enough to meet the growing crop demand particularly in short duration crop like greengram. Therefore, it is hypothesized that foliar nutrition in addition to soil application in commensuration with prevailing weather particularly rainfall will go a long way in meeting crop nutrient need and thereby may help in enhancing productivity. Foliar application of nutrients using water soluble fertilizer is on the possible ways to enhance the productivity of pulses like greengram. Foliar application is credited with the advantage of quick and efficient utilization of nutrients, elimination of losses through leaching and fixation and regulating the uptake of nutrient by plants (Manonmani and Srimathi, 2009). Foliar application of major plant nutrients like nitrogen and potassium was found to be as good as soil application. Deshmukh *et al*., (2013) also reported the 40 per cent higher yield of greengram with foliar spray of 1 per cent urea and 1 per cent DAP before flowering as compared to without foliar spray. Foliar application of nutrients is one of the possible ways to avoid such loss of fertilizer. In addition, several investigators showed the positive response of mungbean yield and chemical composition to micronutrients, i.e., Zn and Fe, and Cu. The recent study conducted to evolve foliar multi nutrition schedule for improving the productivity of green gram.

The two years (2019-2021) field experiments were conducted at Regional Research Station, Paiyur of Krishnagiridistrict as randomized block design replicated thrice to evolve foliar multi nutrition schedule for improving the productivity of green gram under irrigated condition. The soils of RRS farm were Vannapatti soil series of non-calcareous sandy loam Lithic Haplustept. The initial soils were normal in soil pH, non-saline, low in SOC and available N while medium in available P and high in K status. The DTPA extractable Fe, Mn and Cu were sufficient while Zn was deficient. In this site, green gram (var. VBN 4) was grown with following treatments viz.,T_1 –N: P: K (19:19:19) (TNAU-WSF) @ 2%, T_2 –N: P: K (19:19:19) (TNAU-WSF) @ 2% + HA @ 1%, T_3 – Liquid Multi-micronutrient * @ 1%, T_4 – Liquid Multi-micronutrient @ 1% + HA @ 1%, T_5 -T_1+T_3, T_6- T_1+T_4, T_7 - DAP @ 2% - Checkand T_8 - Absolute control. The soil parameters and yield were recorded and subjected to ANOVA for statistical significance.

Among the foliar nutrients, application of TNAU water soluble fertilizer (WSF) @ 2%+ Liquid multi micro nutrient @ 1%+ Humic acid (HA)@ 1% produced higher grain yield of 1102 kg ha^{-1}, which was statistically on par with TNAU WSF 2%+Liquid multi micro nutrient 1%, applied treatment with 44 per cent yield increase over with control. Absolute control plot recorded the lowest yield of 764 kg ha^{-1}. In the case of dry matter production, similar trend was observed as that of seed yield. The significant improvement in yield with these treatments were mainly attributed to significantly higher number of pods per plant and 1000 grain weight over other treatments. These findings are related with those obtained in greengram (Kulkarni*et al*. 2016).

A close observation of crop growth rate revealed that, foliar application of TNAU WSF 2%+Liquid multi micro nutrient 1%+HA1% recorded more crop growth rate (22 g/m^2/day) at 30-60 DAS. The higher SPAD value 54.8 recorded under TNAU WSF 2%+ Liquid multi micro nutrient 1%+HA1% which was comparable with TNAU WSF 2%+LMMN 1% and LMMN 1%+ HA1% applied treatment.Micronutrients such as zinc are essential for enzymes that are involved in many metabolic reactions, necessary for chlorophyll production and for starch formation aids in seed formation. Iron is essential for maintenance of chlorophyll and also an essential component of hemoglobin molecule. Manganese has a role of formation of plants naturally occurring antifungal compounds helping to fight disease infection. It is involved in the enzyme the growth may have biological reactions in plant tissue (Kavya *et al*. 2021).

Table 1. Effect of multi nutrient foliar application on biometric characters, yield attributes and nutrients uptake of green gram

Treatments	Plant height (cm)	CGR ($g/m^2/day$) (30-60 DAS)	SPAD Value	DMP (kg/ha)	Seed yield ($kg\ ha^{-1}$)	N uptake (kg/ha)	P uptake (kg/ha)	K uptake (kg/ha)
T_1-TNAU WSF @ 2%	56.7	16.5	47.5	1620	856	43.9	4.66	36.9
T_2-TNAU WSF @ 2%+ HA @ 1%	56.8	17.3	48.6	1740	864	65.8	5.40	51.3
T_3-LMMN @ 1%	57.3	19.2	49.7	1847	877	67.4	5.89	61.0
T_4- LMMN @1%+HA@1%	59.3	20.1	51.4	1976	939	82.0	6.78	68.1
T_5 TNAU WSF@ 2% + LMMN @1%	61.1	19.2	53.1	2126	1040	91.8	8.00	80.5
T_6-TNAU WSF @ 2%+ LMMN @1%+HA@1%	61.5	22.0	54.8	2323	1102	96.9	9.24	95.0
T_7-DAP @ 2%	56.4	17.5	49.8	1774	837	63.2	5.30	51.7
T_8- Absolute control	54.8	16.3	43.5	1450	764	34.7	3.89	30.0
SEm±	0.91	0.59	1.14	51.3	24.7	2.34	0.14	1.91
CD ($p<0.05$)	2.77	1.79	3.45	156	75.0	7.10	0.43	5.80

The effect of multi nutrient foliar fertilization of green gram on total N, P & K uptake of 96.9, 9.24 and 95.0 kg/ha respectively recorded maximum in the treatment of TNAU water soluble fertilizer (WSF) @ 2%+ Liquid multi micro nutrient @ 1%+ Humic acid (HA)@ 1% which was comparable with TNAU WSF 2%+Liquid multi micro nutrient 1% (table 1).The N,P and K concentrations were higher in grain. This trend may be due to high mobility of the nitrogen from vegetative tissues to reproductive organs after flowering stage. It was evident from the result that the uptakes of N, P and K by grain were significantly influenced due to different treatments. The P uptake by green gram crop was increased of crop and it was significantly influenced by different macronutrients (NPK) applications. The similar results revealed by Mudalagiriyappa *et al*., (2016) that total phosphorus uptake in cowpea increased significantly with 2 per cent DAP spray as compared to water sprayed control and 2 per cent KCl. An uptake of K in green gram crop might be due to foliar application of K resulted into greater availability of K through leaves. Similar findings were recorded by Shashikumar *et al*., (2013).

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YIELD MAXIMIZATION OF RICE THROUGH PLANT GROWTH REGULATORS

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Abstract

Field investigations were carried out at the Experimental Farm, Department of Agronomy, Faculty of Agriculture, Annamalai University to study the effect of Gibberellic acid (GA_3)on growth and yield of rice. The experiments comprised of seven treatments viz., T_1 - Gibberellic acid @ 5 g ha^{-1}, T_2 - Gibberellic acid @ 10 g ha^{-1},T_3 - Gibberellic acid @ 15 g ha^{-1}, T_4 -Gibberellic acid @ 20 g ha^{-1}, T_5 - Gibberellic acid @ 25 g ha^{-1}, T_6 - Triacontanol 0.05% EC @ 250 ml ha^{-1} and T_7 - control. The treatments were conducted in randomized block design (RBD) and replicated thrice, in both the seasons of study. Rice.var BPT 5204 was used as test variety.The effect of Gibberellic acid on growth attributes, yield attributes and yield of crop was critically studied under rice. The growth and yield parameters of rice viz., plant height, number of tillers hil^{-1}, leaf area index, dry matter production, number of panicles m^{-2} and number of filled grains $panicle^{-1}$ were strikingly impressive by Gibberellic acid @ 25 g ha^{-1} in both seasons. In respect of grain and straw yields were also significantly higher in crop raised with application of Gibberellic acid over control. Among the different treatments, Gibberellic acid @ 25 g ha^{-1} recorded the highest grain and straw yields. This was followed by Gibberellic acid @ 20 g ha^{-1}.Triacontanol 0.05% EC @ 250 ml ha^{-1} and Gibberellic acid @ 10 g ha^{-1} were next in order and were on par in their values. The lowest grain and straw yields were recorded in control (no foliar spray).The harvest index was also higher with Gibberellic acid @ 25 g ha^{-1}.The lower harvest index was recorded under control.Based on the above experimental results, it could be concluded that cultivation of rice with application of Gibberellic acid @ 25 g ha^{-1} was found to be an agronomically sound and ecologically safe practice for augmenting higher productivity. Hence this can be recommended to the rice growing farmers of Tamil Nadu.

Key words: Rice, Foliar application,Gibberellic acid, triacontanol, Grain and Straw yield and Harvest index

Introduction

Rice (Oryza sativa L.) is one of the most important cereal crop of the world, grown in wide range of climatic zones more than 90 per cent of the World's rice is grown and consumed in Asia. Rice is grown in 114 countries across the world with an area of 164 million hectares and production of 741.4 million tonnes with the productivity of 4.4 t ha^{-1} (FAO, 2013). In India, rice is grown in an area of 44.10 million hectares with the production of 107 million tonnesand the productivity is 3.58 t ha^{-1}. In Tamil Nadu, rice is grown predominantly with an area of 2.2 million hectares resulting in production of 8.65 million tonnes with the productivity of 3.93 t ha^{-1} (TNAU, 2013).However, the yield is lower, as compared to the average productivity of rice producing countries such as Japan (6.50 t ha^{-1}), China (6.70 t ha^{-1}), Egypt (7.50 t ha^{-1}) and Israel (5.50 t ha^{-1}). At the current growth of population rice requirement increases dramatically and many nations are facing second generation challenging of producing more rice less cost in a deteriorating environment; hence it is challenging task to ensuring food and national security. Thus, improved technologies are required to bridge the gap to feed the increasing population. The introduction of chemical growth regulators has added a new dimension to the possibility for improving the growth and yield of rice corp. Foliar application of plant growth regulators, both natural and synthetic, has proven worthwhile for improving crop growth against a variety of abiotic stresses. Plant hormones play a vital role in coordination of many growth and behavioral process in the plant life. Remarkable accomplishments of plant growth regulators (PGR) such as manipulating plant developments,enhancing yield and quality have been actualized in recent years using new emerging and efficient plant growth regulators. It has long been ascertained that plant hormones including auxins, gibberellins, cytokinin and ethylene etc., GA_3 being well known plant growth promoting hormones has shown to be involved in variety of plant growth and development processes (Frankenberger and Arshad1995). GA_3 is proved to improve effective portioning and translocation of accumulates from source to sink in the field crops (Senthil etal., 2003). GA_3 application was very effective in increasing seed set rate and grain yield through elongation of plant height, promoting panicle and spikelet exsertion, enhancing stigma exsertion and longevity and receptivity in rice and also key to win highergrain yield in rice production. (Gavino *et al* 2008). But research on application of Gibberellic acid on rice crop for improvement of growth and yield is very meager.Considering the above facts, field experiments were conducted to study the influence of gibberelic acid on growth and yield of rice.

Materials and Methods

Field experiments were conducted at the Experimental Farm, Department of Agronomy, Annamalai University, Annamalai Nagar 2014 to evaluate the effect of Gibberellic acid on growth and yield of rice. The weather at Annamalai nagar is moderately warm with hot summer months. The first crop received a rainfall of 1014.9 mm, distributed over 41 rainy days. The Relative humidity ranges from 77 to 92 per cent with a mean of

84.70 per cent. The second crop received a rainfall of 225.6 mm, distributed over 9 rainy days. The Relativehumidity ranges from 72 to 90 per cent with a mean of 84.95 per cent. A long duration rice variety BPT 5204 is selected for the study.The experiments were laid out in Randomized Block Design with three replications. The treatment details are viz.,Gibberellic acid @ 5 g ha^{-1}- (T_1), Gibberellic acid @ 10 g ha^{-1}-(T_2), Gibberellic acid @ 15 g ha^{-1} - (T_3), Gibberellic acid @ 20 g ha^{-1} - (T_4), Gibberellic acid @ 25 g ha^{-1} - (T_5), Triacontanol 0.05% EC @ 250 ml ha^{-1}-(T_6), control (T_7).Gibberellic acid is recommended for foliar application as a diluted spray solution at different concentration according to treatments and the solution was taken for spraying for an area of one hectare. Triacontanol is also another recommended for foliar application as a dissolved and diluted spray solution @ 25ml in 1 litre of water and the solution was taken for spraying for an area of one hectare. Both Gibberellic acid and Triacontanol was uniformly sprayed using hand sprayer (Knapsack) in the evening hours on 20 days after planting. Gibberellic acid was supplied through Progibb 40% WSG. A fertilizer schedule of 150 kg N, 50 kg P_2O_5 and 50 kg K_2O ha^{-1} was followed. The entire dose of P_2O_5, half dose of N and K_2O was applied as basal. Remaining with half the dose of N and K_2O was top dressed in the equal splits at maximum tillering and panicle primodium initiation stages.Thirty days old paddy seedlings were planted @ 2 seedling $hill^{-1}$ with a spacing of 20 ×15 cm to accommodate a plant population of 33 seedlings m^{-2}. The experimental plots were harvested leaving the border rows to avoid border effect. Five sample plants in each plot were selected at random and peg marked permanently for recording biometric observations.The matured crop was harvested from the net plot area and the grain was hand threshed, winnowed and sun dried to bring the moisture content to 14 per cent and the yield was recorded net plot wise and computed to kg ha^{-1}.

Harvest index

The harvest index was calculated by using the following formula suggested by Varma (1973).

$$\text{Harvest index} = \frac{\text{Economic yield}}{\text{Biological yield}} \times 100$$

Statistical analysis

The experiments data were statistically analysed as suggested by Panse and Sukhatme (1978). For significant results, the critical difference was worked out at 5 per cent probability level to draw statistical conclusions. The treatment differences that were non-significant at five per cent were denoted as NS.

Results and Discussion

Growth attributes

In both the experiments, there was perceptible difference observed in rice growth attributes due to effect of Gibberellic acid . Among the various treatment tested, application of Gibberellic acid @ 25 g ha^{-1} (T_5) had significantly registered the highest plant height of 125.72 and 124.84 cm, tillers number of 12.23 and 11.83 $hill^{-1}$, LAI of 7.66 and 7.45 and DMP of 12686 and 12000 kg ha^{-1} (Table 1) during first and second season, respectively.Results indicated that application of Gibberellic acid @ 25 g ha^{-1} (T_5) was theoptimum dose for producing the higher plant height, more number of tillers $hill^{-1}$ and DMP. The result of the present study issimilar to the findings of Prasad *et al*. (2013) who observed that application of GA_3 had significantly registered the higher growth attributes of plant. GA_3application might have increased the translocation ofassimilates to the vegetative organ which resulted in the maximum of plant height, number of tillers $hill^{-1}$. (Khadija *et al*. 2013; PepinurSusilavathi*et al*. 2014).The increased LAI could be attributed to the increased functional leaf area and delayed senescence as spray of growth promoter (Chen *et al*., 1982).The control (T_7) registered the least plant height of 93.69 and 93.02 cm, tillers number of 7.20 and 6.95 $hill^{-1}$, LAI of 5.35 and 5.31 and DMP of 7950 and 7560 kg ha^{-1} (Table 1) during first and second season, respectively.

Yield attributes

Among the treatments, foliar application of Gibberellic acid @ 25 g ha^{-1} (T_5) significantly registered higher number of 394.29 and 377.29 panicles m^{-2} and number of 91.45 and 91.22 filled grains $panicle^{-1}$ during first and second season, respectively and it was significantly superior over other treatments. This treatment was followed by Gibberellic acid @ 20 g ha^{-1}(T_4) and Triacontanol 0.05% EC @ 250 ml ha^{-1} (T_6), Gibberellic acid @ 10 g ha^{-1} (T_2) were on par with each other and ranked next. Foliar application of GA3 registered higher yield attributes viz.,higher number of panicles m^{-2} and number of filled grains $panicle^{-1}$. This might be attributed to the increased supply of photosynthetic materials and its efficient mobilization in plants giving rise to increased stimulation of yield attributes ultimately resulting in increased number of $paniclesm^{-2}$. Similar results were reported by Tiwari *et al*. (2011) and Hedden and Phillips (2000). The plant growth regulators like GA3 might be involved in formation of grain filling and their optimum nourishments have resulted in less number of aborted grains and thus maximized the survival of filled $grainsplant^{-1}$.These results are in conformity with the findings of Prabagaran and Ponnuswamy

(1997) and khadija*et al*. (2015). The control (T_7) registered the least number of panicles m^{-2} of 231.64 and 169.64 and number of 69.49 and 71.51 filled grains $panicle^{-1}$ during first and second season, respectively.

Grain and straw yield

Foliar nutrient management practices significantly influenced the grain and straw yields of rice in both the seasons. Among the treatments, Gibberellic acid @ 25 g ha^{-1} (T_5) significantly registered the higher grain yield of 5530 and 5390 kg ha^{-1} during first and second season, respectively. The grain yield recorded in this treatment was 27 and 25 per cent higher than control (T_7) in first and second season, respectively. Also the same treatment recorded higher straw yield of 7314 and 7274 kg ha^{-1}. This might be due heavier build up of sufficient food reserves diversified towards the developing number of panicles and number of filled grains due to spraying of growth regulators (Elankavi 1999). Besides, higher grain yield might be due to better translocation of photosynthates from source to sink. (Bhatt and Singh, 1997). This treatment was followed by Gibberellic acid @ 20 g ha^{-1} (T_4), Triacontanol 0.05% EC @ 250 ml ha^{-1} (T_6)and Gibberellic acid @ 10 g ha^{-1} (T_2) were on par with each other and ranked next. The treatment T_7 (control) registered the lowest grain yield of 4050 and 3950 kg ha^{-1} andstraw yield of 6177 and 6175kg ha^{-1} during first and second season, respectively.

Harvest index (%)

Among the treatments, the high harvest index of 43.29 in first season and 42.88 insecond seasons was recorded with Gibberellic acid @ 25 g ha^{-1} (T_5). This treatment was followed by Gibberellic acid @ 20 g ha^{-1} (T_4).The treatments T_6 and T_2 were on par with each other and ranked next. The least harvest index of 40.58 and 40.03 in first and second season, respectively was recorded in control (T_7).This might be due to GA_3 application accelerated photosynthetic activity and translocation of photosynthates to sink, which leads to recorded higher harvest index. This result is in harmony with those obtained by Khan, (1998).

Conclusion

Foliar spraying with Gibberellic acid @ 25 g ha^{-1} on 20 DAT could be recommended for farmers of coastal areas of Tamilnadu for early samba andthaladi season to achieve higher production in rice.

Table 1. Effect of Gibberellic acid on growth attributes of rice

Treatments	Plant height		Number of tillers $hill^{-1}$		LAI		DMP (kg ha^{-1})	
	First season	Second season	First season	Second season	First season	Second season	First season	Second season
T_1	106.96	101.70	8.08	7.91	5.83	5.77	9200	8510
T_2	111.19	105.88	8.91	8.77	6.32	6.25	10186	9620
T_3	118.08	117.70	10.56	10.40	6.99	6.86	11474	10850
T_4	122.20	121.79	11.54	11.50	7.35	7.23	12105	11500
T_5	125.72	124.84	12.23	11.83	7.66	7.45	12686	12000
T_6	113.94	108.34	9.63	9.42	6.62	6.45	10796	10050
T_7	93.69	93.02	7.20	6.95	5.35	5.31	7950	7560
SEd	1.73	1.73	0.33	0.29	0.13	0.12	283	296
CD(p=0.05)	3.84	3.63	0.78	0.69	0.32	0.29	623	608

Treatment details - Gibberellic acid @ 5 g ha^{-1} - (T_1), Gibberellic acid @ 10 g ha^{-1}-(T_2), Gibberellic acid @ 15 g ha^{-1} - (T_3), Gibberellic acid @ 20 g ha^{-1} - (T_4), Gibberellic acid @ 25 g ha^{-1} - (T_5), Triacontanol 0.05% EC @ 250 ml ha^{-1} -(T_6),Control - (T_7).

Table 2. Effect of Gibberellic acid on yield attributes, grain and straw yields and harvest index in rice

Treatments	Number of panicles m^{-2}		Filled grains $panicle^{-1}$		Grain yield (kg ha^{-1})		Straw yield (kg ha^{-1})		Harvest index	
	First season	Second season	First season	Second season	First season	Second season	First season	Second season	First season	Second season
T_1	263.64	236.64	75.57	75.00	4500	4330	6177	6175	42.14	41.21
T_2	292.30	236.64	79.61	78.98	4730	4620	6414	6412	42.44	41.87
T_3	346.63	298.63	86.21	84.86	5095	4990	6857	6846	42.62	42.15
T_4	383.29	364.29	90.40	88.88	5425	5295	7104	7070	43.05	42.68
T_5	394.29	377.29	91.45	91.22	5530	5390	7314	7274	43.29	42.88
T_6	313.96	269.63	81.69	81.02	4845	4710	6626	6622	42.23	41.56
T_7	231.64	169.64	69.49	71.51	4050	3950	5930	5917	40.58	40.03
SEd	7.59	6.83	1.75	1.56	56	48	102	98	0.11	0.10
CD (p=0.05)	16.23	14.21	3.75	3.36	120	110	226	216	0.29	0.27

Treatment details - Gibberellic acid @ 5 g ha^{-1} - (T_1), Gibberellic acid @ 10 g ha^{-1}- (T_2), Gibberellic acid @ 15 g ha^{-1} - (T_3), Gibberellic acid @ 20 g ha^{-1} - (T_4), Gibberellic acid @ 25 g ha^{-1} - (T_5), Triacontanol 0.05% EC @ 250 ml ha^{-1} -(T_6), control - (T_7).

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ASSESSMENT OF FARM SAVED SEED QUALITY OF PULSES IN MAJOR PULSE GROWING DISTRICTS IN TAMIL NADU

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Abstract

Quality seed is the most critical and basic input for agricultural output and accounts for 15-20 per cent of yield increase. Of the total seed requirement in the country, more than 70% of the requirement is met by farmers saved seed. To analyze the quality status of farm save seeds of pulses, survey was conducted during 2019-2020 and collected seed samples of blackgram and greengram from Cuddalore District. Seed quality parameters were analyzedand the result revealedthat blackgram seeds recorded the maximum germination (86%) and vigour (2266) but lower physical purity (97.6 %) and greengram also recorded the maximum germination (84%) and vigour (2604) but lower physical purity (96 %) than the Indian Minimum Seed Certification Standards.

Introduction

Farmer saved seed accounts for the greatest proportion of seeds used by farmers worldwide especially in low-income countries. In country like India, it is not possible that public and private sector can fulfill the seed requirements of farmers especially in crops which require large number of seed to raise next season's crop like pulses and oilseeds etc. The percentage of small and marginal farmers who have access to high-quality seed is only 20% (Roy, 2015).Of the total seed requirement in the country, only less than 30% good quality certified seeds are available to the farmers and more than 70% of the requirement is met up by farmers saved seed. But in the maximum cases, the farmer's saved seed is stored in very unhygienic conditions, hence it is prone to seed inhabiting microflora which are capable of deteriorating seed quality. If seeds with low germination are used for planting, the seeding rate could possibly be adjusted according to the expected plant population (Selvamani*et al*., 2021). Based on this background, survey and study on seed quality of farmers' saved seeds of pulses were conducted.

Materials and Methods

Conducted survey during the year 2019-2020 to assess the status of farm saved seeds of blackgram and greengram and collected seed samples from Cuddalore District of Tamil Nadu. Three blocks *viz.,* Nallur, Mangalore and Virudhachalam were selected and three villages from each block were identified and 20 seed samples from each village were collected. Seed quality parameters viz., germination, seedling length, vigour, moisture content and physical purity were analyzed for the collected 180 samples of three blocks each with 60 seed samples in blackgram and greengram.

Result and Discussion

Blackgram

The results revealed that seeds collected from Nallur block recorded 10.6 per cent moisture content, 100 seed weight of 4.21 g, 86 per cent germination, 32.0 cm seedling length, vigour index (2750) and physical purity of 98.4 %. Seeds collected from Mangalore block recorded 9.1 per cent moisture content, 100 seed weight of 4.13 g, 92 per cent germination, 36.1 cm seedling length, vigour index (2750) and physical purity of 98.2 %. Seeds collected from Virudhachalam block recorded 11.7 per cent moisture content, 100 seed weight of 4.22 g,82 per cent germination, 27.9 cm seedling length, vigour index (2266) and physical purity of 97.6% (Table1).

Greengram

Seeds collected from Nallur block recorded 10.6 per cent moisture content, 100 seed weight of 3.48 g, 87 per cent germination, 31.8 cm seedling length, vigour index (2720) and physical purity of 96.3 %. Seeds collected from Mangalore block recorded 8.8 per cent moisture content, 100 seed weight of 3.50 g, 84 per cent germination, 33.8 cm seedling length, vigour index (2843) and physical purity of 96.9 %. Seeds collected from Virudhachalam block recorded 10.4 per cent moisture content, 100 seed weight of 4.67 g, 84 per cent germination, 31.0 cm seedling length, vigour index (2604) and physical purity of 96.0% (Table 2). It is concluded that farm saved **s**eeds of blackgramand greengramrecorded the germination of above Indian Minimum Seed Certification Standards (IMSCS) but the physical purity of below IMSCS.

Table 1. Seed quality parameters of farm saved seeds of blackgram collected from Cuddalore Dt.

S. No.	Name of the block	Name of the village	Moisture content (%)	Germination (%)	Vigour index	Physical purity (%)
1.	Nallur	Kunjanur	10.3	80	2725	98.4
		Valasai	11.3	92	2775	98.7
		Nallur	10.2	88	2752	98.2
		Mean	10.6	86	2750	98.4
2.	Mangalore	Adari	9.5	92	3255	98.7
		Kuyanambadi	8.7	96	3435	98.2
		Angeerakulam	9.0	90	3320	97.6
		Mean	9.1	92.7	3336	98.2
3.	Virudhachalam	Virudhachalam	10.6	84	2250	97.6
		Karunatham	11.9	82	2330	97.4
		Edaichithur	12.6	80	2220	98.0
		Mean	11.7	82.0	2266	97.6

Table 2. Seed quality parameters of farm saved seeds of greengramcollected from Cuddalore Dt.

S. No.	Name of the block	Name of the village	Moisture content (%)	Germination (%)	Vigour index	Physical purity (%)
1.	Nallur	Kunjanur	10.1	88	2830	97.2
		Valasai	11.0	90	2620	96.2
		Nallur	10.6	84	2710	95.6
		Mean	10.6	87	2720	96.3
2.	Mangalore	Adari	9.2	86	2830	97.2
		Kuyanambadi	8.4	82	2620	97.0
		Angeerakulam	8.8	84	2710	96.6
		Mean	8.8	84	2843	96.9
3.	Virudhachalam	Virudhachalam	10.2	82	2600	95.8
		Karunatham	10.6	84	2640	96.4
		Edaichithur	10.3	88	2574	96.0
		Mean	10.4	84	2604	96.0

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EFFECT OF FOLIAR FERTILIZATION OF POTTASIUM, IRON AND UREA ON GROWTH AND DEVELOPMENT Of VARAGU

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Abstract

To improve the productivity of all agricultural crops, only the major nutrients are concentrated. Though the importance of micronutrient realized during past decades in most of the crops but it is not effectively materialized in general crop cultivation practices. The micronutrient deficiencies in soil are not only hamper crop productivity but also deteriorating the produce quality. Nitrogen is highly mobile in the plant. Younger leaves and developing organs with strong sink demands, draw heavily on N in the older or lower leaves. Plants well supplied with K lost less water since K increased osmotic potential and had a positive influence on stomatal closure as well. Iron is used for the synthesis of chlorophyll and is essential for the function of chloroplasts.To overcome these problems foliar spray is being recommended but it is not crop specific or soil specific recommendation.The present investigation was conducted in the Centre of Excellence in millets, Athiyandal, Tiruvannamalai district through foliar spray of micronutrients combination.

Key words: Nutrients, Leaf Area, Crop Growth Rate, RWC (%), yield and Water stress.

Introduction

Survival and reproduction of plants require water, air, light and relatively considerable amounts of nutrients called essential nutrients to carry out photosynthesis and thus produce energy (Wiedenhoeft, 2006). To get the benefit from the crop plant we have to protect them from various kind of stress, especially nutrient stress (deficiency). Plant cannot synthesis required nutrient, so it extracted from soil medium and loaded into the plant parts and which is to be finally entered in to the food chain.

Small millets - a group of six crops / minor coarse cereals, namely finger millet *(Eleusine coracana),* little millet *(Panicum miliare),* kodo millet *(Paspalum scrobiculatum),* foxtail millet *(Setariaitalica),* barnyard millet *(Echniocholafrumentacea)* and proso millet *(Panicum miliaceum),* representing the area grown in that order. These crops have traditionally been the indispensable component of dry farming system.Millets possess several morpho-physiological, molecular and biochemical characteristics whichconfer better tolerance to environmental stresses than major cereals. Primarily, the short lifecycleof millets assists in escaping from stress as they require 12–14 weeks to complete theirlife-cycle (seed to seed) whereas rice and wheat requires a maximum of 20–24 weeks. However,the prevalence of stress conditions and their consequences are circumvented by several traitssuch as short stature, small leaf area, thickened cell walls, and the capability to form dense root system. Also, the C4 photosynthetic trait is highly advantageous to millets.In the C4 system, carbon dioxide (CO2) is concentrated around ribulose-1,5-bisphosphate carboxylase/oxygenase (RUBISCO), which in turn suppresses ribulose 1,5-bisphosphate (RuBP) oxygenation and photorespiration. Drought stress reduces leaf size, stems extension and root proliferation, disturbs plant water relations and reduces water use efficiency. Plants display a variety of physiological and biochemical responses at cellular and whole-organism levels towards prevailing drought stress, thus making it a complex phenomenon.

Several morphophysiological and biochemical studies in millets have shown their stress adaptation strategies. Similarly, increase in biochemical activities such as enhanced levels of antioxidants, reactive oxygen species and their scavenging enzymes, enzyme activity of catalase and superoxide, and synthesis of osmolytes and other stress-related proteins has been reported in response to abiotic stresses in foxtail millet.Being C_4 plants these are more environment friendly with higher water Use Efficiency and low input requirement, but equally responsive to high input management crops.

Potassium is an important nutrient for improving the crop yield per unit area. K+ ions are also involved in the activation of proton-pump ATPases, photosynthesis, osmoregulation, cell expansion, and stomatal movement (Horst, 1995). These various roles explain the symptoms of lesions and necrotic spots in leaves as well as reduction of plant growth.Potassium is vital for physiological processes, water availability, photosynthesis, assimilate transport and enzyme activation with a direct effect on crop production. Potassium deficiency significantly reduces the leaves number and size of individual leaf and as a result photosynthetic activity of the plant was affected. Potassium limits the crop water requirement during drought conditions because K has a dominant role in the opening and closing of stomata, through which transpiration occurs from the leaves and CO2 enters into leaf tissues. If K is inadequate, the stomatal activity decreases and transpiration loss increases. Grain yield increases by enhancing the uptake of potassium under the arid condition.Traditionally, potassium fertilizer directly are applied to soil gets fixed

with clay minerals and becomes unavailable to crop plants.Foliar application of potassium is more suitable, target oriented and economical technique for increasing the fertilizer use efficiency and grain yield over soil application. Impact of micronutrient deficiency in crop production is well documented as loss of yield (Shukla et al., 2009). Thus, micronutrient deficiency has become a limiting factor for crop productivity in many parts of the world (Singh et al., 2009).

Materials and Methods

A field experiment was conducted at Centre of Excellence in millets, Athiyandal, Tiruvannamalai district during *kharif*' (September, 2015-January, 2018). The experiment was laid out in a randomized block design and replicated thrice. Duration of the crop was 120 days. Varagu variety CO3 was sown with a spacing of 45 cm x 10 cm and raised following recommended package of practices.Plant height, Root Length and number of leaves were recorded at Vegetative, Panicle Initiation and Grain filling stage.bio-regulators like Brassinosteroid (BR), Salicylic Acid (SA) and fertilizers / chemicals such as Urea, Ferrous sulphate ($FeSO_4$) and Potassium Chloride (KCl) were used in this study.

T_1-Control
T_2-Water Spray
T_3-1 % KCl Spray
T_4-0.1 ppm BrassinosteroidSpray
T_5-100 ppm Salicylic Acid Spray
T_6-0.1% PPFM Spray
T_7-0.5% $FeS0_4$+ 0.5% Urea Spray
T_8-100 ppm Salicylic Acid+0.5% $FeS0_4$+ 0.5% Urea Spray
T_9-1 % KCl+100 ppm Salicylic Acid+0.5% $FeSO_4$+ 0.5% Urea Spray
T_{10}-1 % KCl+0.1 ppm Brassinosteroid+0.5% $FeS0_4$+ 0.5% Urea Spray

Time of sprays: First spray at Active tillering stage , second spray at Panicle Initiation stage

Foliar spray was given at vegetative, flowering and maturity period. Periodical observations were taken under the cropping period.

Table 1.Impact of foliar spray of nutrients with growth regulators on Leaf Area, No of productive tillers / hill,Crop Growth Rate (g m^{-2} day^{-1}), SPAD Chlorophyll, Chlorophyll Stability Index (CSI %) and Yield Components in Varagu

Treatment	Leaf Area (cm^2 $plant^{-1}$)	No of productive tillers / hill	Crop Growth Rate (g m^{-2} day^{-1})	SPAD Chlorophyll content	Chlorophyll Stability Index (CSI %)	Grain yield (kg/ha)
T_1	531	12	0.179	41	78	1125
T_2	766	13	0.205	46	78	1130
T_3	822	13	0.176	45	88	1277
T_4	757	15	0.225	42	83	1038
T_5	690	14	0.252	44	82	1124
T_6	649	15	0.234	41	84	1032
T_7	835	16	0.286	47	83	1386
T_8	751	14	0.207	45	81	1225
T_9	794	16	0.276	46	86	1357
T_{10}	666	15	0.265	44	86	1226
Mean	726	14	0.230	44	82	1165
SEd	23.48	0.680	0.011	1.170	1.012	70.28
CD (P=0.05)	49.71	1.439	0.024	2.476	2.145	148.79

Results and discussion

Plants require both macronutrients (C, H, O, N, P, S, K, Mg, and Ca), and micronutrients (Fe, Mn, Zn, Cu, B, Mo, Cl, and Ni) in specific concentrations for their growth and development (Horst,1995 and Buchanan*et al*., 2000). These essential elements are components of important molecules and the plant cannot complete its life cycle without them. These nutrients have different functions within the plant body including ionic, enzymatic, structural, and regulatory roles. For example, carbon, hydrogen, and oxygen are important components of carbohydrates, lipids, proteins, and nucleic acids.Iron deficiency in crops, characterised by interveinal chlorosis, is a worldwide problem in calcareous soils. It is often treated by the addition of a commercial chelator. Although iron is more available at pH 5.5, the problem also exists that, in acidic soils, soluble aluminium is abundant, which restricts iron absorption by plants (Salisbury and Ross 1992). In aqueous solution, iron exists in two oxidation states: the ferrous (Fe2+) and ferric (Fe3+) forms. Of these two forms, Fe2+ is more soluble, and thus more easily absorbed by plant roots. However, in well-aerated soils, Fe2+ is oxidised to Fe3+, which precipitates.The foliar application of 0.1 % KCl (T_3) spray maintained higherchlorophyll stability index in all stages than others even under the stress conditions (88%).The morphological parameters such as Leaf Area (LA)(835 cm) and Crop Growth Rate (CGR)(0.286) were greatly influenced by the foliar application of 0.5% $FeSO_4$+ 0.5% Urea Spray (T_7) at grain filling stage. The foliar application of 0.5% $FeSO_4$+ 0.5% Urea spray maintains a higher SPAD value and total chlorophyll content in all the stages than others even under the stress conditions (47).The yield and its components were enhanced by the use of 0.5% $FeSO_4$+ 0.5% Urea Spray, which was closely followed by the foliar spray of 1% KCl+100 ppm Salicylic Acid+0.5% $FeSO_4$+ 0.5% Urea spray with comparable value.This recommendation is effective outreach to farmers to adopt recommendation will improve crop productivity, quality of crop.

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SCREENING OF TENAI (*SETARIA ITALICA* L.) LINES AGAINST MAJOR DISEASES UNDER FIELD CONDITION AS RESISTANT SOURCES

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Abstract
Tenai (Setaria italica L.) is cultivated as dry land crop under marginal and sub- marginallands of tropical and sub tropical Asia. The field experiment was conducted with fifteen tenaigenotypes raised in sandwich method with three replications during kharif and rabi 2017 undernatural epiphytotic conditions at the research farm of Centre of Excellence in Millets,Athiyandal, where the severity of these diseases remain very high during the cropping season.The resistance or otherwise of the test genotypes to blast and rust diseases was assessed using 1-9 rating scale. The lower leaves were severely affected than top young leaves with no symptomsobserved on leaf sheath,nodes, peduncle and panicle. Sharma et al. (2014) found that out of 154accessions of tenai screened for blast resistance under field conditions, 34 were resistant and 96were moderately resistant during 2009; whereas, in 2010, number of accessions in the resistantand moderately resistant categories was 46 and 65 respectively. The mature spots generallymeasured about 1.0 x 1.5 mm in dimension and by their coalescence large irregular patches were formed the similar observation with our studies

Keywords: *Tenai, air-borne, blast, rust and brown leaf spot*

Introduction

Tenai (*Setaria italica* L.) is cultivated as dry land crop under marginal and sub- marginal lands of tropical and sub tropical Asia. The grain is widely used as livestock and poultry feed. It is grown in Tamil Nadu as rainfed crop during June-July and September - October covering the area of western zone of Tamil Nadu and occupies an area of 900 ha with a production of 432 tonnes and productivity of 472 kg/ha (Crop and Season Report, 2016–17) also infested by many diseases like blast (*Pyricularia setariae*), rust (*Uromyces setariae*), brown leaf spot (*Drechslera setariae*), downy mildew (*Sclerospora graminicola*) and smut (*Ustilago crameri*). Among them, blast, rust and leaf spot are the major air-borne and most destructive diseases. The identification of genotypes resistant to these diseases is essential considering the poor purchasing power of rainfed farmers and safer ecology. Therefore, an attempt has been made to identify suitable tenai genotypes, which could be utilized for developing resistant cultivars against important endemic diseases of the region.

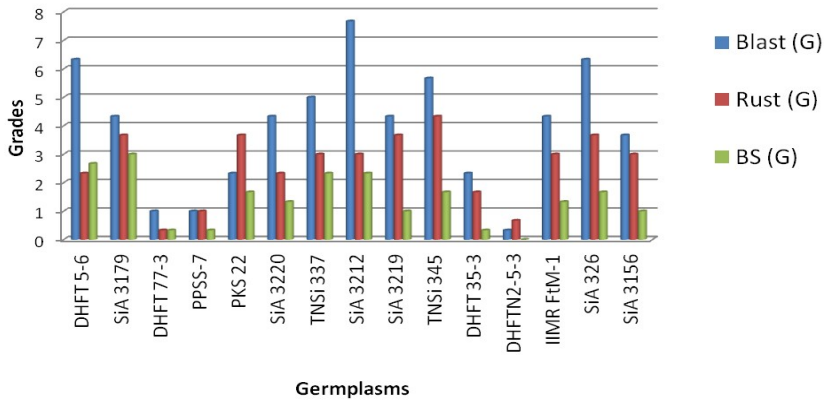
Materials and Methods

In order to find out the resistant sources against air-borne diseases, field experiment was conducted with fifteen tenai genotypes raised in sandwich method with three replications during *kharif* and *rabi* 2017 under natural epiphytotic conditions at the research farm of Centre of Excellence in Millets, Athiyandal, where the severity of these diseases remain very high during the cropping season. The recommended agronomical practices were adopted for better crop growth. Five randomly selected competitive plants were chosen from each genotype and in each replication for recording the observations. It recorded at tillering and panicle emergence stage for diseases symptom on leaves for all the air-borne pathogens. The resistance or otherwise of the test genotypes to blast and rust diseases was assessed using 1-9 rating scale (Proceedings of 27th Annual Group Meeting of AICRP on Small Millets, 2017).

Results and Discussion

The study materials comprised different genotypes from 6 places of 4 states of India. Normally the disease symptoms would be seen on 15 days after sowing. Initially, small yellowish dot appeared that within 2-3 days turned circular to oval with a grayish centre surrounded by brown margin. In severe form, lesions coalesced with tearing off of infected portion. Similar symptoms were reported by Patro *et al*. (2018). The lower leaves were severely affected than top young leaves with no symptoms observed on leaf sheath, nodes, peduncle and panicle. Sharma *et al*. (2014) found that out of 154 accessions of tenai screened for blast resistance under field conditions, 34 were resistant and 96 were moderately resistant during 2009; whereas, in 2010, number of accessions in the resistant and moderately resistant categories was 46 and 65 respectively. Mitra and Mehta (1934) observed the minute oval brown spots on both sides of the leaves. These spots gradually elongated parallel to the axis of the leaf and eventually became dark brown. The mature spots generally measured about 1.0 x 1.5 mm in dimension and by their coalescence large irregular patches were formed the similar observation with our study.

Fig. 1. Grades of blast rust and brown spot diseases of tenai



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FOREWARNING MODEL FOR RAGI BLAST DISEASE MANAGEMENT BY ADJUSTING DATE OF SOWING IN TAMIL NADU

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Abstract
Pyricularia grisea is an important fungal disease in almost all the ragi growingregions of India. The field experiments were conducted at Centre of Excellence in Millets,Athiyandal during 2016 to 2021 to assess the impact of planting dates and varieties on theresultant incidence of blast disease of ragi in the field. Trials were established under rainfedconditions for two consecutive years, 2016-17 to 2020-21 in a randomized block design in3m X 2m plot at a spacing of 25cm X 10cm with three replications of each treatment in allthe years, variety CO14 was sown in at least on fortnightly from 1 st June to 16 th February. Theearly sown and after 16 th January sown crops were free from the all the blast incidences.which recorded minimum temperature of 24.84 0 C and maximum 32.87 0 C, relative humidityof 73.27 per cent and a very high amount of rainfall. Highest finger blast severity of 58.32and 51.37 per cent in genotypes VR-708 and KM-252 respectively in June 16 th sown crop.Similarly highest incidence of leaf blast (Grade-4) was recorded in June 16 th sown VR-708.

Keyword: Ragi, blast disease, forecasting, date of sowing

Introduction

Ragi (*Eleusine coracana* L.) is originally native of the Ethiopian highlands and was introduced into India approximately 4000 years ago. It is cultivated widely in East Africa and tropical Asia, mainly in the rainy slopes. It is also cultivated in the upland area of the Himalayas at an elevation of 2,300 m. India is the largest cultivator of ragi, which is primarily grown in the states of Karnataka, Tamil Nadu, Andhra Pradesh, Maharashtra, Uttar Pradesh, Bihar, Orissa and Gujarat. Of the several fungal diseases blast caused by *Pyricularia grisea* is an important disease in almost all the ragi growing regions of India. The disease is known to occur almost every year during rainy season in all major ragi growing areas and is perceived as one of the major diseases causing recurring yield losses in all the states of India (Seetharam, 1983). Information on the incidence and occurrence of blast in ragi sown at different dates can help to adjust the sowing time to harvest high yields. Therefore, keeping this in mind the field experiments were conducted at Centre of Excellence in Millets, Athiyandal during 2016 to 2021 to assess the impact of planting dates and varieties on the resultant incidence of blast disease of ragi in the field.

Materials and methods

Trials were established under rainfed conditions for two consecutive years, 2016-17 to 2020-21 in a randomized block design in 3m X 2m plot at a spacing of 25cm X 10cm with three replications of each treatment in all the years, variety CO14 was sown in at least on fortnightly from 1st June to 16th February. The crop was raised as per the recommended package of practices and no spray application of any chemical was given for the management of disease. The data on neck and finger blast were recorded at dough stage of the crop was recorded. Neck blast was recorded as the percentage of ears showing infection on the peduncle and finger blast as the percentage of fingers affected (Nagaraja *et al*., 2007).

Results and Discussion

The incidence of leaf blast was recorded from 1st September to 16th January, neck blast recorded in 16th August to 16th November and finger blast incidence recorded in 1st August to 16th November of every year. The early sown and after 16th January sown crops were free from the all the blast incidences. which recorded minimum temperature of 24.84^{0}C and maximum 32.87^{0}C, relative humidity of 73.27 per cent and a very high amount of rainfall (Table1). Patro and Madhuri (2014) recorded the highest incidence of neck blast of 72.67 and 67.00 per cent was noticed in the susceptible genotypes VR-708 and KM-252 respectively in June 16th sown crop, where minimum temperature of 26.1^{0}C, maximum temperature of 32.36^{0}C, relative humidity of 89.9 per cent and a very high amount of rainfall prevailed. Highest finger blast severity of 58.32 and 51.37 per cent in genotypes VR-708 and KM-252 respectively in June 16th sown crop. Similarly highest incidence of leaf blast (Grade-4) was recorded in June 16th sown VR-708.

Table1. Metrological data and blasts (Leaf, Neck & Finger) incidence recorded during experimentation at Centre of Excellence in Millets

S. No.	Date of Sowing	Temperature (^{0}C)		RH (%)	Rain fall (mm)	Leaf blast (G)*	Neck blast (%)*	Finger blast (%)*
		Max.	Min.					
1.	1st Jun. 2019	35.54	27.10	54.64	32.80	0	0	0
2.	16th Jun. 2019	36.67	27.25	52.68	4.00	0	0	0
3.	1st Jul. 2019	34.18	25.88	60.31	96.80	0	0	0
4.	16th Jul. 2019	34.30	27.68	59.26	122.20	0	0	0
5.	1st Aug. 2019	36.27	27.60	68.30	0.00	0	0	3.00
6.	16th Aug. 2019	35.38	27.10	56.80	140.00	0	4.00	8.00
7.	1st Sep. 2019	36.30	27.47	63.51	122.00	3.00	9.50	9.50
8.	16th Sep. 2019	32.87	24.84	73.27	171.00	6.00	11.50	10.00
9.	1st Oct. 2019	33.62	25.92	74.11	90.00	6.33	17.50	19.00
10.	16th Oct. 2019	31.22	23.95	72.73	96.00	6.00	14.50	20.50
11.	1st Nov. 2019	30.14	25.07	73.66	7.70	6.67	9.00	16.00
12.	16th Nov. 2019	27.38	25.42	69.30	53.00	6.33	4.00	10.50
13.	1st Dec. 2019	29.30	24.76	60.31	36.80	7.00	0	0
14.	16th Dec. 2019	28.15	22.10	59.26	0.00	5.67	0	0
15.	1st Jan. 2020	29.50	22.25	68.30	0.00	5.00	0	0
16.	16th Jan. 2020	29.40	23.10	56.80	0.00	3.00	0	0
17.	1st Feb. 2020	31.26	23.10	63.51	0.00	0	0	0
18.	16th Feb. 2020	33.50	23.45	63.27	0.00	0	0	0

* Mean of three replications

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EFFECT OF FOLIAR NUTRIENTS ON THE GROWTH, YIELD AND ECONOMICS OF BLACKGRAM

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Abstract

A field experiment was conducted at Achalvadi village, located at Harur block of Dharmapuri district to study the effect of foliar nutrients on the growth, yield and economics of blackgram. The experiment consisted of ten treatments. The experimental plots were laid out in randomized block design with three replications. Among the various treatments tried, application of RDF + EFYM @ 750 kg/ha+ Foliar application of 2% DAP at 30 DAS + Foliar application of Silixol plus @ 1 ml/litre @ 45 DAS (T8) recorded the maximum growth parameters viz. plant height (35.64 and 40.78 cm at 45 DAS and harvest stages respectively), number of branches/ plant (7.86), leaf area index (5.72) and dry matter production (14.69 g/plant), yield attributes *viz.* number of pods/plant (14.38), number of seeds/ pod(7.27), yield (1247 and 1975 kg/ha of seed and haulm yield respectively) and economics (Rs. 86043, Rs. 57195 and 2.98 of gross income, net income and B:C ratio respectively) of the crop. This treatment was followed by RDF + EFYM @ 750 kg/ha+ Foliar application of 2% DAP twice at 30 and 45 DAS (T_4). The lower values of growth parameters, yield attributes, yield and economics were recorded under the control treatment (T_1).

Keywords: Blackgram, EFYM, foliar nutrients and Silicon

Introduction

Blackgram (Vigna mungo L.) belonging to the family Fabaceae is the most important pulse crop cultivated in India. In India, blackgram is cultivated in the area of 4.47 million hectares with the production of 2.83 million tonnes and productivity of 632 kg/ha (Agricultural Statistics at a Glance, 2019). The United Nations declared the year 2016 as "International Year of Pulses" to increase the public awareness regarding the nutritional benefits of pulses aimed to improve food security and nutrition as part of sustainable food production (Mohanty and Satyasai, 2015). The World Health Organization (WHO) recommends 80 g pulse per day per person and the Indian Council of Medical Research (ICMR) recommends 47g pulse per day per person. Blackgram consists 22.3% of protein, 48.0% of carbohydrates, 154 mg of calcium, 300 mg phosphorus, 9.1 mg of iron, 1.4 g of3.37 g of riboflavin, 0.42 g of thiamin and 2 mg niacin per 100 g of black gram (Asaduzzaman *et al*., 2010). Thoughpulses are rich in protein they are still being cultivated 95 per cent under rainfed condition and more than 78 per cent under energy starved condition. The main reasons for low productivity of blackgram is poor nutrient management practices and cultivation under moisture stress condition (Suhathiya and Ravichandran, 2018). Hence there is
a need to increase the production potentaial of pulses. The growth phase of blackgram is often obstructed by the slow translocation of assimilates, poor pod setting due to flower abscission and lack of nutrient during critical stages of crop growth (Mahala *et al*., 2001). These obstructs can be overcome by the foliar application of the nutrients required for the crop growth. Hence, in this study combined application of various foliar nutrients along with the recommended dosage of fertilizers and EFYM @750 kg/ha to various treatments was imposed to study the effect of the foliar nutrients on the growth, yield and economics of the blackgram.

Materials and Methods

A field study entitled "Effect of foliar nutrients on the growth, yield and economics of blackgram" was conducted at Achalvadi village, Harur block of Dharmapuri district. The experimental field is geographically located in the latitude of 11o59'N and longitude of 78o 29'E with an altitude of 392 meters above mean sea level. The experiment consisted of ten treatments, three replications and the experimental plots were laid out in randomized block design. The treatments include, T1 – Control, T_2 – RDF (44:22:0 kgs of NPK/ha) + EFYM @ 750 kg/ha, T_3 – RDF + EFYM @ 750 kg/ha + Foliar application of 2% urea twice at 30 and 45 DAS, T_4 - RDF + EFYM @ 750 kg/ha + Foliar application of 2% DAP twice at 30 and 45 DAS, T_5 - RDF + EFYM @ 750 kg/ha + Foliar application of 1% KCl twice at 30 and 45 DAS, T_6 - RDF + EFYM @ 750 kg/ha + Foliar application of 3% Panchagavya twice at 30 and 45 DAS, T_7 - RDF + EFYM @ 750 kg/ha + Foliar application of 2% urea at 30 DAS + Foliar application of Silixol plus @ 1 ml/litre @ 45 DAS , T_8 - RDF + EFYM @ 750 kg/ha + Foliar application of 2% DAP at 30 DAS + Foliar application of Silixol plus @ 1 ml/litre @ 45 DAS, T_9 - RDF + EFYM @ 750 kg/ha + Foliar application of 1% KCl at 30 DAS + Foliar application of Silixol plus @ 1 ml/litre @ 45 DAS, T_{10} - RDF + EFYM @ 750 kg/ha+ Foliar application of 3% Panchagavya at 30 DAS + Foliar application of Silixol plus @ 1 ml/litre @ 45 DAS. The field experiment was conducted under the rainfed condition. The experimental field soil was sandy loam in texture which was low in available nitrogen (238 kg/ ha), medium in available phosphorus (19.5 kg/ha) and high in available potassium (292 kg/ha). The blackgram variety ADT 5 was chosen for the study. The recommended dosage of fertilizers (25:12.5:0 kgs of NPK/ha) through urea (46% N) and DAP (18%N and 46%P2O5) were applied as basal. The foliar application of nutrients were sprayed on 30 and 45 DAS as per treatment schedule.

Results and Discussion

Growth parameters

The foliar application of various nutrients had a remarkable effect on the growth of blackgram (Table 1). Application of RDF + EFYM @ 750 kg/ha+ Foliar application of 2% DAP at 30 DAS + Foliar application of Silixol plus @ 1 ml/litre @ 45 DAS (T_8) produced the maximum values of growth parameters viz. plant height of 35.64 and 40.78 cm at 45 DAS and harvest stages respectively, no of branches plant-1of 7.86, leaf area index of 5.78 and dry matter of 14.69 g/plant over the rest of other treatments. It was followed by the application of RDF + EFYM @ 750 kg/ha+ Foliar application of 2% DAP twice at 30 and 45 DAS (T_4). The increase in growth of blackgram might be due to the foliar spray of 2% DAP, silixol plus @ 1ml/litre along with the application of RDF and EFYM @ 750 kg/ha. This combined application of various sources of nutrients favours the crop growth. The growth parameters were significantly influenced due to the foliar spray of 2% DAP + 1% KCl at flowering and pod filling stages of crop growth in pulses (Geetha and Velayutham, 2009). The foliar application of silicon increases the erectness of leaves (Yavarzadesh *et al*., 2008) increasing the light interception and photosynthetic efficiency of the cropwhich eventually lead to the more dry matter accumulation (Jinger *et al*., 2018). The foliar importance of the silicon was earlier reported by Jawahar *et al*. (2019) in rice. The foliar application of DAP supplies nitrogen and phosphorus which are essential for the vegetative growth and nitrogen fixation of the legumes. Chhimpa and Sharma (2018) also reported that foliar application of silicon increases the growth of blackgram. Hence all these factors might have favoured the growth of crop and increase in dry matter production. The minimum values of growth parameters were observed under the control treatment (T_1). These findings are in line withMarimuthu and Surendran (2015), Rakhi Chhimpa and Sharma (2018),Suhathiya and Ravichandran (2018).

Yield attributes and yield

The foliar application of various nutrients significantly influenced the yield attributes and yield of blackgram (Table 2). Among the various treatments imposed in the study application of RDF + EFYM @ 750 kg/ha+ Foliar application of 2% DAP at 30 DAS + Foliar application of Silixol plus @ 1 ml/litre @ 45 DAS (T_8) produced the maximum values of yield attributes viz., number of pods plant-1(14.38) and number of seeds pod-1(7.27) and yield (1247 and 1975 kg/ha of seed and haulm yield respectively). This treatment was followed by the application of of RDF + EFYM @ 750 kg/ha+ Foliar application of 2% DAP twice at 30 and 45 DAS (T_4). The foliar application of 2% DAP supplies the major essential element phosphorus which is necessary for the seed formation. The foliar application of the silicon through the silixol plus increase the photosynthetic efficiency of the crop. The significant increase in yield attributes of black gram might be due to combined foliar spray of 2% DAP and silixol plus along with the basal application of RDF and EFYM @ 750 kg/hawhich play a major role in growth, development and metabolism of black gram.

These combination might have favored better translocation of assimilates from the source to sink resulting in the improvement of yield attributes and yield (Marimuthu and Surendran, 2015). Similar findings regarding the foliar application of 2% DAP in pulse crops were reported earlier by Ramesh *et al*. (2016), Bhaskar Ritika *et al*. (2020) and Sruthi *et al*. (2020). Chhimpa and Sharma (2018) reported that foliar application of 2% silicon produced the maximum yield attributes and yield in blackgram.

Economics

The field investigation on the effect of foliar nutrition on the economics of blackgram reveals that application of RDF + EFYM @ 750 kg/ha+ Foliar application of 2% DAP at 30 DAS + Foliar application of Silixol plus @ 1 ml/litre at 45 DAS (T_8) recorded the maximum values of gross income (Rs. 86043 ha-1), net income (Rs. 57195/ha) and B:C ratio (2.98) over the other treatments. This treatment was followed by the application of RDF + EFYM @ 750 kg/ha+ Foliar application of 2% DAP twice at 30 and 45 DAS (T_4). The lower values of economics were recorded under the control treatment (T_1).

Conclusion

Blackgram is the most important pulse crop cultivated in India. It is cultivated majorly under rainfed condtion in energy starved situation. Hence there is a need to increase the production potential of blackgram under rainfed condition. From the present investigation application of RDF (25:12.5: 0 kg of NPK/ha) + EFYM @ 750 kg/ha+ Foliar application of 2% DAP twice at 30 DAS + Foliar application of Silixol plus @ 1 ml/litre @ 45 DAS can be recommended to produce the economically sound yield under rainfed condition.

Table 1. Effect of foliar nutrients on the growth of blackgram

Treatment	Plant height (cm)		No. of branches /plant	LAI at Flowering stage	DMP at harvest stage (g/plant)
	45 DAS	At harvest stage			
T_1	25.68	28.24	6.18	3.30	9.28
T_2	27.29	30.28	6.45	3.56	10.36
T_3	28.47	31.58	6.59	3.81	10.79
T_4	34.92	39.63	7.53	5.33	14.02
T_5	30.72	32.58	6.95	4.28	11.82
T_6	33.81	38.57	7.21	4.70	12.95
T_7	29.56	33.46	6.79	4.05	11.23
T_8	35.64	40.78	7.86	5.72	14.69
T_9	31.62	36.38	7.02	4.50	12.32
T_{10}	32.74	37.45	7.38	5.01	13.45
SEd	0.51	0.52	0.12	0.13	0.31
CD (p=0.05)	1.08	1.16	0.27	0.30	0.63

Table 2. Effect of foliar nutrients on the yield attributes and yield of blackgram

Treatment	No. of pods/ plant	No. of seeds/pod	Test weight (g)	Seed yield (kg/ha)	Haulm yield (kg/ha)
T_1	9.08	5.84	3.72	767	1462
T_2	10.18	6.08	3.73	803	1587
T_3	10.97	6.15	3.75	879	1632
T_4	13.88	7.02	3.78	1201	1895
T_5	11.30	6.14	3.76	925	1739
T_6	12.80	6.79	3.77	1148	1830
T_7	11.16	6.49	3.77	842	1630
T_8	14.38	7.27	3.79	1247	1975
T_9	12.10	6.54	3.79	960	1800
T_{10}	13.58	6.76	3.76	1004	1845
SEd	0.22	0.11	0.02	21.03	23.89
CD (p=0.05)	0.46	0.24	NS	43.80	74.65

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PERFORMANCE OF SAMAI BASED CROPPING SYSTEMS UNDER RAINFED CONDITIONS FOR SUSTAINABLE AGRICULTURE

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Abstract

Field experiment was conducted to investigate the relative performance and the effects of redgram intercropping system on productivity of samai with three different row ratios (4:1 , 6:2 and 8:2) during kharif seasons 2018 at the Centre of Excellence in Millets, Athiyandal, Dry Land Agricultural Research Station - Chettinad and Regional Research Station – Paiyur. Among the intercropping system, Samai+Redgram- horsegram (8:2) or Samai +Redgram- mothbean (8:2) sequence produced plant height, DMP, productive tillers / plant, thousand grain weight, grain yield, stover yield and little millet grain equivalent yield (LMGEY). Sequential crop (horsegram and mothbean) yield was also highly influenced by redgram intercropping under system. The highest gross returns (Rs. 53798/ ha), net returns (Rs. 28393/ ha) and benefit cost ratio (2.12) were recorded by samai intercropped with pigeonpea at 8:2 ratio with Succeeding crop of horsegram/ mothbean as sequence crop.

Key words: Samai, Intercropping, Yield, SGEY and Economics

Introduction

Littlemillet (*Panicum sumatrense*) crop is well known in Tamil Nadu and grown quite extensively in many parts of the country. Its colloquial names are kutki, samai, samalu etc., The crop is strongly associated with tribal agriculture and grown as an important catch crop in view of its earliness and resistance to adverse agro climatic conditions. Intercropping is an age old practice being followed by subsistence farmers to achieve their domestic needs.

The main advantage of the intercropping is that the component crops are able to use the growth resources differently and make better overall use of growth resources than grown separately (Willey, 1979). It was considered as part of holistic farming designed to meet diverse domestic requirement. It is now generally perceived that the main advantage of intercropping is stability and risk aversion. Among food and feed crops, millets as a group figure prominently especially in the dryland farming system. Small millets are often grown mixed with legumes/pulses viz., pigeonpea, dolichos, green gram and black gram and oil seeds like niger, mustard and castor.

In intercropping system, the competitive effects between main and intercrop depends on the rooting pattern, canopy structure and days to maturity. The intercropping system of cereals + pigeonpea/legumes were tested and found to be profitable systems (Patil, 2003).

Materials and Methods

A field experiment was conducted at Centre of Excellence in Millets, Athiyandal, Tiruvannamalai , Dry Land Agricultural Research Station - Chettinad and Regional Research Station – Paiyur district during kharif, 2016, 2017 and 2018, . The soil of the experimental field was sandy clay loam in texture of three centres. The experiment was comprised of 9 treatments, viz., T_1-Samai +Redgram (4:1)- Moth bean , T_2-Samai +Redgram (4:1) – Blackgram , T_3-Samai +Redgram (4:1) – Horsegram , T_4-Samai +Redgram (6:2) – Moth bean , T_5- Samai +Redgram (6:2)-Blackgram , T_6- Samai +Redgram (6:2) – Horsegram, T_7-Samai +Redgram (8:2) – Moth bean , T_8-Samai +Redgram (8:2) – Blackgram , T_9-Samai +Redgram (8:2) – Horsegram .The experimental was laid out in randomized block design with three replications, the little millet variety Co (Samai) 4, was sown with Pigeonpea (Co (Rg) 7), moth bean (TMV (Mb) 1C) followed by sequential crops of moth bean TMV (Mb)1, horse gram (Paiyur2) and Black gram (T9).

Basal application of 44:22:0 kg NPK / ha was given for base crop of little millet uniformly to all the plots at the time of sowing and no additional dose of fertilizers was used for intercrops. For comparison between treatments, the yields of all intercrops were converted into little millet equivalent yield on price basis.

Results and Discussion

Growth and yield attributes

Pooled mean analysis of three centre data of CEM, Athiyandal, DARS Chettinad and RRS,Paiyur during kharif, 2016, 2017 and 2018 were given below. Growth attributes like plant height and dry matter production was significantly affected by intercropping. Plant height of samai was found to be higher at all the stages under the treatment, little millet + pigeonpea - mothbean at 8:2 ratio (T_7) (96.6 cm at harvest) followed by littlemillet + pigeonpea - horsegram at 8:2 ratio (T_9) (94.2 cm at harvest) (Table. 1). Similar results were also obtained by Sharma (2017) in wheat based intercropping system. The yielding ability of a crop is reflected through its yield attributing characters. The yield attributes of littlemillet like number of productive tillers per hill,panicle height ,pani and test weight is found to be increased when intercropped with pigeonpea at 8:2 ratio (Table.1).This might be due to development of better complementary relationship and non-renewable resources like water, nutrients and incoming sunlight. Tripathi and Kushwaha (2013) also reported that plant height and number of leaves per plant of pearl millet under intercropping system were either higher or statistically at par with sole pearl millet, which might be due to

better utilization of space and light interception coupled with nutrient contribution of leguminous crop to cereal crop.

Yield

The grain yield of little millet was significantly influenced by various intercrops at harvest and the grain yield ranged from 803 to 1602 kg / ha (Table 2). The highest grain and straw yields were recorded little millet + pigeonpea - Blackgram at 8:2 ratio (T8) (652 kg / ha grain yield and 1676 kg / ha straw yield, respectively) and it was on par with littlemillet + pigeonpea - horsegram at 8:2 ratio (T9) (648 kg / ha grain yield and 1652 kg / ha straw yield, respectively). Higher grain yield of pigeonpea in 8:2 row ratios could be attributed to higher yield attributes and least competition due to better planting arrangement. These results are in close conformity with the findings of Rathore and Gautam (2003) revealed significant increase in yield components when foxtail millet was intercropped with pigeonpea at 5:1 ratio as compared to 1:1 row ratio. Succeeding crop of Horsegram yield were significantly higher at samai + Redgram at 8 : 2 ratio was on par with mothbean sequence relayed in samai + redgram 8:2 row ratios. Similar finding was reported by Kumar et al., (2008). The highest little millet grain equivalent yield (1516 kg ha) was recorded in 8:2 row ratio of samai+ redgram-horsegram sequence which was closely followed by 8:2 row proportion of little millet + pigeonpea - mothbean sequence (1501kg / ha). Ansari et al., (2011) reported that pearl millet intercropped with pigeonpea recorded significantly higher pearl millet equivalent yield as compared to sole stand of component crops. It was due to almost similar yield of intercropped pearl millet as that of its sole stand and additional yield of pigeonpea as a bonus in intercropping system. Kumar et al., (2008) reported that the higher little millet grain equivalent yield in 8:2 row ratio and horsegram sequence was due to higher yield of samai and redgram coupled with better utilization of the natural resources by the component crops in intercropping system.

Economics of intercropping

The highest gross returns (Rs. 53798/ ha), net returns (Rs. 28393/ ha) and benefit cost ratio (2.12) were recorded by samai intercropped with pigeonpea at 8:2 ratio with horsegram as sequence crop (Table 2). Samai intercropped with redgram at 8:2 ratio with moth bean as sequence crop was found to be the second best. According to Seran and Brintha (2009) the intercropping system provides higher cash return to smallholder farmers than growing the monocrops. Based on these results, it may be summarised that to increase the productivity per unit area in littlemillet intercropping system under rainfed conditions of Tiruvannamalai district, growing of samai and pigeonpea in 8:2 row ratio with horsegram or mothbean in sequence have been found superior over other intercropping systems under rainfed conditions.

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EFFECT OF INTEGRATED USE OF ORGANIC MANURES AND MICROBIAL NUTRIENT SPRAY ON GRAIN YIELD OF ORGANIC RICE (*ORYZA SATIVA* L.)

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Abstract

A field experiment was carried out in Northern Block farm of Agricultural Research Station, Bhavanisagar, Tamil Nadu Agricultural University during Summer 2021 with rice variety of medium duration *viz*., Improved White Ponni to study the effect of organic manure and microbial nutrient spray on yield attributes and yield of organic rice. The experiment was deliberated in Factorial Randomized Block Design (FRBD) with three replications. The experiment comprised of two factors, factor I consists of organic manures with 6 levels *viz*., Farm Yard Manure (OM_1), Enriched Farm Yard Manure (OM_2), Vermicompost (OM_3), Composted Poultry Manure (OM_4), Neem oil cake (OM_5) against absolute control (OM_6). Factor II consists of microbial nutrient spray with 3 levels *viz*., 3% Panchagavya (MNS_1), 1% Pink Pigmented Facultative Methylotrophs (MNS_2) against water spray (MNS_3). From the results, vermicompost @ 2.08 t ha^{-1} + 3% Panchagavya spray (OM_3 + MNS_1) recorded higher grain and straw yield (5715 and 9144 kg ha^{-1}, respectively).

Keywords: Organic Rice, Organic Manure, Microbial Nutrient Spray, Grain Yield, Straw Yield

Introduction

Rice (*Oryza sativa* L.) is life for almost half of the global population and majority (60%) of Indian population. It is grown in more than 100 countries, especially in Asia and 90% rice is produced and consumed by Asian countries. In world, India and China accounts for half of the total area under rice cultivation. Among the rice growing countries, India ranks first in area (44.4 M ha) and second in production (121 MT), next only to China. However, the average productivity of rice in India is only 4.1 t/ha against the global average of 4.67 t/ha (FAS, 2021). Organic farming in recent years is gaining momentum due to realization of inherent advantages. It confers in sustaining crop production and also in maintaining dynamic soil nutrient status and safe environment (Lokanath and Parameshwarappa, 2006). In world, organic agriculture is practised in 69.8 M ha. In India, total area under organic certification process is 3.56 M ha (FiBL, 2019). Balanced use of nutrients through organic sources like FYM, vermicompost, green manuring, neem cake and biofertilizers are prerequisites to sustain soil fertility, to produce maximum crop yield with optimum input level. In the view of above facts, field experiment on "Effect of Integrated Use of Organic Manures and Microbial Nutrient Spray on Grain Yield of Rice" was conducted. To study the effect of organic manures and microbial nutrient spray on yield attributes and yield of organic rice.

Materials and Methods

The field experiments were directed during summer 2021 in Northern Block Farm of Agricultural Research Station (ARS) Bhavanisagar, Tamil Nadu Agricultural University. The field experiment was deliberated in Factorial Randomized Block Design (FRBD) with three replications. The experiment comprised of two factor, Factor I contained 6 treatments viz., Farm Yard Manure (FYM), Enriched Farm Yard Manure (EFYM), vermicompost, composted poultry manure, neem oil cake and absolute control. The Factor II contained 3 treatments *viz*., 3% panchagavya, 1% Pink Pigmented Facultative Methylotrophs (PPFM) and water spray. Based on equal N basis, required quantity of organic manures were calculated and incorporated in the soil before transplanting of rice. The microbial nutrient spray of 3% panchagavya sprayed on 15, 30 and 45 DAT and 1% PPFM sprayed on 30 and 45 DAT of rice. The organic farming responsive variety, Improved White Ponni was raised. Transplanting was done in the main field with a spacing of 20×15 cm at two seedlings/hill. Other management practices were adopted as per the recommendation of the crops. The enriched farm yard manure compost is prepared by using 10 kg of rock phosphate, 2 kg of biomineralizer and 10 kg of each biofertilizers *viz*., Azospirillum, Azotobacter and Phosphobacteria were thoroughly mixed with one ton of well decomposed and powdered FYM on dry weight basis and made into a heap like structure. Biomineralizer is used to accelerate the decomposition rate. Periodical watering should be done once in 2 days and turning should be given on 15th day of composting. The heap was kept for 30 days for composting under the shade with 60 per cent moisture. For composted poultry manure, a known quantity of fresh poultry droppings is to be collected and mixed thoroughly with chopped rice straw. The moisture content to the heap should be maintained at 50-60%. Periodical watering should be done once in 2 days and turning should be given on 15th day of composting, within a period of 30 days, materials are converted to mature compost. Grain and straw yield were recorded by following standard procedure. The data on various characters studied during the course of examination were statistically analysed for factorial randomized block design.

Result and Discussion

Productive tillers

Application of different source of organic manure, microbial nutrient spray and their interaction significantly influenced the number of productive tillers per m^2 . The treatment with application of vermicompost @ 2.08 t/ha (OM3) recorded more number of productive tillers per m^2 (380) and was statistically superior over with

other treatments *viz*., OM5 and OM4 (Table 1). Significantly lower number of productive tillers per m2 (286) were recorded for control. Application of 3% panchagavya showed more number of productive tillers per m^2 (365) than PPFM and water spray. It was significantly influenced by the interaction of organic manure and microbial nutrient spray.

Test weight

Organic manure influenced the test weight significantly over control. Maximum 1000 grain weight was recorded (19.6 g) for the treatment in which vermicompost @ 2.08 t/ha (OM3) was applied. Significantly lower 1000 grain weight was produced by control.

Grain yield

Grain yield was significantly differed among the organic manures and microbial nutrient spray while their interaction were remained non significant (Table 2). Significantly higher grain yield (5236 kg/ha) was obtained in plots where vermicompost @ 2.08 t/ha (OM3) was applied. The next in order was application of neem oil cake @ 1.2 t/ha (OM5) and composted poultry manure @ 3.47 t/ha (OM4) (Table 4). Significantly lower grain yield (2814 kg/ha) was noted for absolute control (OM6). While the application of microbial nutrient spray, 3% panchagavya recorded highest grain yield (5008 kg/ha) against water spray. Combined application of Vermicompost @ 2.08 t/ha + 3% Panchagavya spray (OM_3 + MNS_1) recorded higher grain yield (5715 kg ha^{-1}). Vermicompost contains high level of plant growth hormones and soil enzymes, while enhancing the microbial population in soil and retaining its nutrients over a longer period of time without having an adverse effect on environment. The highest grain yield might be due to the availability of all essential nutrient in soil enriched with vermicompost. Application of NPK 50% + vermicompost + panchagavya 3% + jeevamrutha 5% gave the significantly higher grain yield in rice by Shardha and Sujathamma (2018). These findings also supported by Amitava *et al.* (2008) who reported that treatment with vermicompost imparted maximum grain yield compared to all other treatment. The results are in line with the observations of Sailajakumari and Ushakumari (2002) and Vasanthi and Kumarasami (1999) who found increased rice yield after treatment with vermicompost plus NPK and enhanced nutrient uptake and yield by cowpea after application of vermicompost enriched rock phosphate. Jeyabal and Kuppuswamy (2001) reported that integrated nutrition comprising vermicompost, fertilizer N and biofertilizers could be applied to rice – legume cropping system to achieve higher yields and sustain soil health. Su lin lim *et al*. (2014) found that vermicompost should be applied at moderate concentrations in order to obtain maximum plant yield.

Straw yield

There was significant difference in the straw yield among the organic manure, microbial nutrient spray and their interaction remained non significant. Significantly higher straw yield (8640 kg/ha) was obtained in plots where vermicompost @ 2.08 t/ha (OM3) was applied. The next in order was application of neem oil cake @1.2 t/ha (OM5) (Table 4). Significantly lower straw yield (5105 kg/ha) recorded for control. Application of 3% panchakavya recorded higher straw yield (8016 kg/ha) than PPFM and water spray. Combined application of vermicompost @ 2.08 t ha^{-1} + 3% Panchagavya spray (OM_3 + MNS_1) recorded higher straw yield (9144 kg ha^{-1}). Vermicompost has nitrogen, potassium, phosphorus and other micro and macronutrients, so that the plants are fed continuously. This also improves the fertility of soil in which crops are planted. The higher the straw yield was might be due to continuous availability of macro and micronutrients. Shardha and Sujathamma (2018) reviewed that application of 50% NPK + vermicompost + 3% panchagavya + 5% jeevamrutha recorded significantly higher straw yield. Suchitra Rakesh *et al*. (2017) showed that increase in growth and yield parameter at 3% foliar spray of panchagavya. Application of panchagavya enhance the biological efficiency and it ultimately Increased yield of crop plants by Natarajan (2002).

Conclusion

The study could be concluded that the combined application of vermicompost on equal N basis (2.08 t/ha) + microbial nutrient spray 3% panchagavya recorded higher yield attributes such as number of productive tillers per hill, grain yield and straw yield of organic rice.

Table 1. Effect of organic manure and microbial nutrient spray on number of productive tillers and test weight of organic rice

Treatment	Productive tillers m^{-2}				Test weight (g)			
	MNS_1	MNS_2	MNS_3	Mean	MNS_1	MNS_2	MNS_3	Mean
OM_1- FYM	356	327	325	336	19.2	18.4	18.3	18.6
OM_2 – EFYM	361	342	321	341	19.6	18.6	18.1	18.8
OM_3 - Vermicompost	398	385	358	380	20.1	20.0	18.7	19.6
OM_4 – Composted PM	359	356	327	347	19.8	19.5	18.8	19.4
OM_5 – Neem oil cake	416	355	316	362	20.5	18.9	19.1	19.5
OM_6 – Absolute control	305	294	261	286	18.1	18.2	17.5	17.9
Mean	365	343	318	342	19.5	18.9	18.4	19.0
Factor	OM	MNS	OM × MNS		OM	MNS	OM × MNS	
SEd	5.4	3.8	9.3		0.50	0.35	0.87	
CD (P=0.05)	11	7.7	19		1.02	0.72	NS	

Table 2. Effect of organic manure and microbial nutrient spray on grain yield and straw yield of organic rice

Treatment	Grain yield (kg/ ha)				Straw yield (kg/h)			
	MNS_1	MNS_2	MNS_3	Mean	MNS_1	MNS_2	MNS_3	Mean
OM_1- FYM	5016	4620	4135	4590	8035	7531	7094	7553
OM_2 – EFYM	5086	4621	4165	4624	8064	7534	7193	7596
OM_3 – Vermicompost	5715	5230	4765	5236	9144	8643	8135	8640
OM_4 – Composted PM	5385	4827	4363	4858	8894	8013	7427	8111
OM_5 – Neem oil cake	5598	5056	4635	5096	8543	8173	7645	8120
OM_6 – Absolute control	3250	2835	2357	2814	5417	5175	4723	5105
Mean	5008	4531	4070	4536	8016	7511	7036	7521
Factor	OM	MNS	OM × MNS		OM	MNS	OM × MNS	
SEd	169	119	293		239	169	415	
CD (P=0.05)	344	243	NS		487	344	NS	

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IMPACT OF GREENGRAM CO 8 IN YIELD ENHANCEMENT AND CHANGING THE SOCIO ECONOMIC STATUS OF FARMERS IN TIRUVALLUR DISTRICT

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Abstract

Greengram is an important pulse crop cultivated in Tiruvallur District mainly during Rabi (Dec-Jan) season. It is an important crop in the cropping pattern Rice-Rice-Greengram and enhances the food and livelihood security of the farmers. To improve the productivity from the existing level of 850kg /ha, the new high yielding short duration variety CO 8 was introduced in Tiruvallur District through Front Line Demonstrations and Cluster Front Line Demonstrations during the years 2014-15, 2016-17 and 2017-18 in Ellapuram, Kadambattur, Ekkadu, Sholavaram and Pallipet blocks. Awareness on Integrated Crop Mangagement Practices was created through trainings, method demonstrations and technical extension materials among the farmers. The average productivity of the demo variety CO 8 across demonstrations was 11.39q/ha which is significantly higher than the check variety KM 2 by 24.07%. The Net return and BCR was also found to be Rs. 56437 and 3.8 respectively with the demo variety CO 8. The variety was found to be resistant to Yellow Mosaic Virus and fetches good market price. The variety was very much acceptable by the farmers because of its synchronous maturity and non shattering nature.

Key words: Greengram, Front Line Demonstration, IPM, ICM, Tiruvallur

Introduction

Pulses are important food crops owing to their high protein (20 to 25 per cent) and carbohydrate content (55 to 60 per cent). Pulses can contribute significantly in achieving the twin objectives of increasing productivity as well as improving the sustainability of the rice and wheat based cropping system (Yadav *et al*. 1998). India shares 35% of the World Pulse area (281.70 lakh ha) and 25% of the World Pulse production (183.11 lakh tonnes) (Tiwari and Shivhare, 2016**)**. However India has to import 3-4 lakh tonnes of pulses annually to meet out the domestic requirement. With the area under pulses cultivation in India stagnating from 1950s and showing least signs of improvement, there is a vast scope for improvement of pulse production by way of improving its productivity. There are more constraints of improving the pulse production in India and few of them may be attributed to non availability of improved variety seeds, lack of knowledge on improved package of practices, input use *i.e*., lack of fertilizers / minerals use, lack of irrigation facilities. Since Pulses are mainly grown under rainfed conditions, varieties with-better yield advantage and desirable characteristics to suit the varied agro-climatic conditions need to be developed in pulses (Prem Narayan and Sandeep Kumar, 2015).

The study by Ali and Gupta, 2012 indicated that the adoption of improved technologies can increase pulse production by at least 13–42 per cent in the country. Improved varieties of different pulse crops hold promise to increase productivity by 20-25 per cent, whereas package technology comprising improved varieties and integrated management of nutrients and pests have shown 25-42 per cent yield advantage over the farmers' practices in a large number of frontline demonstrations conducted across the country.

Tamil Nadu shares 3% (7.54 lakh ha and 4.9 lakh tonnes) to India's pulse area and production with a productivity of 650kg/ha. Greengram is cultivated in an area of 1.82 lakh ha with the production of 1.23 lakh tones and productivity of 675kg/ha (Tiwari and Shivhare, 2016**)**. Tiruvallur is one of the potential greengram cultivating Districts in Tamil Nadu with an area of 9188ha mainly during Rabi (Dec-Jan) season in the cropping pattern Rice-Rice-Greengram. The average productivity of this crop is 850kg /ha in Tiruvallur. KM 2 is the popularly cultivated variety in the District. This is a very old variety released 20 years back with indeterminate growth habit and needs several picking. This variety is also susceptible to pod borer leading to yield losses upto 25% and is shattering in nature at the time of maturity leading to additional yield losses. Moreover acute labour scarcity is noticed in Tiruvallur District which is adjacent to the Metropolitan city Chennai. Hence a variety suitable for mechanized harvesting and synchronous maturity is very much needed at this point of time to sustain farmers in Greengram cultivation.

With a vision of improving the greengram production and productivity in Tiruvallur District, the new variety CO 8 released from TNAU during 2013 as a replacement to KM 2 has been promoted among the farmers. It is a short duration variety maturing in 55-60 days, with determinate growth habit, synchronous maturity, non shattering nature and therefore is highly suitable for mechanized harvesting. This is also tolerant to Yellow Mosaic Virus and Pod borer. Hence the variety CO 8 was introduced among the farmers of Tiruvallur District through Front Line Demonstrations in the years 2014-15 & 2017-18 and Cluster Front Line Demonstrations in 2016-17 & 2017-18 with the objective of replacing the low yielding older variety.

Materials and Methods

Front Line Demonstrations in Greengram CO 8 was conducted by Krishi Vigyan Kendra, Tirur in Ellapuram, Kadambattur, Ekkadu, Sholavaram and Pallipet blocks of Tiruvallur District during 2014-15 (10 demos) and 2017-

18 (10 demos) and Cluster Front Line Demonstrations during 2016-17 & 2017-18 (50 demos each). Awareness was created among the farmers on seed treatment, pulse wonder spray, use of yellow sticky trap and pheromone traps, Seed Production techniques, ICM and IPM practices through trainings, method demonstrations and field days. Technical handouts on ICM and IPM in greengram were also provided to the farmers during trainings.

For seed production, Foundation seeds of CO 8 was procured and distributed from the Department of Agriculture, Tiruvallur. Seeds produced by the farmers were procured as certified seeds by the Dept. of Agriculture. Farmers also provided seeds to fellow farmers.

Technologies Demonstrated

Demonstration on seed treatment with Imidacloprid @5ml/kg of seeds for control of Yellow Mosaic Virus; seed treatment of *Trichoderma viride* @ 4g/kg or *Pseudomonas fluorescence* @ 10g/kg of seeds for control of root rot, growing of sorghum in the borders as trap crop, installation of yellow sticky trap @ 12/ha for controlling and monitoring white flies and pheromone traps @ 12/ha, spraying of Pulse wonder @5kg/ha during 30th and 45th DAS for improving flower formation, flower retention and pod set, spraying of neem oil 30 ml/lit and spraying of thiamethoxam @ 0.4 g/lit to control sucking pests of green gram were done by KVK, Tirur to the farmers of various blocks.

Results and Discussion

Economic gains

The economic gains obtained by the farmers in various demonstrations were given in Table 1. The average yield and productivity of the demo variety CO 8 was 11.39q/ha which is significantly higher than the check variety KM 2 by 24.07%. The Net return and BCR was also found to be on the higher scale *viz*., Rs. 56437 (47% higher than the check) and 3.78 (50% higher) respectively. The varietal comparison in terms of yield, Net Returns and BCR is expressed in Fig. 1. The demo variety Co 8 has shown consistent performance across demonstrations and over years. During 2014-15, CO 8 registered yield of 11.24 q/ha which was 26.3% increase over the check variety KM 2 (8.90q/ha). During 2016-17, it recorded 11.23q/ha as against the check variety showing 9.20q/ha. During 2017-18, it has shown 26.10% and 21.80% yield advantage over the check variety in FLD (11.45 q/ha) and CFLD (11.62 q/ha) respectively. The net income and BCR ratio were also on higher scale during 2014-15 (Rs. 69885 and 3.9 respectively); 2016-17 (Rs. 50386 and 3.86 respectively) and 2017-18 (Rs. 59175 and 4.3 in FLD and 46300 and 3.06 in CFLD respectively)

CO 8 is a synchronous maturing variety and hence can be harvested in a single time. Hence the labour requirement is less for this variety compared to the traditional variety which requires 2-3 pickings periodically. Reducing the labour requirement by way of synthesizing new varieties with synchronous maturity amenable for mechanized harvesting has been the research priority in green gram over years. This objective is now accomplished by way of this variety. This variety is also non shattering in nature. Owing to this character the yield loss at the time of harvest is minimized and this is reflected in the improved productivity of this variety. Moreover it is resistant to pod borer and yellow mosaic virus which are the major pest and diseases hampering the yield and quality of the produce. The reduction in cost of cultivation may be attributed to the less no. of sprays for pest and disease control. The marketability of this variety was also found be good. By seed production, this variety fetches Rs. 63 /kg and as grain it fetches Rs. 52/kg.

Hence, Greengram variety CO 8 can be recommended among the farmers of Tiruvallur District for further spread. The average productivity of Tiruvallur District got improved form 800kg/ha to 850kg/ha in the past two years as this variety is spread over 30 % greengram growing area in Tiruvallur District.

Success Story of a Farmer

Mr. Harikrishnan, a progressive farmer from Perambakkam village of Tiruvallur District has achieved 526kg (1315kg/ha) of seed yield by cultivating CO 8 in one acre of his farm during Rabi, 2017-18. This was the highest yield obtained during 2017-18 among the 50 one acre cluster front line demonstrations conducted in Tiruvallur District. He raised a certified seed production farm out of the 8kg Foundation seed received from KVK, Tirur. He adopted all the package of practices and got a gross income of Rs.33138/-. The Dept. of Agriculture procured the certified seeds from the farmer @ Rs.63/kg and he obtained net income of Rs. 23354 and BCR of 3.37 (Table 2.).

Conclusion:

The greengram variety CO 8 was found to be the best alternate to KM 2 and horizontal spread of this variety during 2021-22 was found to be 3200ha during Rabi season in Tiruvallur District. In coming years more horizontal spread is expected in Tiruvallur District.

Impact:

1. CFLD and FLD on Greengram CO (Gg) 8 resulted in high yield and profit. BCR is on an average of 3.78 in CO (Gg) 8 which is 50% higher than the check.
2. The new variety is resistant to yellow mosaic virus and pod borer.
3. Marketability of the produce is good.
4. The variety is non shattering in nature and also has synchronous maturity

Table 1. Economic Gains obtained from Green gram CO 8 by the farmers in various Demonstrations

Parameters	FLD (2014-15)		FLD (2017-18)		CFLD (2016-17)		CFLD (2017-18)	
	Check	Demo	Check	Demo	Check	Demo	Check	Demo
Yield (q/ha)	8.90	11.24	9.08	11.45	9.20	11.23	9.54	11.62
Yield increase (%)	-	26.3	-	26.10	-	18.07	-	21.80
Cost of cultivation (Rs.)	18055	17785	22250	15250	18660	17450	24,200	22,500
Gross income (Rs.)	69240	87860	59020	74425	55320	67836	52,470	68,800
Net income (Rs.)	51365	69885	36770	59175	36660	50386	28,270	46,300
BCR	2.84	3.9	2.12	4.3	2.95	3.86	2.17	3.06

Table 2. Cost of Cultivation and Net Returns obtained by Th. Harikrishnan of Perambakkam Village, Thiruvallur District.

Particulars of Cost and Income in one acre	Amount (Rs.)
Land preparation	2000
Seed Cost	1024
Sowing	200
Seed treatment	60
Fertilizer	900
Weed control	700
Pulse wonder/ DAP spray	600
Pest and Disease Management	300
Harvesting and Processing	4500
Gross Cost	9784
Gross Income	33138
Net Income	23354
Benefit Cost Ratio	3.37

Fig 1. Comparison of Yield, Net Returns and BCR of the Demo (CO 8) and Check (KM 2) variety over years and across Demonstrations

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IDENTIFICATION OF ROOT KNOT NEMATODE, *M. graminicola* RACES INFECTING RICE IN TAMIL NADU

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Abstract

The rice root-knot nematode, *Meloidogyne graminicola* belongs to the family Heteroderidae and is one of the most economically important nematodes affecting rice. It has been reported to cause significant yield losses of 20-50 per cent**.** The root knot nematode, *M. graminicola* infecting rice in Tamil Nadu was identified as race 'b' by North Carolina host differential test. The results of North Carolina host differential test showed that *M. graminicola* reproduced on both rice and wheat. But the rate of reproduction was highest in rice compared to wheat. Number of second stage juvenile (J_2) per gram of root in rice was recorded as 992.69 compared to wheat (409.92). Similarly number of galls on rice roots were significantly higher (72.69) compared to wheat (37.62). Number and size of galls were bigger in case of rice compared to wheat.

Introduction

The rice root-knot nematode, *M. graminicola* belongs to the family Heteroderidae and is one of the most economically important nematodes affecting rice. It has been reported to cause significant yield losses of 20-50 per cent in many regions of rice production. *M. graminicola*, like other root-knot nematodes causes swellings and galls in the root systems. Infected rice root tips show swollen and hooked like symptoms. The nematode can retard plant growth and cause unfilled spikelets, reduced tiller development, chlorosis and wilting symptoms under upland and intermittently flooded conditions.

M. graminicola has a wide host range (Ou, 1972) that includes many of the common weeds of rice fields and can also be damaging to agricultural crops that are grown in rotation with rice, onion, cabbage and tomato. The recent adoption of labour and water conserving practices eg. direct seeding, aerobic rice etc. increase the pest potential of *M. graminicola*. The objective of this study is to identify races of root knot nematode, *M. graminicola* isolates infecting rice in Tamil Nadu by North Carolina host differential test.

Materials and Methods

The North Carolina host differential test (Pokharel *et al.,* 2010) was conducted by using rice cv. Co 43 and wheat cv. CoW 1 as differential hosts. The seeds of these plants were grown in 5 kg capacity pots filled with sterilized soil. Similarly, rice cv. Co 43 and wheat cv. CoW 1 were inoculated with *M. graminicola.* All plants were inoculated at the rate of $2IJ_2$ /g soil in a glasshouse and replicated 13 times. The experiment was terminated 75 days after planting and the experiment was repeated once. At the end of the experiment the following parameters *viz*., shoot length, root length, shoot weight, root weight, number of J_2, number of females, number of egg masses, number of J_2 in soil, number of galls and number of *M. graminicola* eggs/root system were recorded.

Results

The host differential test to identify the races of *M. graminicola* was made under glasshouse. The root knot nematode, *M. graminicola* infecting rice in Tamil Nadu was identified as race 'b' by North Carolina host differential test. The results of North Carolina host differential test showed that *M. graminicola* reproduced on both rice and wheat. But the rate of reproduction was highest in rice compared to wheat. Number of J_2 / g of root in rice were 992.69 compared to wheat (409.92).

The observation on number of root knot females revealed that rice (36.30) was highly preferred by *M. graminicola* than wheat (27.77). In rice, number of egg masses and eggs (59.92, 2162.23) were significantly higher compared to wheat (31.46, 933.46). Soil population in rice and wheat were 821.54 and 619.69 respectively. Similarly number of galls on rice roots were significantly higher (72.69) compared to wheat (37.62) (Table 1 and Fig. 1).

Discussion

The root knot nematode, *M. graminicola* infecting rice in Tamil Nadu was identified as race b based on North Carolina host differential test. Pokharel *et al*., (2010) reported that race 'a' reproduced only in wheat and race 'b' reproduced in both wheat and rice which confirmed the existence of atleast two races in this species. In the present investigation, *M. graminicola* reproduced on both wheat and rice. But the rate of reproduction was highest in rice compared to wheat. It was confirmed with previous reports by Pokharel *et al*. (2012) who reported that *M. graminicola* reproduced six times more on rice than wheat.

Higher reproductive factor of *M. graminicola* was observed on rice than wheat indicating higher rate of reproduction of this nematode in rice. The higher reproduction of the nematode might be due to the genetic make-up of the plants and or the available root biomasses for nematode growth and reproduction (Pokharel *et al.*, 2011). Rice has a greater root mass than wheat, thereby supporting higher nematode reproduction (Gaur and Sharma, 1999).

Number and size of galls were bigger in case of rice compared to wheat (Taya and Dabur, 2004). These findings were in line with the observations of the present study. Large rooted plants will allow more nematode reproduction and tolerate more damage than small rooted plants despite the latter having fewer invasion sites for the

nematode (Elkins *et al*., 1979). Results of the variety x isolate interaction in rice and wheat (Pokharel *et al*., 2005) provided further support to the hypothesis that genetic make-up of plants plays a greater role in the reproduction of *M. graminicola*

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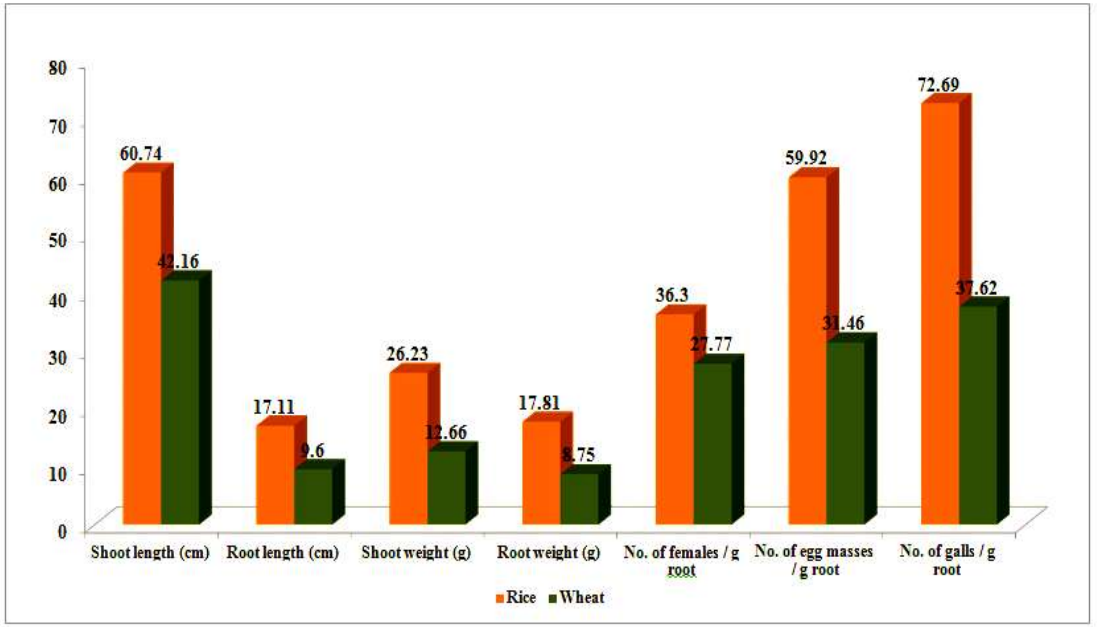
Table 1. Host differential test for identification of *M. graminicola* races

Differential hosts	Shoot length (cm)	Root length (cm)	Shoot weight (g)	Root weight (g)	No. of J2 / g root	No. of females /g root	No. of egg masses / g root	No. of eggs / g root	No. of J2 / 250 cc soil	No. of galls / g root
Rice	60.74	17.11	26.23	17.81	992.69	36.30	59.92	2162.23	821.54	72.69
Wheat	42.16	9.60	12.66	8.75	409.92	27.77	31.46	933.46	619.69	37.62
t - value	9.38	3.92	5.01	2.67	15.54	2.15	9.07	4.81	5.69	13.91

Fig. 1 Identification of *M. graminicola* races by North Carolina host differential test



HISTOLOGICAL CHANGES CAUSED BY RICE ROOT KNOT NEMATODE, *MELOIDOGYNE GRAMINICOLA* IN RICE

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Abstract

Rice root-knot nematode (*Meloidogyne graminicola*) has emerged as a major threat throughout the world and it is a major constraint in successful rice cultivation leading to significant loss to the rice grower. The present study was carried to know the histological changes caused by *M. graminicola*. The results revealed that the rice plant infested with *M. graminicola* showed presence of multinucleate giant cells with dense cytoplasm.

Keywords: Histological changes, *Meloidogyne graminicola*, Rice, Giant cells

Introduction

Meloidogyne graminicola commonly named as rice root knot nematode is considered as one of the most important damaging parasites for upland, lowland and deep-water rice cultivation throughout the world, particularly in South and Southeast Asia. The second-stage juveniles (J2) of rice root-knot nematodes penetrate the roots and migrate intercellularly thereby establish a feeding site in the zone of differentiation of the vascular cylinder and develop into sessile and swollen females. During feeding site formation some cells become hypertrophied, with intense cellular multiplication and hyperplasia leading to giant cells and gall formation (Williamson & Hussey, 1996). Compatibility between a host plant and a nematode during root penetration and feeding site formation is vital for the establishment of a successful host-parasite relationship. Better understanding of the host plant-parasite interactions is important in order to use in resistant breeding programmes. Hence, the present study was carried to know the histopathological changes caused by rice root knot nematode, *M. graminicola*.

Materials and methods

M. graminicola infested rice roots (variety Co 43) were collected from a pure culture maintained in the glasshouse, Department of Nematology, TNAU to obtain inoculum for this study. Three weeks old seedlings of the rice variety Co 47 (susceptible) were transplanted and inoculated with 500 eggs of *M. graminicola* extracted by Barker's technique (1985). One set of uninoculated rice plants was also maintained for comparison.

Segments of infested roots sampled 10, 20, 30 and 40 days after inoculation of the nematode were fixed in TAF (Triethanolamine - 2ml, Formalin - 7ml and distilled water - 9ml) at 70°C for 24 hours. The root segments were dehydrated through ethyl alcohol series and embedded in paraffin wax for microtomy sectioning. Fixed root segments were then sectioned at 10 - 15µm, stained in safranin and fast green on gelatine coated glass slides and mounted in Dummar xylene (Johansen, 1940). Root sections of healthy and infested rice were observed at 45 days after inoculation (DAI).

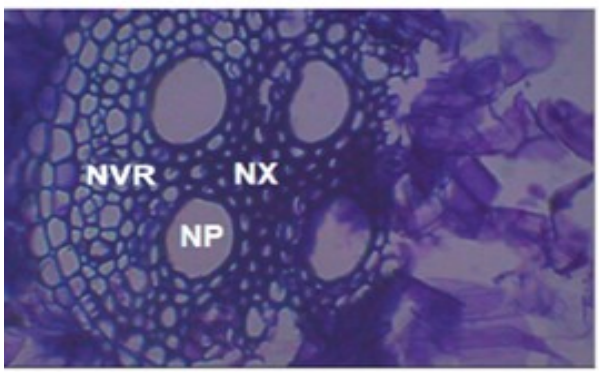
Results

Studies on histological changes caused by *M. graminicola* in rice revealed that the presence of giant cells, pre adult and several mature females were found in the vascular region. The formation of giant cells disrupts the vascular region. In uninoculated healthy plant, well defined vascular region was found (Fig. 1).

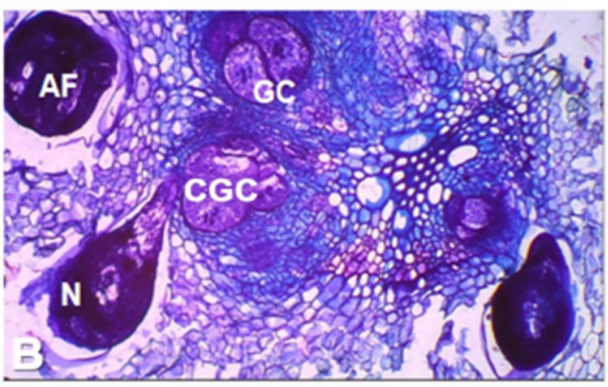
Discussion

The present finding supports the observation of Cabasan *et al*., 2012 that in maize and cotton genotypes infected with *M. incognita*, soybean with *M. arenaria*, potato with *M. fallax* and rice with *M. graminicola*. The feeding cells are rapidly turned into multinucleate giant cells which act as metabolic sinks and compete with rice (Singh *et al*., 2006)

Fig. 1. Histological changes caused by *M. graminicola* in Rice



Cross section of Healthy root



Cross section of *M. graminicola* infested root

NVR – Normal vascular region; NX – Normal Xylem; NP – Normal Pholem
AF – Adult Female; GC – Giant cell; CGC – Complex Giant cell; N – Nematode

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IMPACT OF RICE ROOT KNOT NEMATODE, *Meloidogyne graminicola* INFESTATION ON CATALASE ACTIVITY IN RICE

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Abstract

Root-knot nematode, *Meloidogyne graminicola* is capable of disturbing the host metabolism. In the present study, catalase activity was triggered in roots of susceptible varieties than resistant ones. Higher catalase activity was recorded in TN 1 (18.58) followed by Co 43 (18.44) and Co 47 (18.36). Among the resistant rice varieties, the catalase activity was ranged from 12.72 to 13.44 at 1 min interval. It was gradually decreased at 2, 3 and 4 min interval. Lowest catalase activity was observed in PY 1 (12.72) followed by Swarna (12.76).

Introduction

As sedentary endoparasites, rice root knot nematode enter into the root as second stage juveniles (J_2) and then migrate to developing vascular cells. After choosing their feeding site, J_2 stimulate the formation of the giant cells i.e large feeding cells that are formed by repeated nuclear division in the absence of cell division (Jones and Goto, 2011). The nematodes feed on these giant cells throughout their lives.

Production of Reactive Oxygen Species (ROS) is common in plants even under optimum growth conditions. Under biotic stress, enhanced generation of ROS disturbs the normal redox environment of plant cells and damages the cellular components causing cell dysfunction (Tewari et al., 2007). Therefore, one of the most rapid defense responses of plants against rice root knot nematode infection is the production of ROS (called oxidative burst) at the site of invasion (Nikoo *et al*., 2014).

Hydrogen peroxide is a common ROS involved in plant responses not only against nematodes but also against bacterial and fungal pathogen infections (Sahebani and Hadavi, 2009). Catalase (CAT) is one of the most important ROS – scavenging enzymes of plants (Demidchik, 2015). The CAT activity was used as indexes of the antioxidative defense.

The main physiological role of CAT is H_2O_2 degradation. Therefore, an inhibition of such activities enhances the cellular level of H_2O_2, which is presently recognized as a diffusible signal for gene activation in HR, as a trigger for hypersensitive cell death as well as a strong antimicrobial molecule (Levine *et al*., 1994).

Materials and Methods

Five hundred mg of leaf and root samples from resistant and susceptible varieties was weighed and macerated with 10 ml of phosphate buffer. The contents were centrifuged at 3000 rpm for 10 minutes. One ml of supernatant was taken in each 5 different beakers. To this, 5 ml of 1.5% sodium perborate and 1.5 of phosphate buffer was added. Finally 10 ml of 2 N sulphuric acid was added at 1 minute, 2 minutes, 3 minutes and 4 minutes interval in first four beakers after the enzyme extract was added. In the final beaker, 10 ml of sulphuric acid was added before addition of enzyme extract. The final beaker was kept as a blank for comparison. The contents in the beakers were titrated against 0.05 N KMnO4. The pink color was developed and persisted for 30 seconds was the end point. The volume of the consumed KMnO4 was noted. 1 ml of KMnO4 was equal to 0.85 µg of the H2O2. The activity of the enzyme was expressed as µg of H2O2 / g/ minute (Barber, 1980).

Results

The catalase activity in shoots and roots of resistant and susceptible rice varieties infested by *M. graminicola* were analysed at various time intervals. Generally catalase activity was highest in roots compared to shoots. The catalase activity in shoots of susceptible varieties infested by *M. graminicola* was found to be higher as a result of nematode infestation. The highest catalase activity in shoots was observed in TN 1 (8.57) at 1 min followed by Pusa Basmathi (8.52) and it was gradually decreased subsequently. Among the resistant rice varieties, the lowest catalase activity was recorded in PY 1 (5.52) followed by ADT 45 (6.36).

Catalase activity in rice varieties infested with *M. graminicola* was measured as changes in µg of H_2O_2 $g^{-1}min^{-1}$. It is clearly evident that catalase activity was triggered in roots of susceptible varieties than resistant ones. Higher catalase activity was recorded in TN 1 (18.58) followed by Co 43 (18.44) and Co 47 (18.36). Among the resistant rice varieties, the catalase activity was ranged from 12.72 to 13.44 at 1 min interval. It was gradually decreased at 2, 3 and 4 min interval. Lowest catalase activity was observed in PY 1 (12.72) followed by Swarna (12.76) (Table 1).

Discussion

Catalase is an active enzyme that specifically destroys H_2O_2 in cells. The high levels of H_2O_2 required for hypersensitivity response (HR) may be obtained by a strong inhibition of catalase, which must be specific to the tissues involved and limited temporarily.

The present investigation showed a decreased catalase activity recorded in resistant varieties and increased catalase activity in *M. graminicola* infested susceptible rice varieties. This is due to the nematode infected tissues of susceptible varieties maintaining a lower level of superoxide anion and lipid peroxidation. This reflects an efficient scavenger system to the site of superoxide production in susceptible plants. Thus,

increased activity of the susceptible varieties clearly indicates maximum increase of catalase activity in shoots and roots recorded 45 days after inoculation.

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Table 1. Impact of rice root knot nematode, *Meloidogyne graminicola* infestation on catalase activity

Catalase activity at different interval (µg of H_2O_2 / g / min)																	
Moderately resistant varieties	**Shoot**				**Root**				**Susceptible varieties**	**Shoot**				**Root**			
	1min	**2min**	**3min**	**4min**	**1min**	**2min**	**3min**	**4min**		**1min**	**2min**	**3min**	**4min**	**1min**	**2min**	**3min**	**4min**
ADT 41	6.86	4.86	3.40	1.84	13.22	11.82	10.08	5.30	Co 47	8.22	6.60	5.54	3.32	18.36	16.78	14.96	11.9
ADT 45	6.36	4.94	3.12	1.56	13.10	11.30	10.24	5.36	Pusa Basmathi	8.52	6.72	4.72	3.64	18.1	16.56	15.28	12.1
TRY 1	6.78	4.66	3.38	2.10	13.44	10.90	10.62	4.66	ASD 19	7.96	6.36	4.72	4.28	17.92	16.84	14.8	11.36
TPS 3	7.04	4.64	3.64	1.36	13.06	11.68	10.38	5.32	Co 43	8.48	6.54	5.04	2.92	18.44	17.38	14.24	11.06
Swarna	6.44	4.92	3.58	2.06	12.76	11.10	9.60	5.24	Co 2	7.98	6.52	4.62	3.18	18.28	16.58	15.12	11.08
GEB 24	6.66	4.66	3.34	1.92	13.32	11.14	10.58	5.02	Co 19	8.28	6.32	5.40	3.60	17.72	16.78	15.32	11.42
PY 1 (Resistant check)	5.52	4.86	3.40	1.66	12.72	11.48	10.40	5.22	TN 1 (Susceptible check)	8.57	6.62	5.28	4.02	18.58	16.34	15.20	11.32
SEd	0.22	0.23	0.21	0.15	0.25	0.18	0.20	0.18	SEd	0.18	0.20	0.17	3.84	0.38	0.17	0.27	0.20
CD (P = 0.05)	0.45	0.48	0.44	0.31	0.52	0.37	0.41	0.38	CD (P = 0.05)	0.36	0.42	0.35	7.87	0.78	0.35	0.56	0.41

EVALUATION OF TRAPS IN STORED PRODUCT INSECT PEST MANAGEMENT IN STORAGE GODOWNS

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Abstract

The presence of insect pests in grain storages throughout the supply chain is a significant problem for farmers, grain handlers, and distributors world-wide. Insect monitoring and sampling programmes are used in the stored grains industry for the detection, estimation and management of pest populations. This study provides an overview of insect trapping and interpretation of trap captures for stored-product protection in bulk grain, food processing, and retail environments. Insect traps *viz.*, UV light trap, probe trap and pheromone traps were used for monitoring and detection of insect pest population in various storage godowns. Early pest detection and control can significantly reduce pest numbers throughout the supply chain, with a resultant improvement in management costs and grain quality.

***Key words*: Warehouses, arthropods, traps, detection and monitoring.**

Introduction

Insects are characterized by their ability to increase in numbers rapidly and are able to colonize a new environment with a few individuals. (Pacavira, 1998). The presence of arthropods in dry stored products is a serious risk of contaminations that make the products and its derivatives unacceptable and can lead to the rejection of contaminated lots, with consequent qualitative reduction. Development and implementation of monitoring methods are essential to detect early pest infestation on stored products to prevent quality and economic losses (Arbogast *et al*.,1998) . For this several devices can be used to sample grain and check for insects. Hence it is proposed to detect and monitor the insect pests found infesting stored agricultural produce in selected storage godowns in Tiruvallur.

Material and Methods

The detection of insect pests was conducted in seven storage godowns, and a rice milling factory. Initially pilot survey on occurrence of insect pests by direct visual observations and also done by collecting samples of product (rice, paddy, pulses and rice flour and from residues samples. Ten grain samples of approximately 10 grams each were taken by inserting a open-ended grain trier to a depth of 50 cm. Insect pests' occurrence/emergence was identified and counted after two weeks using microscope and hand lens. Each grain sample was weighed and insect density was expressed as the number of adult insects / 100 grams grain sample. Monitoring of insect pests was followed utilizing various traps *viz.,* UV-Light traps, Probe traps and dome traps (with pheromone and kairomone for beetles during 2017-2018 in two loctions and trapped insects were collected and counted and identified at weekly interval. All insects that were known to be stored product pests were collected and identified (Gorham *et al.*, (1987)) Species richness and abundance was calculated, analysed for every location and for various trap capture and based on the results, the best trap for monitoring specific insect pests has been recommended.

Results and Discussion

Detection of insect pests: Eleven insect species belonging nine families belonging to four orders had been detected. The presence of seven insect species of Coleoptera, *viz., Rhyzopertha dominica,* Bostrichidae; *Oryzaephilus surinamensis,* Silvanidae; *Tribolium castaneum, Tribolium confusum,* Tenebrionidae; *Sitophilus oryzae,* Curculionidae; *Typhaea stercorea,* Mycetophagidae; *Lasioderma serricone,* Anobiidae was registered. Two species of Lepidoptera was identified namely, *Sitotroga cerealella*, Gelichiidae and *Corcyra cephalonica,* Pyralidae. Insects of the orders Psocoptera and Hemiptera, *Xylocoris flavipes*(Reuter), Anthocoridae were also identified.

Monitoring of insect pests: UV light trap catches at storage godown, were recorded from September 2017 to April 2018 at weekly interval and population was expressed as total number of insects trapped in a month. In both the places *Sitotroga cereallela* population was high, reaching its peak during the month of Feb'2018 (5160 and 6324) and was low during Sep'2018 (420) Oct'2018 (70) respectively. Similarly gradual increase in population of *Rhyzopertha dominica* was recorded from Sep'2017, reaching maximum during Mar'2018 (353 and 370). In light trap only *Sitotroga cereallela* and *Rhyzopertha dominica* population dominate in both the storage units and limited number of fungus beetle, red flour beetle and rice moth was observed. (Figure.1). Insect population showed various seasonal population dynamics and patterns in the study conducted by Arthur *et al.,* 2014 in paddy storage godowns. Similarly, light traps attract a wide variety of adult stages of flying insects, including stored product insects, but have limited utility for detection and monitoring of key economically important stored product insect species.

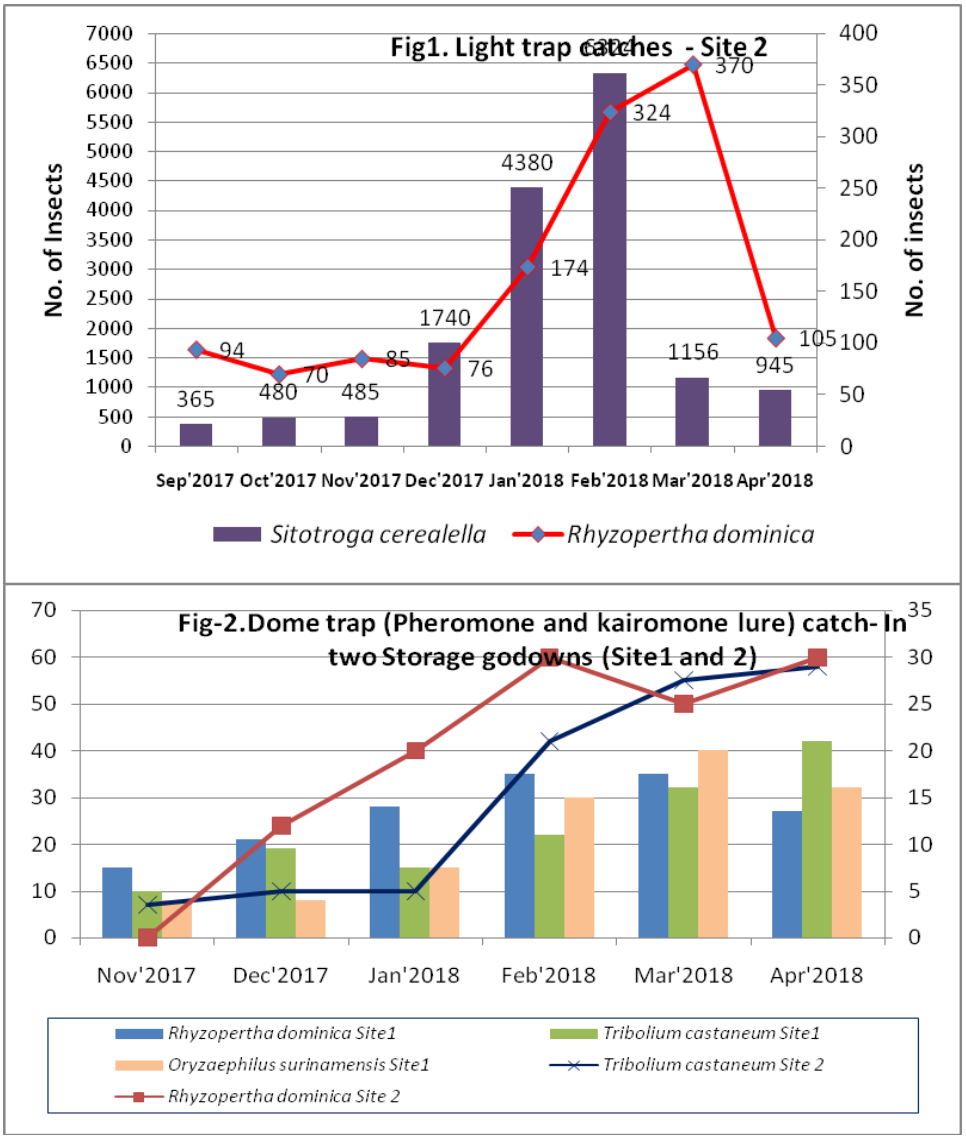

Table.1. Probe trap catch (Number of insects trapped / month)

Species	*Rhyzopertha dominica*		*Tribolium castaneum*		*Oryzaephilus surinamensis*		*Sitotroga cerealella*	
Site	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2
Sep'2017	11	18	0	0	0	0	0	0
Oct'2017	15	15	0	0	0	0	0	0
Nov'2017	8	20	0	0	0	0	0	5
Dec'2017	10	10	5	2	7	0	0	0
Jan'2018	22	12	10	15	9	0	0	7
Feb'2018	31	45	22	32	16	0	0	13
Mar'2086	40	66	34	24	15	0	0	0
Apr'2018	46	40	30	26	18	0	0	0

Probe trap catches revealed that infestation of *Tribolium castaneum* was in both the places and *Oryzaephilus surinamensis* only in site 1. Population of *Rhyzopertha dominica* and *Tribolium castaneum* trapped were gradually increasing from Nov'2017 and was peak during Apr'2018 and Mar'2018 respectively. In both the locations *Sitotroga cereallela* was low in probe trap (Table.1). In probe trap more number of insect species were collected compared to sampling method similarly probe trap detect insect when no insects are detected by standard grain sampling methods. Six insect species were detected in the dome traps (with pheromone and kairomone for beetles). Dome traps were installed in both the places and observation was recorded from Nov'2017 to Apr'2018. Insects *viz, Tribolium castaneum* and *Oryzaephilus surinamensis* were detected earlier during Nov'2017 itself when compared to probe traps. Dome trap with pheromone lure catch was high in both the places compared to probe trap. *Tribolium castaneum* and *Oryzaephilus surinamensis* catch were high during Mar'2018 and *Rhyzopertha dominica* in Feb'2018 (Fig.2.). When compared to probe trap more number of coleopteran species were trapped in dome trap with pheromone lure as documented by Wakefield *et al*., 2006, where the increase in trap catches were modest and lure was more effective floor traps than in grain bulk.

Conclusion

The UV light traps are efficient in capturing Lepidoptera adults, probe traps for beetles and weevils and Dome traps with mixed pheromone lure for beetles. Use of the pheromone traps has resulted in detection of insect infestations earlier when compared to light and probe traps. Early pest detection and control can significantly reduce pest numbers throughout the supply chain, with a resultant improvement in management costs and grain quality.

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EFFECT OF GROWTH SUBSTANCES ON THE GROWTH AND YIELD OF GOLDEN ROD (*Solidago canadensis* L.)

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Abstract

An investigation was undertaken to study the effect of exogenously applied growth substances on the growth and yield of Golden rod. The growth substances used were Gibberellic acid (GA_3 at 100, 150, 200 ppm), Chlormequat (CCC at 1000, 1500, 2000 ppm), Maleic hydrazide (MH at 500, 1000, 2000 ppm) and Salicylic acid (SA at 25, 50, 100 ppm). The growth substances were applied as foliar sprays at three stages *viz*., 15 days, 30 days and 45 days after transplanting with water spray as control. The growth parameters *viz*., plant height (66.93cm), flower stalk length (42.27cm), early flowering (71.07 days after transplanting) was significantly increased by the application of GA at 150 ppm also drastically reduced the number of days taken for 50 per cent flowering (74.30 days after transplanting). The yield parameters *viz*., number of flower stalks per meter square (36.50) and number of suckers per plant (8.60), maximum duration of flowering (33.13 days) and flower yield per meter square (1333.53 g) was significantly increased by the application of GA at 150 ppm whereas individual stalk weight (61.13g) was increased by the application of CCC at 1500 ppm. The application of GA at 200 ppm enhanced the vase life by 7.33 days.

Key words: Golden rod - Gibberellic acid - growth substances – growth - yield

Introduction

Golden rod (*Solidago canadensis* L.) belongs to the family Compositae. It is native to North America and an important floricultural crop, basically used as a filler material. This hardy perennial herb grows well in all types of soil and climate. It produces large panicles of attractive yellow flowers for several months in a year. It is cultivated for cut flowers all over the world, besides it is grown in beds, borders, rock garden etc. It is also used for indoor decoration in vases and used either singly or with other flowers in flower bouquets. Golden rod has got promising and untapped export potential, besides local demand. Growth regulators at an optimum concentration are known to modify the growth and development of plants without causing any malformation. Regulation of plant growth has been studied extensively in many flower crops to enhance the production of quality flowers.

Materials and Methods

The experiment was carried out to study the effect of certain growth substances on the growth and yield of golden rod (*Solidago canadensis* L.) in the Department of Floriculture, Horticulture College and Research Institute, TNAU, Coimbatore. The trial was laid out in randomized block design with three replications. The experiment comprised of 13 treatments *viz*., T_1- GA_3(100 ppm), T_2- GA_3(150 ppm), T_3- GA_3(200 ppm), T_4- CCC (1000 ppm), T_5- CCC (1500 ppm), T_6- CCC (2000 ppm), T_7- MH (500 ppm), T_8- MH (1000 ppm), T_9- MH (2000 ppm), T_{10}- Salicylic acid (25 ppm), T_{11}- Salicylic acid (50 ppm), T_{12}- Salicylic acid (100 ppm) and T_{13}- Control (water spray). The experiment was laid out in randomized block design with three replications and the growth substances were applied as foliar spray to the plant at three stages of crop growth viz., 15, 30 and 45 days after transplanting. The suckers were transplanted during October at a spacing of 30 x 30 cm . FYM is added at the rate of 5 kg per square meter. A fertilizer dose of 140:175:150 kg per hectare was applied as a basal and half of the N as top dressing after 30 days after transplanting. The panicles were harvested in the morning hours when about 25 per cent of the flowers have been opened in all the treatments.

Results and Discussion

Growth parameters

In the present study the growth parameter, plant height was greatly influenced by the chemical treatments. The growth promoter, GA provided the maximum increase in height of the plants over a period of time. The plants treated with GA at 150 ppm recorded the maximum plant height whereas the plants treated with MH at 500 ppm recorded the minimum plant height. The increase in plant height might be attributed by rapid elongation of internodes which is again due to increased cell division and enlargement, and this mostly confined to sub apical meristem. The plants treated with GA had higher mitotic index in the sub apical meristem and there might have enhanced cell division in this region. GA application reduced the duration of the cell cycle by 30 per cent and it caused a change in the plane of cell division i.e. the mitotic spindle of dividing cells becomes reoriented towards longitudinal direction, as a result of which the plane of the cell division becomes transverse. This results in vertical files of cells being added on. Hence, the new cells that are formed contribute to the length rather than to the girth of the stem. Therefore, the stem of GA treated plants increases in length. This has also been confirmed by the findings

of Moore (1966) in Chrysanthemum, Mittal (1967) in Dahlia, Reddy (1977) in China aster, Nagarjuna *et al*.(1988) in Chrysanthemum. Reddy and Sulladmath (1983) also noticed a direct correlation between the concentrations of GA sprayed and the increased China aster plant height. The present findings confirms the earlier reports that GA at 150 ppm increased the plant height of Shadeed *et al*. (1991) in China aster, Padma priya *et al.* (2003) in Chrysanthemum followed by the application of GA at 200 ppm increased the plant height in Golden rod which is in confirmation with the findings of Nagarjuna *et al*.(1988) in Chrysanthemum, Prabhat *et al*.(2003) in China aster, Anil *et al*.(2004) in French Marigold and Patil *et al*.(2004) in Golden rod. Maleic hydrazide sprays reduced the plant height and internodal length significantly and the reduction was more with higher doses. The reduction in the plant height could be because of its inhibitory effect on cell division both in the apical and the sub apical meristem. These results are in line with the findings of Reddy (1977) and Reddy and Sulladmath (1983) in China aster, Singh and Rathore (1992) in Marigold, Aswath *et al*.(1994) in China aster, Yadav (1997) in African marigold, Khandelwal *et al*.(2003) in African marigold.

The application of SA at 50 ppm followed by GA at 200 ppm concentration resulted in higher number of leaves. The increase in number of leaves might be due the effect on shoot elongation which produced more number of leaves. Similarly, increased stem length with GA has been reported in China aster by Prabhat *et al*. (2003) and Patil *et al*. (2004) in Golden rod.

The application of GA at 150 ppm induced early flowering as compared to the control. The early flowering might be due to the fact that such plants have built up sufficient food reserves at the initial stages and also attributed to the raise in endogenous GA level. This is in confirmation with the findings of Ramesh (1999). The delayed flowering was observed in the treatment CCC at 2000 ppm. Such delayed flowering is supposed to be due to its inhibitory effect on the plant growth. These findings were in line with Narayana Gowda and Jayanthi (1992); Yadav (1997); Khandelwal *et al*. (2003) in African marigold. The shortest duration to 50 per cent flowering was recorded in the treatment with GA at 150 ppm which is in conformity with the results of Ramesh (1999) while the longest duration of flowering was registered by CCC at 2000 ppm .These findings were similar to the results of Nagarjuna *et al*.(1988) in Chrysanthemum. Gibberellic acid application increased the length of the flower stalk which might be attributed to the increased internodal length and length of the branch. These findings were similar to the results of Dutta and Seemanthini Ramadas (1997) and Rakesh *et al*. (2004) in Chrysanthemum, Ramesh (1999) in China aster. The shortest flower stalk length was noticed in the treatment with CCC. These results are corroborated with the findings of Dutta and Seemanthini Ramadas (1997) in Chrysanthemum.

Yield parameters

The treatment with GA recorded the highest number of flower stalks. This findings was in confirmation with Prabhat *et al*.(2003) in China aster followed by the treatment with CCC. The results are in consonance with the findings of Aswath (1991) and Prabhat *et al*. (2003) in China aster, Singh and Rathore (1992) and Khandelwal *et al*. (2003) in African marigold, Patil *et al*.(2004) in Golden rod. The treatment with GA recorded the maximum number of suckers followed by CCC and SA. This is in line with the findings of Patil *et al*. (2004) in Golden rod, Padmapriya *et al*.(2003) in Chrysanthemum. The maximum individual stalk weight was observed with CCC at 1500 ppm followed by SA at 50 ppm. The present findings confirm the earlier reports of Khandelwal *et al*. (2003) in African marigold. The minimum stalk weight was recorded with MH application. The results are in consonance with the earlier findings of Khandelwal *et al*.(2003) in African marigold.

The highest yield was recorded with the application of GA at 150 ppm. This is in confirmity with the results of Dutta *et al*. (1993) in Chrysanthemum. The maximum flower yield and flower stalk yield were recorded when the plants were treated with GA at 200 ppm in China aster (Prabhat *et al*., 2003); (Rakesh *et al*., 2004) in Chrysanthemum; (Varma *et al*., 2004) in African marigold; and (Patil *et al*., 2004) in Golden rod. The lowest yield was recorded with MH application by Khandelwal *et al*. (2003) and Varma *et al*.(2004) in African marigold also recorded the similar results for the yield. The maximum duration of flowering was noticed with the spray of GA at 150 ppm followed by GA at 100 ppm. These findings are in affirmation with the reports of Ramesh (1999). The minimum duration of flowering was noticed with MH at 2000 ppm which had taken relatively lesser duration of flowering which is in confirmation with the works of Ramesh (1999).The longest vase life was observed with GA application. Similar observations were made by Dutta *et al*. (1993) in Chrysanthemum, Goyal *et al*. (1994) in Rose. The shortest vase life was observed with the spray of MH at 500 ppm was also recorded.

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Mean performance of growth and yield parameters of Golden rod (*Solidago canadensis* L.)

Treatment	Plant height (cm)	No.of leaves/ plant	No.of days for Ist flowering (days)	No. of days for 50% flowering (days)	Length of flower stalk (cm)	No. of flower stalks/m^2	No. of suckers /plant	Weight.of Individual stalk (g)	Yield of flower stalks/m^2 (g)	Duration of flowering (days)	Vase life (days)
T1- GA_3(100 ppm)	60.60	54.93	73.60	78.13	40.27	25.07	6.27	22.80	571.95	32.40	4.80
T2 - GA_3(150 ppm)	66.93	52.13	71.07	74.30	42.27	36.50	8.60	38.67	1333.53	33.13	5.07
T3 - GA_3(200 ppm)	63.73	56.67	71.17	77.53	41.60	28.53	7.13	38.33	1094.67	28.60	7.33
T4 - CCC (1000 ppm)	53.53	42.33	74.43	77.40	37.13	34.40	8.27	28.87	999.43	21.47	4.73
T5- CCC (1500 ppm)	51.27	34.67	74.13	79.23	30.80	15.47	3.87	61.13	944.53	23.67	5.00
T6 - CCC (2000 ppm)	49.80	36.33	79.20	84.20	24.97	12.80	3.20	58.67	750.93	20.47	4.00
T7 - MH (500 ppm)	46.87	31.67	78.43	79.20	37.60	13.60	3.33	33.73	459.52	18.67	3.40
T8 - MH (1000 ppm)	47.13	28.33	78.10	79.23	36.03	12.27	3.07	39.53	485.07	17.67	5.00
T9 - MH (2000 ppm)	54.67	28.53	75.17	78.13	31.67	10.40	2.60	19.27	193.07	16.20	6.00
T10 - Salicylic acid (25 ppm)	51.20	46.50	78.00	81.33	34.60	11.00	2.07	45.33	498.67	29.00	6.00
T11 - Salicylic acid (50 ppm)	62.67	57.93	75.33	78.20	36.27	32.00	8.00	59.60	1267.63	24.20	6.67
T12 - Salicylic acid (100 ppm)	56.87	44.53	72.37	79.87	39.47	12.27	3.07	45.93	563.20	21.40	5.40
T13 – control (water spray)	54.07	21.50	77.40	82.33	21.93	10.00	2.00	19.60	196.00	18.60	3.00
SEd	1.31	1.40	1.03	0.84	0.78	0.62	0.15	1.04	30.72	0.37	0.15
CD at 5%	2.71	2.89	2.13	1.73	1.62	1.29	0.31	2.15	63.40	0.78	0.31
CD at 1%	3.70	3.95	2.90	2.36	2.21	1.76	0.42	2.94	86.40	1.06	0.42
CV%	2.91	4.18	1.68	1.30	2.75	3.92	3.91	3.26	5.23	1.98	3.61

TRANSLOCATION OF NUTRIENTS IN MUSTARD

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Abstract

Studies were undertaken at the Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore in mustard cv. GM-2 to elucidate the information on Position of siliqua on top, middle and bottom portion of the mother plant did not show any influence on the quality of seeds. The study on the effect of siliqua position on seed quality revealed non significant influence on physiological parameters *viz.,* germination, shoot and root length, vigour index, dry matter production and biochemical parameters like α- amylase activity, oil and protein content for seeds collected from the top, middle and bottom portion of the mother plant.

Introduction

Indian mustard (*Brassica juncea* (Linn.) is one of the important oilseed crops grown in rabi season contributing 25 per cent of the oilseed production of the country. It occupies a prominent place next to groundnut in meeting the oil requirement of about 50 per cent population. It has been found that in many plant species the fate of the next generation or generations, as the germination is concerned, is dependent, at least to a certain degree, on the maturation conditions of the seeds when they are still on the mother plant and the position of that inflorescence / seed in that crop.

Methodology

Ten plants were randomly tagged and seeds were collected from different positions as detailed below. The total plant height was equally divided into three positions as P_1-Top; P_2 - Middle; P_3 - Bottom; and recorded Germination (ISTA 1999), Total seedling length (cm), Vigour index (Germination % x Total Seedling length), Oil content (AOAC, 1960) and Protein content (Alikhan and Youngs, 1973).

Result and discussion

Seeds with maximum germination and vigour are the basic requirements in achieving maximum crop yield. In different plant species maternal factors such as position of inflorescence on the mother plant or the position of seeds in the inflorescence or in the fruit influence the germinability of seeds (Patil and Jadhav, 1977; Thomas *et al.,* 1979; Jacobson and Globerson, 1980; Grey and Thomas, 1982).

Positional influence on germination percentage, shoot and root length (cm) vigour index Oil content and Protein content in mustard cv. GM-2

Treatments	Germination (%)	Shoot length (cm)	Root length (cm)	Vigour index	Oil (%)	Protein (%)
Top	93 (74.66)	10.4	20.7	2903	37.6	13.9
Middle	93 (74.66)	10.4	20.0	2839	37.7	14.2
Bottom	95 (77.08)	10.4	20.5	2930	37.6	14.1
Mean	93.9 (75.70)	10.4	20.4	2891	37.6	14.1
SEd	0.611	0.063	0.459	48.074	0.095	0.249
CD(P=0.05)	NS	NS	NS	NS	NS	NS

(Figures in parenthesis are arc sine values)

In the present study the seeds were collected from the siliqua of top, middle and bottom positions on the mother plant. The seed quality attributes like germination, shoot length, root length, vigour index, oil and protein content did not have significant influence due to the siliqua position on mother plant. Similar results were obtained by Balamurugan (1993) in sunflower, Uslu (2003) and Chandrasekar (2004) in safflower.

Conclusion

The study on the effect of siliqua position on seed quality revealed non significant influence for seeds collected from the top, middle and bottom portion of the mother plant.

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CONSTRAINTS EXPERIENCED BY PADDY FARMERS IN ADAPTATION TO CLIMATE CHANGE

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Abstract

The study examined the constraints experienced by paddy farmers in adaptation to climate change. Among the 29 districts of Tamil Nadu, Nagapattinam district was selected to conduct the present study. This is situated in coastal area and very often subjected to natural calamities which were mainly reflected on the rice cultivation to the worst status. Total sample size of 200 respondents was selected through simple random sampling technique. The study revealed that High cost of farm land, non availability of water storage facility, non-availability of farm labour, poor information on early warning systems and high cost of farm inputs were the major constraints experienced by the paddy farmers in adaptation to climate change. Extending long term loans to small and marginal farmers on easy terms to purchase and develop agricultural land, creating on- or off-farm water storage systems, evolving labour saving techniques, proper early warning system and providing subsidies for farm inputs to the local farmers in the vulnerable areas would help the respondents to cope with climate change.

Key words : Climate change, Adaptation, Constraints, Paddy farmers

Introduction

Climate change is a long-term change in the statistical distribution of weather patterns over periods of time that range from decades to millions of years. Climate change refers to a statistically significant variation in either the mean state of the climate or in its variability, persisting for an extended period. Climate change may be due to natural internal processes or external forces, or to persistent anthropogenic changes in the composition of the atmosphere or in land use. (IPCC:2007). The growing problems of climate change are becoming more threatening to sustainable economic development and the totality of human existence (Adejuwon, 2004). The most obvious manifestation of climate change is the rising of average worldwide temperature, popularly termed as global warming. The average annual temperature of the Earth's surface has risen over the last century. Not only the temperature rising, but the rate of warming itself is increasing too. The mean global annual temperature increased between 0.4 to 0.7^{o} C (Singh, 2008). In ecological terms, this is a very rapid change.

Most of the countries are facing the problems of rising temperature, melting of glaciers, rising of sea-level leading to inundation of the coastal areas, changes in precipitation patterns leading to increased risk of recurrent droughts and devastating floods, threats to biodiversity, an expansion of plant diseases and a number of potential challenges for public health. Adverse impact on agriculture due to climatic changes will have effect on national economy and livelihood. Several global studies have indicated that India is particularly vulnerable to climate change, and is likely to suffer with damage to agriculture, food and water security, human health and cattle populations. Like most other developing countries, people in India are depending to a large extent on its natural resources for livelihood and economy. Any adverse impacts on these natural resources will have repercussion on the nation's livelihood security, economy and widen the gap between the rich and the poor. Though research initiatives are afoot in physical and biological sciences, it is imperative to assess the constraints experienced by paddy farmers in adaptation to climate change. An indepth probing to the constraints faced by the paddy farmers in the adaptation to the climate change and suggestions given by them would bring focus on the needed ameliorative measures.

Materials and methods

The choice for selection of the district had fallen on coastal ecosystems of Tamil Nadu state for the conduct of the present study. Coastal belts are more prone to devastating impact of climate change. The geographical setting of Tamil Nadu makes the state vulnerable to natural disasters such as cyclones, floods and earthquake-induced tsunami (Mascarenhas & Jayakumar, 2007). Among the 29 districts of Tamil Nadu, Nagapattinam district is very often subjected to natural calamities which were mainly reflected on the rice cultivation to the worst status. Since 10 years, the district has high range of variability in rainfall and temperature .The district is one among those districts having more area under rice cultivation. The district has eleven blocks, of which five blocks viz, Thalainayar, Kuttalam, Mayiladuthurai, Kilvelur and Sembanar Koil were selected based on the maximum area covered under rice cultivation and high range of variability in rainfall and temperature. In order to select the villages for the study, the list of revenue villages in each of the five selected blocks was collected. Five villages from each of the selected blocks were identified purposively based on the maximum area under rice cultivation. The respondents for the present study were rice farmers from the selected villages. A sample size of 200 was fixed for the study. Sample of 40 rice farmers were selected from each of the 5 blocks by adopting simple random sampling method.

According to UNDP (2005), Adaptation is a process by which strategies to moderate, cope with and take advantage of the consequences of climatic events are enhanced, developed and implemented. The data on constraints experienced by the paddy farmers while taking adapting measures to climate change were collected under seven dimensions viz., land constraints, poor information on climate change, constraints on farm inputs, irrigation, credit, labour, and technological constraints. To analyse the collected data the percentage analysis was used.

Results and discussion

Constraints faced by the paddy farmers while taking adapting measures to climate change were gathered and the results are presented in Table 1.

Table 1. Distribution of respondents according to constraints experienced in adaptation to climate change

(n = 200)

S.No.	Constraints	Yes	Per cent	No	Per cent
I	**Land constraints**				
1.	Limited availability of land for farming	114	57.00	86	43.00
2.	High cost of farm land	132	66.00	68	34.00
3.	Land tenure status decelerate the adaptation measures	108	54.00	92	46.00
II	**Poor information on climate change**				
1.	Lack of access to weather forecast technologies	148	74.00	52	26.00
2.	Poor information on early warning systems	151	75.50	49	24.50
3.	Poor agricultural extension service delivery	54	27.00	146	73.00
4.	Lack of capacity of extension personnel to build resilience capacity of farmers on climate change	32	16.00	168	84.00
5.	Poor access to information source relevant to climate change adaptation	96	48.00	104	52.00
6.	Lack of information on short term climate variations	110	55.00	90	45.00
7.	Lack of information on long term climate change	118	59.00	82	41.00
III	**Constraints on farm inputs**				
1.	High cost of farm inputs	132	66.00	68	34.00
2.	Non-availability of timely farm inputs	110	55.00	90	45.00
3.	Lack of information for input management	90	49.50	101	50.50
IV	**Constraints on irrigation**				
1.	Non availability of water storage facility	185	92.50	15	7.50
2.	High cost of efficient irrigation systems	152	76.00	48	24.00
3.	High cost of water management infrastructure	125	62.50	75	37.50
4.	Poor supply of electricity	168	84.00	32	16.00
V	**Credit constraints**				
1.	Adaptation to climate change requires more money	153	76.50	47	23.50
2.	Taking more time to get crop loan from the banks	152	76.00	48	24.00
3.	Low price for the produce in the market	107	53.50	93	46.50
4.	Delay in settlement of crop insurance claim	136	68.00	64	32.00
VI	**Labour constraints**				
1.	Non-availability of farm labour	179	89.50	21	10.50

2.	Labour wage rate is high	149	74.50	51	25.50
3.	Minimum working hours per day	107	53.50	93	46.50
VII	**Technology constraints**				
1.	Recommended rice cultivation technology with respect to climate change does not fit into the needs of the farmers	99	49.50	101	50.50
2.	Lack of technical guidance	85	42.50	115	57.50
3.	Difficulty in technology adoption	75	37.50	125	62.50

Land constraints

High cost of farm land (66.00 %), limited availability of land for farming (57.00 %) and land tenure status (54.00 %) were the major constraints faced by the paddy farmers in the climate change adaptation measures.

High cost of farm land was considered as the foremost constraint expressed by the respondents. Similar constraint was also reported by Benhin (2006). The reason might be due to the fact that the world population is continuously growing at a rapid rate and at the same time the need for the habitat is alarmingly increase. Hence, to meet out this need the farmland is converted into urban uses which resulted in farm land shrinkage and high cost of farm land.

Poor information on climate change

From the Table 56, it could be understand that nearly three fourth of the respondents reported that poor information on early warning systems (75.50 %), lack of access to weather forecast technologies (74.00 %) were the major constraints in the climate change adaptation measures and more than fifty per cent of the respondents felt that lack of information on short term climate variations (55.00 %) and lack of information on long term climate change (59.00 %) were the barriers to adaptation measures.

In the present information age, information problems could pose serious challenges to farmers coping strategies as they may not be aware of recent developments regarding climate change adaptations and the necessary readjustments needed. The lack of adaptive capacity due to constraints on resources such as the poor information on early warning system and lack of access to weather forecasts create serious gaps between the farmers and useful information that should help them in their farm work. Weather forecasts are supposed to guide farmers on climate variability so that they can make informed decisions and useful farm plans. However, the absence of the facility will undoubtedly make the farmers become ignorant of the weather situations and hence become vulnerable to the impact of changes in the climate and weather. This finding is in line with the findings of Ozor *et al*. (2010).

Constraints on farm inputs

The data in Table 56 revealed that 66.00 per cent of the respondents felt that high cost of farm inputs was the major constraint followed by non-availability of timely farm inputs (55.00 %). Purchase of inputs such as high yielding variety seeds, herbicides, fertilizers, *etc.,* requires a considerable amount of money and as stated elsewhere most of the farmers belonged to medium farmer category, so the cost of these inputs may really be very high to them. This could pose threats to the coping strategies of the farmers. Reilly (1996) noted that climate change might constitute significant addition to the stresses already borne by farmers such that adapting to it might be beyond their resource capabilities.

Constraints on irrigation

Under the dimension of adaptation constraints on irrigation, non availability of water storage facility (92.50 %), poor supply of electricity (84.00 %) and high cost of efficient irrigation system (76.00 %) were the major constraints experienced by the respondents.

Most of the farmers felt that there was no storage facility for irrigation water. Most of the paddy farmers are resource poor and cannot afford to invest on irrigation technology for climate change adaptation so as to sustain their livelihood during harsh climate extremes such as flooding and drought. This finding is in line with the findings of Deressa (2008).

Credit constraints

It could be seen from the Table 56 the respondents reported that climate change adaptation measures requires more money (76.50 %), taking more time to get crop loan (70.00 %), and delay in settlement of crop insurance claim (68.00 %) were the constraints experienced under the credit dimension.

Climate change adaptation measures perceived as cost effective by the respondents because most of the paddy farmers had medium level of annual income. Lack of money hinders farmers in getting necessary resources and technologies which facilitated adaptation to climate change. Getting crop loan and crop insurance claim process has to undergo a series of systematic process which would consume considerable amount of time. Furthermore the process involves the participation of both Governments at Central and State. This might lead to delaying of the process. This finding in accordance with the findings of Senthilkumar (2009).

Labour constraints

Non-availability of farm labour (89.50 %), higher labour wage rate (74.50 %) and minimum working hours per day (53.50 %) were the constraints faced by paddy farmers.

The reason for non-availability of farm labour and higher labour wage rate might be rural people were migrated to urban areas due to various reasons like climatic disasters, job, education etc leads to labour scarcity. Because of labour scarcity, wages for labour were also increased. Mahathma Gandhi National Rural Employment Guarantee Act program is another important reason for labour problem because it was providing enough wage to rural people with minimal work compared to agricultural works. This finding in accordance with the findings of Bhuvaneswari (2012).

Technology constraints

Recommended rice cultivation technology with respect to climate change does not fit to the needs of the farmers (49.50 %), lack of technical guidance (42.50 %) and difficulty in technology adoption (37.50 %) were the constraints experienced in climate change adaptation measures as reported by the respondents.

Technological constraints are low in the climate change adaptation measures compared to other constraints. The probable reason for this result might be due to some of the respondents in the study area had minimum educational status and low level of adaptation behaviour which might have hindered them to comprehend the technology adoption. This finding in accordance with the findings of Shanmugasundaram (2007).

Conclusion

From this study it could be concluded that high cost of farm land, non availability of water storage facility, non-availability of farm labour, poor information on early warning systems and high cost of farm inputs were the major constraints experienced by the paddy farmers in adaptation to climate change. Extending long term loans to small and marginal farmers on easy terms to purchase and develop agricultural land, creating on- or off-farm water storage systems, evolving labour saving techniques, proper early warning system and providing subsidies for farm inputs to the local farmers in the vulnerable areas would help the respondents to cope with climate change.

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SOURCE-SINK RELATIONSHIP IN AEROBIC RICE BY DRIP BIOGATION WITH SEAWEED EXTRACT AND HUMIC ACID

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Rice (*Oryza sativa* L.) is the important primary cereal crop in the world. It is the staple food for more than two-third of the world's population (Dowling *et al.*, 1998). Although rice ranks second in the context of most extensively grown crop in the world, it is the largest irrigated crop in the world. More than 75 per cent of the world's rice supply comes from 79 million ha of irrigated land in Asia. Thus, the present and future food security of Asia depends mainly on the irrigated rice production system. This system is a major user of fresh water. The available amount of water for irrigation is, however, increasingly getting scarce. Since several parameters concerning the physical frame of the plants are almost exhaustively studied, it is likely that the manipulation of functional traits might enhance the yield plateau especially under low-yielding aerobic environment. Realizing this aspect, attempts are being made to elucidate the 'source' (leaf) and 'sink' (panicle) inter-relationships to improve the functional efficiency of the rice plants intended for aerobic environment.

Key words: Aerobic rice, drip fertigation, source strength, sink strength, grain filling rate.

Material and Methods

Field experiment was conducted in Wetland, Tamil Nadu Agricultural University, Coimbatore, India. Source characteristics, four hills from each replication and treatment were removed and the leaf was counted as a single unit. The number of leaves per hill was presented by calculating the mean of four hills. Leaf area duration (LAD) was determined by using the formula of Power *et al.*1967 and the values expressed in days. The individual leaf size (ILS) was calculated by using the formula of ILS = Total leaf area (cm^2 $hill^{-1}$) / Total leaf number $hill^{-1}$ and expressed in cm^2 $leaf^{-1}$. Sink characteristics, for Panicle growth modeling, at the time of flowering, about one hundred panicles in the each treatment in three replications were tagged. The panicles were sampled periodically and their dry weights collected at the chosen time interval were plotted as a function of time from anthesis until the final harvest. Panicle data from the treatments were fitted with different panicle growth models as proposed by Thornley(1976). Models are exponential (negative), Gompertz, logistic, cubic, polynomial, quadratic and Richard's and a hybrid of Richard's-Quadratic model. Among them, the hybrid model of Richard's - Quadratic was found to be best fit. Statistical analysis, the data collected were subjected to statistical analyses in the randomized block design using ANOVA (AGRES version 7.01) following the method of Gomez and Gomez (1984).

Result and Discussion

1. Source characteristics`

The source strength was the major factor influencing the sink strength (Yoshida, 1981). Source strength has got two components, viz., source size and its activity. The source activity is further split into two sub-components i.e., photosynthetic efficiency (in terms of leaf photosynthetic rate) and longevity of source (LAD). It was noticed that the source size (in terms of both leaf number and ILS at FF stage) increased with the hybrid than the variety. For the irrigation treatments, number of leaves increased with increase in water availability (Table 1), while the values were lower for moderate and excess moisture supply situations. Source sizes were favorably influenced by the sub-surface drip system and with the seaweed extract biogation.

Interestingly, the percentage reduction in leaf photosynthetic rate noticed at lower water availability (100 % PE) was found to be 21.1 and 12.4 in comparison with 125 and 150 % PE level of drip irrigation respectively. Similar trend was evident with LAD also with higher magnitude of reduction in the case of lower water availability situation. Parameters of source activity were higher for the sub-surface drip system than surface drip or conventional method of irrigation. In the case of biogation treatment, seaweed extract registered higher leaf photosynthetic rate by 13.7 and 17.5 per cent than humic acid and unbiogated control respectively. Similar increase was recorded with seaweed biogation for LAD parameter also.

In general, hybrid culture generally exhibited faourable trend for all the traits of source strength than the variety tested.

Thus, components of source strength were better placed for the hybrid under sub-surface drip irrigation scheduled at 125 % PE level and also with seaweed biogation treatment.

Table 1. Components of source strength due to drip biogation treatments in rice

Treatment	Source size		Source activity	
	Leaf No. $hill^{-1}$	ILS (cm^2 $leaf^{-1}$)	Leaf photosynthesis (µmol CO_2 m^{-2} s^{-1})	LAD (Days)
Genotype				
TNRH 180	87.3	13.2	19.6	142.7
PMK (R) 3	77.9	12.8	16.2	128.8
Irrigation regime				
100 % PE	70.5	14.0	16.1	126.5

125 % PE	86.0	12.3	19.5	134.7
150 % PE	96.0	11.7	18.1	141.8
Irrigation method				
Surface drip	78.0	13.4	17.6	132.0
Sub-surface drip	90.3	11.9	18.2	136.7
Conventional practice	73.0	15.2	18.1	144.3
Biogation treatment				
Humic Acid	65.9	15.4	21.9	144.4
Seaweed Extract	71.4	14.9	24.9	152.0
Control	70.7	13.0	21.2	127.2

These trends implied that under water stressed environment at 100 % PE level, fair crop productivity could be achieved when optimum leaf area was maintained with greater and sustained photosynthetic efficiency. This association appeared valid only when proper efficient varieties and good agronomic management practices (such as the sub-surface drip biogation at optimum PE level) were ensured for the stressed scenarios.

2. Sink characteristics

Sink strength consists of two major components, *viz*., sink size and sink activity (Wareing and Patrick, 1975). The sink activity is again sub-divided into two components as the Grain Filling Duration (GFD) and Grain Filling Rate (GFR) (Pearson and Hall, 1984). The GFD and GFR are calculated as follows:

Panicle dry weights collected at the chosen time interval were plotted as a function of time from anthesis until the final harvest. Panicle data were used to test the different panicle growth models (Thornley, 1976) as mentioned elsewhere in the study; of which, the hybrid model of Richard's - Quadratic function was found to be the best fit (Mohandass *et al*., 1988). From these models and functions, observations were made of the dates on which 90 per cent of the final panicle weights were attained and the relative duration of the GFD of each treatment was calculated as the length of the time interval from zero flowering (anthesis) to the predicted date of 90 per cent of final grain yield (Jones *et al*., 1979). The dry matter accumulated by the panicle at 90 per cent of the final panicle weight was divided by the GFD to arrive at the GFR.

The data on different components of sink strength are furnished in Table 2, which indicated that the sink capacity, calculated as the product of spikelets per m^2 and test weight (Yoshida, 1981), was higher for PMK (R) 3 than the hybrid culture TNRH 180. The values increased by 4.4 and 2.9 per cent under 125 % PE in comparison with 100 and 150 % PE level of drip irrigation respectively.

But, the sub-surface drip system (886.90) showed marginal increase in sink capacity than conventional irrigation (882.66) or surface drip (870.06). Nevertheless, increase (4.9 %) in the sink capacity was evident with seaweed biogation than the control treatment.

The sink size, i.e., the spikelet number per unit area (Yoshida, 1981) was also reduced with deficit situation (100 % PE) by 4.1 and 2.9 per cent as against 125 and 150 % PE levels of drip irrigation respectively but compensated fairly well with seaweed biogation practice.

Grain filling, a crucial determinant of grain yield in rice crop, is characterized by duration and rate of filling (Yang *et al*., 2008). The GFD showed little variations with the values ranging from 22.3 (100 % PE) to 23.4 (150 % PE) for water treatments. Similar was trend observed for methods of irrigation as well as biogation treatments. Nevertheless, water stress at grain filling induced early senescence and shortens the GFD but increased the remobilization of assimilates from source to the sink (Plaut *et al*., 2004) This is in agreement with the present findings especially under deficit and moderate water supply situations.

Table 2. Components of sink strength due to drip biogation treatments of rice

Treatment	Sink size		Sink activity	
	Sink capacity ($g\ m^{-2}$)	Sink size ($No.\ m^{-2}$)	GFD (days)	GFR ($mg\ panicle^{-1}\ day^{-1}$)
Genotype				
TNRH 180	824.62	42.05	22.7	55.23
PMK (R) 3	933.54	38.48	23.4	66.59
Irrigation regime				
100 % PE	871.67	39.31	22.3	48.00
125 % PE	910.25	40.98	22.8	63.73
150 % PE	884.34	40.48	23.4	60.81
Irrigation method				
Surface drip	870.06	39.99	22.6	58.24
Sub-surface drip	886.90	40.52	23.0	63.45
Conventional practice	882.66	40.35	24.5	41.31
Biogation treatment				

Humic Acid	938.16	40.47	23.5	68.04
Seaweed Extract	953.88	40.65	23.9	72.28
Control	909.53	39.89	23.1	55.41

However, the reduction in GFR was found to be phenomenal with the lesser water availability treatment (100 % PE: -14.0 %) in comparison with the moderate water supply (125 % PE). The GFP was shortened in the stressed plants with much reduction in the GFR, which might be due to lesser rate of transpiration required for mobilization of photoassimilates. It seems possible, in the present investigation, that controlled moisture supply in the 125 % PE level at later grain filling stage might promote whole-plant senescence, leading to increased re-translocation of the free stored carbon reserves in the stem, leaf and root (as mentioned previously) as indicated by Yang and Zhang (2006). Many processes were likely to be involved including the hydrolysis of stored carbohydrate, phloem loading, long-distance translocation and phloem unloading into the grains. Mild soil drying (as in the case of 125 % PE) might not seriously disrupt phloem function. It has been shown that phloem translocation might be less susceptible to such scenario than leaf photosynthesis (*ibid*, 2006).

Influence of biofertigation was phenomenal in safely narrowing down such reduction in GFR values as seen with the stressed plants. In this regard, seaweed biogation excelled the unbiogated control by 30.4 per cent. This signified that between the two components of sink activity, the GFR played a vital role for panicle weight and grain yield increase especially in less water applied and unbiogated practice. Similar association of GFR and grain yield was also established with the drip fertigation studies by Vanitha (2008).

Nevertheless, performance of the hybrid culture TNRH 180 was always superior than the variety PMK (R) 3 for all the components of sink strength.

Summarizing the influence of drip biogation system on sink characteristics in aerobic rice, it could be inferred that the yield variations as observed under varied water levels and methods of irrigation as well as biogation treatments, could be mainly due to the alterations in sink capacity and its activity. Again, the major component of sink activity, viz., GFR was greatly influenced by the levels and methods of water application besides the biogation treatments. Yang *et al*. (2008) further suggested that genotypic variation in GFR is an intrinsic genetic characteristic. It is therefore indicated that further crop improvement and management strategies intended for stabilizing the yield under aerobic environment should be aimed at stabilizing the sink capacity and GFR.

5.8.2.3. Source-sink limitation

An attempt has also been made to explain yield variations in the chosen drip biogation treatments in terms of source- and / or sink-limitation. Sink-limitation was worked out as spikelet number x 1000 grain weight / Leaf Area at flowering (Rao and Murty, 1976). Approximate source-limitation (Sa) was calculated by taking into account the changes in dry weight of panicle (ΔY) and the biomass (ΔB) due to variation in treatments as: Sa = (ΔY) / (ΔB) (Gifford *et al*., 1973).

The results (Table 3)indicated that though the sink-source ratio was higher for the variety (0.951) with limited (0.871; 100 % PE) and moderate (0.869; 125 % PE) level of water and with surface drip system (0.856). Nevertheless, the differences due to two drip biogation treatments for sink / source ratio were less appreciable.

Table 3. Variations in source-sink limitation due to drip biogation treatments in aerobic rice

Treatment	Sink / Source Ratio (Sink limitation)	Sa (Source limitation)
Genotype		
TNRH 180	0.729	0.951
PMK (R) 3	0.951	0.910
Irrigation regime		
100 % PE	0.871	0.856
125 % PE	0.869	0.925
150 % PE	0.800	0.983
Irrigation method		
Surface drip	0.856	0.910
Sub-surface drip	0.837	0.988
Conventional practice	0.799	0.782
Biogation treatment		
Humic Acid	0.929	0.912
Seaweed Extract	0.904	0.985
Control	1.011	0.785

On the contrary, the Sa values increased steadily from 100 (0.633) to 150 % (0.983) PE level of drip irrigation. In the case of irrigation methods, higher Sa (0.988) was observed at sub-surface drip method. The hybrid culture TNRH 180 (0.951) had an edge over PMK (R) 3 variety (0.910). Regarding the biogation treatments, seaweed extract application was superior (0.985) than humic acid (0.912).

Thus, the values of Sa was centering around 1 especially for the hybrid with moderate and excess water supplying (125 and 150 % PE levels) treatments given under sub-surface drip system biogated with seaweed extract, which in the scale of 0 - 1 of Gifford *et al*. (1973) explained that all additional assimilates produced was fully used up by the developing grains, and thus the grain growth was entirely source-limited in the chosen genotypes.

These findings also suggested that in this source-limited rice crop, future strategies should be aimed at developing efficient plant type and management options ensuring the capability of not only synthesizing more biomass under aerobic environment but also to partition it more towards 'sink', i.e., the developing grains.

To summarize, the performance of the aerobic rice grown with the sub-surface drip system scheduled at 125 % PE along with seaweed biogation was found to be superior for most of the characters related to the source and sink strength.

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THEME II MEDICAL SCIENCES

ATHEROGENIC INDEX OF PLASMA IN POSTMENOPAUSAL WOMEN COMPARED TO PREMENOPAUSAL WOMEN

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Abstract

Background: Menopausal health in our social environment have received minimal awarenessamong women. Dyslipidaemia, which has been more prevalent among postmenopausal womenhavemadethem moresusceptible to cardiovascular risks and data's of association between c-reactive protein and dyslipidaemia is scarce.

Objective: To estimate and to find the association between serum C-reactive protein and atherogenic index of plasma in postmenopausalwomen and determine the risk of cardiovascular disease with increasing years, compared with premenopausalwomen.

Material and methods: This case control study involved 60 postmenopausal women (cases)aged between50 -70years and30 pre-menopausal women (controls)aged between 30–40years.Totalcholesterol(TC),and their subfractions: highdensity lipoproteincholesterol(HDL-C),verylowdensitylipoproteincholesterol(VLDL-C),lowdensitylipoproteincholesterol (LDL-C) and triglycerides (TG) were analysed. Atherogenic index of plasma (AIP);log(TG/HDL–C)wascalculatedand comparedbetween the casesandcontrol group. These parameters were also compared with 5 yrs post menopausal and 10 yrs postmenopausal women, to assess the cardiovascular risks with increasing years.

Results: There was statistically significant increase in TC, TG, LDL-C, VLDL-C and AIP withpvalue:<0.05anddecreaseinHDL-Cinpostmenopausalwomencomparedwithpremenopausalwomen. There was statistically significant derangement of lipid subfractions as the duration ofmenopauseincreased.

Conclusion: Menopause, undoubtedly alters lipid profile. Atherogenic index of plasma beingtriglyceride based index definitely can add significant value in assessing atherosclerosis andcardiovascularrisks in postmenopausal women.

Keywords:

Postmenopausal women, Atherogenic index of plasma(AIP),Lipid profile, Dyslipidaemia.

EFFECT OF BODY MASS INDEX ON VISUAL EVOKED POTENTIAL AMONG UNDERGRADUATE MEDICAL COLLEGE STUDENTS IN PUDUCHERRY

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Abstract

Introduction: Obesity is one of the most common public health problem prevalent in India. Obesity, being a condition of overnutrition, is not just concerned with weight related health issues but also with systemic inflammation triggered by excess of adiposity. Obesity has impact on all vital body systems including the nervous system as reflected by delayed neural conduction. Visual evoked potentials (VEP) are reported to have delayed conduction time among higher BMI(Body mass index) individuals. As evidences for the involvement of neurophysiology of vision in higher BMI ranges is less pronounced unlike the involvement of cardiovascular or metabolic outcomes, this pilot study has been designed to elucidate the impact of BMI on visual evoked potential.

Aim & objectives: To determine the effect of BMI on VEP among undergraduate medical college students.

Material & Methods: This pilot study was carried out in 90 medical students (30 students in each group)at Sri Venkateshwaraa medical college hospital and research centre, after the approval of institutional ethical committee. After getting written consent volunteers of age 18-21 yrs of both genders without history of hypertension, diabetes or any other hormonal disorders were included. Participants were divided based on Indian-specific BMI as group 1: BMI <23, group 2: BMI 23-25, and group 3: BMI >25. Recording of Visual evoked potential was carried, in the research lab of department of Physiology using Neurostim, Medicad system by placing scalp electrodes relative to bony landmarks according to the International 10/20 system. SPSS version 23.0 was used for data analysis.

Results: Out of 90 participants (30 in each group) the BMI observed was 21.22 ±1.08in group 1 followed by 23.57 ±0.45 in group 2 and 27.10 ±2.34 in group 3, the BMI differedsignificantlybetween the groups. There was a significant difference in VEP parameters among all the three groups.The prolongation of VEP latencies significantly correlated with the increasing order of BMI ranges.

Conclusion: The findings observed suggest thathigher BMI, in the long run, not only have metabolic consequences but also leads to neurological impairment. Therefore the vulnerable population must be encouraged to be involved in regular physical activity to maintain the BMI in normal range and thus their health and wellbeing.

Keywords: Body Mass Index, Obesity,Visual Evoked Potential.

ROLE OF mecA, PVL AND SCCMEC TYPES AMONG MRSA ISOLATES FROM A TERTIARY CARE HOSPITAL

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Abstract
Introduction: Methicillin resistant staphylococcus aureus (MRSA) is the major cause of most of the nosocomial as well as the community acquired infections.Methicillin resistance which is conferred by the presence of *mecA* gene is carried on the mobile genetic element known to be as the SCC*mec.* Panton Valentine Leukocidin (PVL) is a cytotoxin, one of the β-pore forming toxins, which has the ability to form pores in the membrane by its synergistic actions and is associated with both community as well as hospital acquired MRSA infections. Association of PVL gene and MRSA severely impacts the clinical outcome of patients.SCC*mec* typing serves as an important epidemiological tool in the characterization of MRSA infections.
Aim and Objective: To characterize *mecA* gene responsible in methicillin resistance by SCCmec typing and to characterize *pvl* genein MRSA isolates using Polymerase chain reaction.
Material & Methods:A total of 50 MRSA isolates were included for the study. Molecular detection of mecA and pvl was carried out as per the *Karmakar et al* protocol and SCC*mec* typing (type I-V) was done by using M-PCR (*Boyeet al.,* 2007) protocol.
Results and Conclusion: In our study, all the isolates were amplified for the mecA gene. There was a prevalence of 24% of PVL positive MRSA isolates and five different SCC*mec* types namely SCC*mec* type I, type II, type III, type IV and type V were detected among the clinical isolates of MRSA. SCCmec typing along with *pvl*gene detection is helpful in determining the contribution of the isolates in antibiotic resistance which in turn helps in designing effective surveillance and control strategies in management of MRSA infections.
Keywords: Antibiotic resistance, Panton-valentine gene, Staphylococcal cassette chromosome, methicillin resistance

SCOPE OF VIRTUAL REALITY [VR] AND AUGMENTED REALITY [AR] IN MEDICAL EDUCATION SYSTEM

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Abstract
Background: The goal of the current Medical Education in India is to produce well trained and skilled medical professionals. This has been achieved by good clinical exposure and skill lab training. This can be further enhanced by implementing the latest technologies in medical education. The concept of virtual reality and augmented reality in medical education teaching has long been explored in the Western world. This paper will highlight the scope of virtual reality and augmented reality in Indian Medical Education system.
Discussion: Virtual reality (VR) and augmented reality (AR) are two contemporary simulation models that are currently upgrading medical education. VR/AR provides a 3D and dynamic view of structures and the ability of the user to interact with them.VR/AR techniques are currently being used in technical training skills likeintubation, cardiopulmonary resuscitation, suturing etc. Studying clinical cases in form of virtual patients [examination, interpretation of reports and management]. The recent technological advances in haptics, display systems, and motion detection allow the user to have a realistic and interactive experience for the students. VR/AR techniques are also used in developing communication skills with the patients
Conclusion: VR/AR based teaching increases the skills and accuracy thus reducing of errors of learners. It bring about more involvement and Positive psychological effect on learners. Disadvantages include the cost of the equipment's, accessibility, reliability and trained personnel's. VR/AR can never replace the real environment training, but when coupled with the standard teaching methods the outcome will be better.
Keywords: Virtual reality, augmented reality, medical education, simulation and advanced learni

NEVUS LIPOMATOSUS CUTANEOUS SUPERFICIALIS- A RARE CUTANEOUS HAMARTOMA

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Abstract

Introduction

Nevus lipomatosus cutaneous superficialis (NLCS)is a rare benign cutaneous hamartoma defined by the presence of aggregates of mature adipose tissue among the collagen bundles of dermis. We report a case of 43 year old female with solitary subtype of Nevus Lipomatosus cutaneous superficialis.

Case Report

A 43year old female presented with single, asymptomatic, skin coloured growth over left side of buttock for the past 3 months, it appeared spontaneously which gradually progressed to attain the present size in 3 months. Cutaneous examination showed Single,Hypopigmented, Pedunculated nodule of size 5cm x 3cm, cerebriform surface, softconsistencyover left side of gluteal region near thegluteal cleft. Excision biopsy was done and the specimen was sent for histopathological examination which showed features of Basket weave orthokeratosis, irregular acanthosis and sheets of matureadipocytes in the dermis.Based on the abovementioned characteristic features, a diagnosis of Nevus Lipomatosus cutaneous superficialis was made.

Discussion:

NLCS includes two clinical subtypes, the classical type & the solitary type.The classical subtype occurs at birth and most commonly occurs on the posterior surface of thigh, gluteal region and lower back. The solitary subtypeoccurs after two decades consists of single skin coloured nodule or papule without specific sites of distribution.

Conclusion:

Solitary subtype of NLCS is rare and high degree of clinical suspicion is required to confirm the diagnosis. In our case the lesion was mimicking a giant achrochodron. The diagnosis was confirmed by histopathology .

CORRELATION BETWEEN HIGH RESOLUTION CT-THORAX SEVERITY SCORE AND CLINICAL OUTCOME AMONG COVID INFECTED PATIENTS

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Abstract

Introduction: CT has a reported high sensitivity in patients infected by sars-cov-2. The aim of this retrospective study is to determine the correlation between a CT -based semi-quantitative score of pulmonary involvement with clinical staging of disease and to assess the role of CT in predicting short-term mortality. For each of the patients, CT images will be taken and evaluated for: (1) presence of ground-glass opacities (ggo), consolidation, interstitial thickening or reticulation, fibrous stripes and air bronchograms, (2) severity of opacifications, (3) other manifestations, such as the location of the lesion (peripheral, central, both central and peripheral), pleural effusion, mediastinal lymph node changes (enlargement or increased number of lymph nodes).

Aims and Objectives: To correlate the CT severity score with clinical outcome among COVID infected patients and to correlate the CT severity score with CRP levels and spo2 levels. tp predict mortality with initial CT severity score.

Materials and Methods: retrospective cohort study 72 cases during the period of data collection patients satisfying the eligibility criteria will be included in the study. Duration of the study: March 2021- June 2021. Data will be entered in excel and analysed by using SPSS 23.0 version software. Statistical variables will be reported as frequency, percentages and continuous variables as mean + 0r - standard deviation (sd). Chi-square test will be used for categorical variables. The p value <0.05 will be considered significant.

Results: Results are being compiled and will be presented in the conference.

KNOWLEDGE ATTITUDE PRACTICE AND ACCEPTABILITY TOWARDS COVID 19 VACCINATION AMONG MEDICAL PROFESSIONALS- A CROSS SECTIONAL STUDY

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Abstract

Introduction: Coronavirus disease 2019 (COVID-19) is still spreading worldwideand caused severe disruptions in and unprecedented challenges for healthcare system. Currently, India is battling the third wave due to Omicron- the variant of COVID-19. As of January 30th2022 only50.8% of population has been vaccinated with 2 doses and 0.8% of population is vaccinated with Booster doses.As medical professionals are frontline workers managing the COVID-19 pandemic, this study aimed to evaluate their knowledge, attitude and practice and acceptability towards COVID-19 vaccination among them in our Institution.

Aim and Objectives: To assess the knowledge, attitude, practice and acceptability of COVID 19 vaccination among medical professionals in Sri Venkateshwaraa Group of Institutions in Puducherry.

Material and Methods: A Questionnaire based Cross sectional study was conducted on 276 Medical professionals (Doctors, Postgraduates, CRRI's, Dentists, Pharmacists& Nurse) working in Sri Venkateshwaraa Group of Institutions (SVGI) using convenience sampling method. Demographic characteristics, knowledge, attitude, practice and acceptance towards vaccination were collected using self-administered questionnaire and statistical analysis were done using SPSS 28.0software. Descriptive statistics, such as frequency and percentage, were calculated. Chi square test was done to elicit the difference between the groups.

Results: Among the 276 respondents, 71(25.7%) were affected with COVID 19 infection, 274 (99.2%) got vaccinated. Two doses of vaccination were taken by 250 (90.6%) and 24 (8.7%) had received one dose of vaccination during the study period. Among the25.7%who were affected with COVID19, 22.8 % have received two doses and 2.8 % have received one dose of vaccination.

Majority (97.8%) of them have adequate knowledge about the mode of transmission of COVID19and the available vaccines in India(67.8%), but there was poor knowledge about the efficacy of the vaccine(27.9%) and the dosing interval for the vaccines(17.8%). Statistically significant good knowledge was seen (P=0.029) among the Doctors, Dentists, Post graduates and CRRIs when compared to Nurses and Pharmacist.

79%of the study participants experienced common side effectsdue to vaccination.95.3% participants agreed that they follow Government COVID-19 vaccine guidelinesin order to protect the public health. 83.3%participants were willing to take Booster dose and their choice of vaccine wasCovishield 76.4%,Covaxin 21.7% and only 12% endorsed Sputnik V.

Conclusion: Our study concluded that there is a positive Knowledge and attitude among Medical Professionals regarding COVID-19 vaccination.As Vaccination among medical professionals is directly linked to the general population this study help to achieve maximal vaccination rates among general population.

STUDY THE CORRELATION OF THE CARDIAC RISK INDICES AND HIGH SENSITIVE CRP IN PREDIABETES AND DIABETES PATIENTS IN PUDUCHERRY

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Abstract

Introduction: Diabetes Mellitus and pre Diabetes are associated with Dyslipiemia and contributing to cardiovascular events leads to likelihood of CVD in future. Inflammatory marker like hs-CRP and cardiac risk indices measurement will improve the risk prediction of these events.

Aim and Objectives: This study it is aimed to assess the Cardiac risk indices and determine their correlation with hs-CRP in diabetic and pre-diabetes patients

Materials and Methods: This study included 125 patients in diabetic, 125 pre diabetes patients and 125 control groups. The anthropometric parameters like BMI, WHR and blood parameters like fasting glucose, HbA1c, lipid profile (Total cholesterol, TGL, HDL, LDL) and hsCRP, were measured. Cardiac risk indices derived from lipid profile.

Results: High Cardiac risk indices were observed in pre-diabetic and Diabetes subjects. The indices were shows significant positive correlation with BMI, fasting sugar, HbA1c, Total Cholesterol, TGL and LDL and negative correlation with HDL. The hsCRP showed significant positive correlation with cardiac risk indices in type 2 DM patients and pre-DM patients.
Conclusion: The cardiac risk indices values are increased in diabetic and pre-diabetic patients, shows increasing the CVD susceptibility of these patients in future. The correlation of indices values with hs CRP shows that association of inflammation with CVD risks in type 2 DM and pre-DM patients. Screening of these indices among study population will help the prevention of future development of CVD and that can be reduced by encouragement of healthy lifestyle.

Exserohilum rostratum CAUSING KERATOMYCOSIS REQUIRED EVISCERATION - A RARE CASE REPORT

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Abstract
Introduction: Fungal keratitis is common in tropical and subtropical climates constituting around half of all culture positive infective keratitis. Filamentous fungi and Candida are the commonly isolated fungal pathogens. Dematiaceous fungi like *Exserohilum* spp., *Curvularia* spp., *Alternaria* spp. can also rarely cause fungal keratomycosis. We, hereby, report a rare case of perforated corneal ulcer secondary to exserohilum keratitis.
Case Report: A 85-year-old man presented to the Ophthalmology OPD with a right eye perforated corneal ulcer which subsequently needed surgical evisceration. Gramstain, 10% potassium hydroxide wetmount , and bacteriological culture of ocular samples were negative. Sabouraud dextrose agar tubes showed grey to olivaceous black floccose growth, while lactophenol cotton blue preparation showed phaeoid/dematiaceous septate hyphae that was phenotypically identified as *E.rostratum,* and further confirmed by Matrix Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry. Patient was initially discharged on topical antibiotics. Topical antifungal (5% Natamycin) eyedrop was added subsequently based on positive fungal culture report.
Discussion: Keratomycosis is commonly caused by hyaline moulds like Fusarium, Aspergillus, Acremonium, Penicillium etc. following injury with vegetable matter. Exserohilum species are commonly found as environmental moulds in hot and humid climatic regions. *Exserohilum* species usually cause phaeohyphomycosis involving the skin, subcutaneous tissue, nose, paranasal sinuses etc. Incidence of *Exserohilum* species related keratomycosis is very low- 1.3% to 6.6% among all mycotic keratitis. Incidence of *Exserohilum* keratitis associated descemetocele or corneal perforation is still lower. Our case presented with such a rare finding of *Exserohilum* keratitis associated corneal perforation which needed evisceration.
Conclusion: Exserohilum is a new emerging corneal pathogen, albeit rare. Exserohilum keratomycosis should be considered as a differential in all clinically suspected fungal keratitis, especially in hot and humid climates. Early diagnosis, identification of fungal species and timely treatment are key to successful clinical outcomes in fungal keratitis.

A COMPARATIVE STUDY OF SEVERITY STATUS OF COVID -19 INFECTION IN DIABETIC AND NON DIABETIC PATIENTS

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Abstract
Introduction: Covid 19 infection has emerged as a rapidly spreading communicable disease all over the world. Type 2 DM is a common health problem all over the world especially in India. Several studies have suggested higher susceptibility to some infections especially COVID in diabetes patients due to dysregulated immune response. Diabetes patients presented with higher inflammatory serum markers than non diabetic patients indicating the severity of infection. Also diabetics were at a higher risk of intensive care which usually means invasive ventilation.
Aim and Objectives: The aim of this study is to prove that type 2 DM patients were more susceptible to COVID-19 infection than non diabetic and disease severity and hospitalisation of COVID 19 was more in type 2 DM patients.
Materials and Methods: A total of 100 RTPCR confirmed COVID 19 patients were taken for the study. FBS, PPBS, HBA1C and RBS values were assessed to categorise COVID 19 patients as diabetic and non diabetic individuals. The severity of COVID 19 infection was assessed based on the D dimer levels, CT severity score, the need for oxygen support, duration of hospital stay and outcome of the disease.

Results: It was a retrospective analysis of 100 patients with RTPCR positive COVID 19 infection admitted in the hospital and on oxygen support .Out of this 65% were diabetic and 35% were non diabetic. The percentage of death, severity of infection, need for invasive ventilation and higher D dimer values were found in diabetic than non diabetic individuals. Also the association of diabetes with severity of infection, need for invasive ventilation, higher D dimer values and poor disease outcome were found to be statistically significant

Conclusion: From this study we conclude that the severity of COVID 19 infection is higher in diabetic than non diabetic patients.

ASSOCIATION OF NUTRITIONAL STATUS WITH TYPE OF NUTRITIONAL SUPPLEMENTS - A COMMUNITY BASED CROSS SECTIONAL STUDY

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Abstract

Introduction: Integrated Child Development Scheme (ICDS) was launched in India during 1975, to tackle the high prevalence of malnutrition among under 5 children. NFHS 5 (2018-2019) reports that only 41.2 % of ICDS services were utilized by them. To ensure the effectiveness of the scheme, nutritional status of under 5 children and the form of nutritional supplement - either Hot Cooked Meals (HCM) or Take Home Ration (THR), preferred by the beneficiaries must be assessed.

Aim and Objectives: To determine the prevalence of under nutrition among the study participants and to compare the nutritional status of 1-5 years children using Take Home Ration (THR) or Hot Cooked Meals (HCM).

Materials and Methods: A Cross sectional study was done among under 5 children for a period of 2 months, in three randomly selected villages, Rural Field Practice Area, Sri Venkateshwaraa Medical College Hospital & Research Centre, Puducherry. Weight and Height were measured for all the eligible children using standard procedures. World Health Organization (WHO) growth standards were used to assess their nutritional status. The mothers were enquired about the nutritional supplement utilization, type of nutrition supplement (HCM/THR) utilized by their children and the reasons for preference. Statistical significance was assessed by chi-square test.

Results: Among 153 children enrolled, 138 (90%) were consuming HCM and 15 (10%) were THR utilizers. The overall prevalence of under nutrition was 59%. The prevalence of underweight, stunting and wasting were 48%, 48% and 65.5% respectively and it was found significantly (p value <0.05) higher among THR utilizers (60%, 53% and 73% respectively) than those receiving HCM (25%, 25% and 35%).

Conclusion: HCM was preferred more than THR. The children receiving HCM were better nourished than THR utilizers.

SANITATION AND STUNTING- A COMMUNITY BASED CROSS-SECTIONAL STUDY

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Abstract

Background: Under nutrition is the major health problem among under five children. Growth of the children is not only related to dietary deficiency, but also to surrounding environmental condition.

Objectives

To assess the nutritional status of 1-5 years aged children

To determine the proportion of children growing under poor environmental condition

To determine the association between stunting and environmental sanitation

Methods: A Cross sectional study was done among children aged between 1 to 5 years for a period of 2 months in three randomly selected villages of rural field practice area of a tertiary care hospital, Puducherry . Weight and Height were measured and WHO growth standards were used to assess their nutritional status. The mothers were enquired about the environmental sanitation, using a content validated questionnaire. Chi-square test was used to determine the association between sanitation and stunting. Multivariate logistic regression analysis was used to determine influence of selected factors associated with under nutrition

Results: Out of 153 children, around half (56%) of the children were under nourished. The prevalence of underweight, stunting and wasting among the study participants were 28%, 26% and 37% respectively. Nearly two-third (61%) of the children were exposed to poor sanitation practices. Children growing under poor environmental condition was found to be

stunted, and the association was significant ($p<0.005$).Among the various selected influencing factors, adolescent pregnancy was found to be significantly associated with under nutrition.

Conclusions: In spite of various measures to combat nutritional deficiencies, mortality and morbidity ofunder five children due to the malnutrition still exists. Proper environmental sanitation will help to avoid recurrent infection in children and hence malnutrition.

CLINICAL CHARACTERISTICS OF MORTALITY OF COVID19 PATIENTS IN A TERTIARY CARE CENTRE IN PUDUCHERRY

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Abstract

Introduction

A worldwide outbreak of a respiratory illness, first detected in December 2019 in Wuhan city, Hubei province, China is ongoing. The rapid spread of the COVID 19 infection has un-doubtedly become a burden to health systems in several countries, as a significant proportion of elderly, immunosuppressed and those with underlying metabolic, cardiovascular or respiratory diseases continue to develop severe forms of COVID-19 and are at an increased risk for adverse outcomes.

Aims and Objectives

To asses the clinical profile of patients died of covid 19 infection in a tertiary care hospital in Puducherry.

Materials and Methods

This was aretrospective observational study conducted in SriVenkateshwaraa Medical college and Research Centre, Puducherry. The case sheets of people who died of COVID19 infectionconfirmed either by covid RTPCR or CT THORAX from 1st April 2021to 30thJunewere obtained and required data like age, sex, risk factors, symptoms with their duration, day of starting oxygen therapy , duration of hospital stay were collected from the medical records department and entered in Microsoft excel and analysis done by SPSS VERSION 23.0

Results

Total of 119 patients were considered for the study in which 68% were male and 32% were female. The mean age of study was 58 (SD 13.1). 25% of the patients were in the age group 40-50 years and 50-60 years each. Fever was thepredominant symptom inour study accounting for 37% which was followed by cough in 29% and dyspnea in 15% of patients. The mean duration of the symptom was 5 days (SD2.69). Among risk factors, 61% of patients had diabetes, 30% had hypertension and other risk factors accounted for 9%. On arrival to casualty, 95% of the patients required oxygen therapy and of these 10% required invasive ventilation. Patients on an average received 18 days of medical attention before death.

Conclusion

In this study, predominantly middle aged and those with diabetes and hypertension succumbed to the disease. Also, 95% of people seeked medical attention at a later stage of the disease requiring oxygen therapy at admission. 18 days was the average duration of hospital stay . Hence public health campaigns can be aimed at reducing the risk factors and also people should be educated to seek medical attention at the earliest in future.

Key words : COVID 19 mortality, age, gender, co-morbidities.

A STUDY ONCLINICAL AND BIOCHEMICAL PROFILE OF COVID-19 PATIENTS RECEIVING OXYGEN THERAPY REQUIRING VENTILATORY SUPPORTIN A TERTIARY CARE CENTRE

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Abstract

Introduction

Coronavirus 2019 (COVID -19) is a contagious disease caused by the recently discovered severe acute respiratory syndrome. Several studies have demonstrated that age, co morbidities and abnormalities in different clinical biomarkers can be important to understand disease severity. This study was conducted to document different clinical and biochemical profiles in srivenkateshwaraa medical college, Ariyur

Aims and objectives:

To study the clinical and biochemical profile of covid-19 patients receiving oxygen therapy who requires ventilator support

To assess various parameters like vaccine status, time of admission, clinical status on admission, initiation of anti viral,steroid, CRP, FERRITIN D-DIMER levels, HRCT-CORADS scoring of patients on oxygen therapy later requires ventilator support.

Materials and Method:

This is a retrospective study of all patients admitted to Sri Venkateshwaraa Medical college hospital between March 01, 2021 and June 30, 2021, with a diagnosis of COVID-19 receiving oxygen support later becomes ventilator dependent. From a common database prepared for COVID-19, we retrieved the relevant data and compared the clinical findings,biochemical parameters and outcomes of covid ward and covid ICU patients.

Results

The results under statistical analysis

Conclusion

In this study the most common presentation followed by dry cough and fatigability in covid patients. Diabetes and hypertention are the common co morbidities. CRP,serum FERRITIN,D-Dimer, HRCT,SGOT and SGPT should be monitored to differentiate between mild and severe cases.

RISK STRATIFICATION OF PATIENTS ADMITTED WITHCOVID 19 TO A TERTIARY CARE HOSPITAL IN PUDUCHERRY USING ISARIC - 4C MORTALITY SCORE.

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Abstract

Introduction

Risk stratification of patients is the most important aspect in the management of COVID-19 as there is need to prioritize critical care services in situations of overwhelming numbers of patients. Globally many scoring systems have been specifically developed for risk stratification in COVID-19 patients.

Materials and Method

This study is conducted with the objective of finding out the association of various clinical and laboratory parameters as used by 'International Severe Acute Respiratory And Emerging Infections Consortium / World Health Organisation - Coronavirus Clinical Characterisation Consortium (ISARIC/WHO 4C)'Mortality score in predicting high risk patients of COVID-19.

Results

It is observed that the Covid disease present differently in different regions of the world. Hence the applicability of the ISARIC -4C score in the local population needs to be studied. Ascertaining this ISARIC-4C risk score in our set up would help in triage of patients of severe disease at the outset, and shall prove beneficial in improving the standard of care.

Conclusion

A positive correlation would help us in triage of patients of severe disease at the outset, and prove beneficial in improving the standard of care during the subsequent wave of covid infection.

ASSESSMENT OF EUTHYROID TYPE 2 DIABETICS WITH HAEMOGLOBIN AND SERUM BILIRUBIN LEVELS RELATING TO GLYCAEMIC CONTROL – A RETROSPECTIVE STUDY

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Abstract

Background: Diabetes can subsidize to anaemia through reducing absorption of iron laterally with diabetic complications. Thyroid dysfunction may turn influence the glucose metabolism in diabetes, changes in serum TSH were found to be related with changes in glycated haemoglobin (HbA1c). In this way, our review is to examine the relationship between glycated haemoglobin with the degree of haemoglobin and serum bilirubin in euthyroid T2DM patients.

Methodology: The study was a retrospective analysis of euthyroid type 2 diabetic patients (FBS≥126) who had attended the clinics in the department of General Medicine namely diabetic clinic. Collection of data approximately 1100 following analysis for a period of four years (2018 to 2021). The hospital records (case history from MRD) were used as the sources of information.

Results: PBS, HbA1C, LDL, TSH were highly significant with fasting blood sugar whereas RBC and MCV with HbA1C and total bilirubin with haemoglobin of p value 0.000. When HbA1c ≥ 7 greatly significant with LDL and Hb with Cholesterol, total protein, bilirubin, T4 and WBC whereas HbA1c ≤ 7 correlated with FBS alone. Multivariate Binary Logistic Regression analyses showed Age and Hb is strongly associated with TSH as dependent variable of p value 0.000 and 0.027respectively

Conclusion: Thus, our findings suggest that complete blood count and thyroid status could be considered as a routine screening parameter forT2DM patients.

Keywords: T2DM, Euthyroid, Complete Blood Count, Haemoglobin, Bilirubin.

DEEP PENILE ULCER- A DIAGNOSTIC DILEMMA

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Abstract

Introduction

Deep Penile ulcer can be of venereal and non-venereal origin. Venereal causes include primary syphilis, chancroid, granuloma inguinale and non-venereal causes include tuberculous chancre, malignant ulcer, crohn's disease, amoebic ulcer etc. Here we present an uncommon infection in a diabetic leading to penile ulcer.

Case Report

A 45 year old diabetic male presented with painful deep penile ulcer. He was thoroughly investigated for common causes of genital ulcer and HIV screening was also negative. On detailed evaluation, it was found that ulcer was secondary to enterococcal infection.

Discussion

Enterococcus faecalis is a commensal of genital flora. However, it has been established as a pathogen in diabetic trophic ulcers. In this case, enterococcus has been isolated as a pathogen from a penile ulcer. This also highlights the possibility of sexually acquiring the infection from his partner. Approximately 11% of women of reproductive age group harbor the organism in the genital tract.

Conclusion

After ruling out common causes of penile ulcer, rare causes like enterococcal infection should be considered especially when the patient is diabetic.

KNOWLEDGE ATTITUDE PRACTICE AND ACCEPTABILITY TOWARDS COVID 19 VACCINATION AMONG GENERAL POPULATION- A CROSS SECTIONAL STUDY

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Abstract

Background: Coronavirus disease 2019 (COVID-19) has spread worldwide resulting in excess mortality, serious social and economic disruptions. Now the new variant called NeoCov has been identified recently. As of 27th January 2022, 50.3% of population has been fully vaccinated in India. Poor knowledge about vaccine, vaccine hesitancy and disinformation about vaccine poses major challenge to achievement of coverage and population community. Hence this communitybased study will provide an insight about the people's Knowledge, Attitude, Practice and Acceptability towards COVID 19 vaccines and it will help the Government to address the barriers and help them to promote and improve vaccine awareness among general population.

Objective

To assess the knowledge, attitude, practice and acceptability of COVID 19 infection and COVID 19 vaccination among General population in Puducherry.

Materials and Methods
A questionnaire based cross sectional study was conducted among general population in & aroundPuducherry. The survey was conducted on 2345 participants residing in Ariyur and Villianur using probability proportion to size sampling technique. Astructured questionnaire with five sections (i.e., demographics, knowledge, attitude, practice and acceptability) were collected from the general population. Statistical analysis weredone using SPSS 28.0 software. Descriptive statistics, such as frequency and percentage were done. Chi-square analysis were performed to compare the socio-demographic variables with the knowledge and attitude score variables.

Results
Among 2345 respondents,759(32.3%) from Ariyur and 1586(67.7%)from Villianur. 55.1% were males and 44.8% were females.25.5% participants had comorbidities and 5.9% had COVID 19 infection.Two doses of vaccination were taken by 44.3%, one dose of vaccination by 38.8% and 16.9% were not vaccinated during the study period.The mean score of knowledge and attitude were 6.19±2.0 and 8.092 ± 1.24 respectively.54.9% and 35.9% of the respondents knew about the name of the vaccines and interval between two doses of vaccines respectively.Only 60.6% of the study participants will have or had the vaccination without any hesitation and most common cause of the hesitation was the side effects (59.2%) and 98% believed the vaccine should be available at free of cost. Among the respondents who received the vaccination, 54.8% experienced side effects. 96.1% followed the protective measures against COVID 19. 69.7% believed that COVID 19 vaccines will protect them against infection.
Conclusion
Our findings showed adequate knowledge andpositive attitudes towards COVID-19 vaccine among the general population. The most important factor for vaccine hesitancy is the occurrence of mild or serious adverse effects following immunization. In order to improve knowledge, immediate and periodic health education programs need to be strengthenedregarding COVID 19 vaccinations.
Keywords: COVID 19, Knowledge, Attitude, Practice, Acceptability

IMPACT AND ACCEPTANCE OF INFECTION CONTROL NURSES OF HOSPITAL INFECTION CONTROL COMMITTEE AMONG HEALTH CARE WORKERS

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Abstract
Background
Healthcare acquired infections (HCAIs) is associated with increased morbidity and mortality among hospitalized patients and predisposes healthcare workers (HCWs) to an increased risk of infections. The study explores the acceptance and the impact of having Infection Control Nurses (ICNs) and practices of infection control among HCW in a tertiary referral centre in Puducherry.
Materials and Methods
This is a cross sectional study. A self-administered structured questionnaire was distributed to the study group (of doctors and nurses). Data on soft skills, knowledge and practice of infection control were obtained and analyzed before and after ICNs were recruited. Study population were selected by convenience sampling.
Results
A total of 200 responses were analyzed, 100 were nurses while 50 were doctors. Most of the respondents have correctly answered to the questionnaire that their knowledge and practices have increased after they were continuously monitored by ICNs on a daily basis. The overall correct answers rate to the selected questionnaire before and after recruitment of ICNs was 52% & 84% respectively. The HCW also answered that the acceptance of ICNs in the initial stages was very difficult and slowly developed the trust and the scenarios are much different after a certain period of time.
Conclusion
ICNs play a very major role in implementation of Infection control practices and thus have to be well trained and teach them various knowledge ,practices and skills including soft and communication skills to have good interaction, develop trust and at the same time to make sure the works are being carried out ina smooth fashion.

INCIDENCE AND PROGNOSTIC INDEX OF SECONDARY BACTERIAL INFECTION IN COVID 19 PNEUMONIA

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Abstract:
Introduction: Coronavirus disease is the greatest ongoing global pandemic of our generation .Co infections can modify the virulence of the virus and cell death, therefore altering disease severity.They lead COVID 19 patient into a vicious infectious circle of life and are substantially associated with significant mortality and morbidity.Diagnosing secondary infection in COVID 19 patients will help to improve the clinical outcome and mortality and to warrant extra caution to prevent the occurrence of secondary bacterial infection and to practice appropriate antimicrobial stewardship**.**
Aim: to determine incidence of secondary bacterial infection in covid 19 pneumonia and to find the outcome of covid 19 patient with concomittent infection.
Methodology: It is a retrospective cohort study done by observation of 70 case sheets who have admitted in covid ward during the time period of June 2021 to june 2022. All case sheets with age more than 18yrs with both gender were included in the study. RT PCR negative and lack of documents in case sheets were excluded from the study. Collected date were statically analysed.
Results:A total of 70 patients were included. 14 patients (19.5%) were culture positive. Among 14 patients 2 patients (14.2%) were found to death.
Conclusion: Secondary bacterial growth is a predictor of in-hospital mortality in patients with COVID-19. In settings with limited resources, efforts to reduce mortality in COVID-19 should focus on early identification of secondary bacterial growth and early treatment with appropriate antibiotics.
Keywords:Covid19, secondary bacterial infection.

A RETROSPECTIVE STUDY ON MORBIDITY AND MORTALITY IN COVID ICU PATIENTS

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Abstract
Introduction
Studies have consistently described poor clinical outcomes and increased ICU mortality in patients with severe coronavirus disease 2019 (COVID-19) who require mechanical ventilation (MV). This study is to describe the clinical characteristics and outcomes of patients with severe COVID-19 admitted to ICU in sri Venkateshwara medical college and hospital puducherry.
Aim &Objective
To determine the morbidity and mortality of patients with severe COVID-19 in the intensive care unit (ICU) in relation to age, gender, co-morbidities, ventilatory status, and length of stay (LOS).
Material and methods
Study design : Retrospective study.
Study setting : Academic hospital.
Sample size : 100
During the period of data collection patients satisfying Eligibility criteria will also be included in the study
Participant timeline: 4 months (April2021 – July 2021)
Eligibility criteria:
Inclusion crirteria:
1.severe covid pneumonia patients includes
-respiratory rate >30breaths/min
-severe respiratory distress
-spo2<90% on room air
2.Acute resapiratory distress syndrome
3.sepsis
4.septic shock.
Exclusion criteria:
Mild and moderate category patients

Results
Between April 2021-july 2021,around 40 % the patient had severe COVIDpneumonia.Among severe COVID pneumonia patient 57% had comorbidities. The length of stay in COVID ICU was increased among patient with comorbidities.The mortality was higher in population with comorbidities.
Conclusion
In our study morbidity and mortality due to COVID 19 was higher in COVID ICU patients.In order to decrease the morbidity and mortality, vaccination should be encouraged among the population with comorbidities.

CORRELATION OF SERUM C-REACTIVE PROTEIN (CRP) LEVELS AND CLINICAL OUTCOMES IN PATIENTS WITH COVID-19 IN A TERTIARY CARE HOSPITAL

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Abstract
Introduction
The systemic inflammatory response to the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection is a hallmark of the 2019 coronavirus disease (COVID-19), and most hospitalized patients with COVID-19 have abnormal inflammatory biomarkers.C- reactive protein (CRP) is a type 1 acute phase response protein whose synthesis in the liver is regulated by the pro-inflammatory cytokines IL-6,IL-1 and TNF. Elevated plasma levels of CRP have been reported to be marker of endothelial cell dysfunction. **AIMS AND objective:**
The aim of this study is to explore the associations between CRP concentrations at initial hospital presentation and clinical outcomes of patients who were covid 19 positive.
Materials and methods
A single Centre, Retrospective Medical Record based Observational Study done in Department of General Medicine at Sri Venkateshwara Medical College Hospital and Research Centre, Ariyur, Puducherry,inpatients with COVID RTPCR positive in SVMCH&RC in a patients of 72 in number, During the period of data collection patients satisfying eligibility criteria will be included in the study.
Results and conclusion
The level of plasma CRP was positively correlated to the severity of COVID-19 pneumonia. Our findings could assist to discern patients of moderate to severe COVID-19 pneumonia from the mild ones. Our findings may be useful as an earlier indicator for severe illness and help physicians to stratify patients for intense care unit transfer.
KEY WORDS: COVID-19,CRP.

OUTCOME OF VACCINATED AND UNVACCINATED COVID 19 PATIENTS IN A TERTIARY CARE HOSPITAL AT PUDUCHERRY

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Abstract
Introduction
Breakthrough infections after adequate vaccinations are matter of concern but adequate data regarding these infections are not available in real world setting. Vaccines have effectiveness in decreasing risk of getting COVID19 infections by 70-90%, and also shield from severe infection. It is possible, therefore, some people who are fully vaccinated against COVID-19 may get COVID19 infection.These breakthrough infections are either asymptomatic or mild in nature.Breakthrough infection in India after complete dose of vaccination should be prime area of research.This brief report describes vaccination status,morbidity and mortality vaccinated and unvaccinated patients & breakthrough infections in Covid 19 patients admitted in a tertiary health care hospital.

Aims and Objectives
Aim and Objective of this study is to compare the clinical characteristics, morbidity and mortality among vaccinated and unvaccinated Covid 19 patients admitted in a tertiary health care hospital at Puducherry between June 2021 and September 2021.
Result
The results are under statistical analysis and will be published during the conference.
Discussion
Reinfection with SARS-CoV-2 has been documented, but the likelihood of getting reinfection among vaccinatedpatients is lesser when compared to unvaccinated COVID-19 patients and these reinfection are mostly either asymptomatic or mild in nature.
Conclusion
By promoting the public for vaccination against COVID-19 ,we can reduce the disease severity and by knowing the COVID-19 vaccination status at the time of hospital admission, we can anticipate the disease severity and outcome.

ZOSTERIFORM CUTANEOUS METASTASIS- A CLANDESTINE THREAT

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Abstract
Introduction
Cutaneous metastasis is an uncommon presentation of internal malignancies and zosteriform cutaneous metastasis(ZCM) is very rare. Here we present a treatedcase of invasive ductalbreast carcinoma presented as zosteriform cutaneous metastasis.
Case report
A 55 years old female presented with four weeks history of painful, pruritic lesions over left upper back and chest. She had invasive ductal carcinoma of left breast two years back and was treated with modified radical mastectomy and adjuvant chemo- and radio-therapy. Cutaneous examination revealed multiple, grouped, firm, erythematous pseudo-vesicular papules, nodules, plaques along the distribution of left T4 to T6 dermatomes. Skin biopsy showed features of cutaneous metastasis from invasive ductal breast carcinoma.
Discussion
ZCMis rare with very few case reports. It has two aspects; one is zosteriform lesions and the other islesions in zosteriform distribution. Many of these metastases have initially been diagnosed as herpes zoster and treated with antivirals. ZCM might be due toKoebner-like reaction or spread via perineurallymphatics or via dorsal root ganglion veins or coincidentally during surgery due to implantation.
Conclusion
ZCM may be the first sign of an underlying primarycarcinoma or recurrence thereof. Hence,any case of non-healing herpes zoster-like presentation should always raise the suspicion of cutaneous metastasis and biopsy must be considered for early diagnosis and management of underlying malignancy. This case highlights the importance of being vigilant about zosteriform cutaneous metastasis and is presented for its clinical rarity.

SLEEP HYGIENE INTERVENTION, ITS EFFECTIVENESS IN REDUCTION OF INSOMNIA AND OBESITY

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Abstract
Background
Adequate amount and quality sleep is the basic need for any human but is often neglected. Recently, strong relationship between the improper sleep pattern and obesity is been observed.

Objectives
To assess the prevalence of Insomnia based on Insomnia Severity Index (ISI) grading
To determine the efficacy of Sleep Hygiene Intervention Programme in reducing Insomnia and Obesity
Materials and methods
This Interventional study was conducted among602 Private Medical college students in Chennai. It was conducted in 2 phases, In phase 1, all the participants were surveyed using ISI scale and parameters like Height, weight were noted.Those with ISI scores between 8-21 and the BMI ≥30 were eligible for Phase 2.In the Phase 2 using, Stratified Random sampling method,100 participants were selected, 50 each for CONTROL & INTERVENTION group.Periodical Sleep hygiene Intervention was given for Intervention group, insisting to follow 10 simple non-pharmacological behavioral measure to promote sleep hygiene.Followup assessment of weight(Kg) was done at Day 1 and at the end of 1, 2 and 4 months in Intervention group and ISI survey was done at Day 1 and at end of 4 months in both the groups.The statistical tests such asPaired t test, RMANOVA, Bon ferroniPosthoc test was used for significance.
Result
The prevalence of No clinical significant Insomnia, Sub threshold insomnia, Moderate Clinical Insomnia, Severe Clinical Insomnia were 151(25%), 289(48%),144(24%) and 18(3%) respectively. On comparing the weight and ISI change from Day 1 to 4 months in Control and Intervention group it was found that Intervention group had better reduction in weight (62.48 ± 10.62 kg to 51.48 ± 6.88 kg)and insomnia severity, determined by ISI score (13.70 ± 3.62 to 10.34 ± 2.43) than Control group, where the weight and ISI score change was from 61.58±10.35 kg to 62.48 ±10.66 kg and from 12.56±3.78 to 13.50±3.63 respectively.
Conclusion
Simple Non-pharmacological intervention seem to have greater benefits in improving sleep hygiene and reducing obesity. Hence such Behavior change communication based Sleep Hygiene Intervention programmes should be provided on regular basis to achieve maximum benefit.
Keywords: Sleep, ISI, Obesity, Insomnia

MORPHOMETRIC STUDY OF THE INFRAORBITAL FORAMEN – A DRY BONE ANALYSIS

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Abstract
Introduction
The infraorbital foramen (IOF) is one of the most important facial foramina, which is located on the anterior face of the body of the maxilla, around 1 cm below the infraorbital border (IOB). The infraorbital artery and nerve are responsible for the vascular and nervous supply to important areas of the face passes through this foramen. It is important for operating surgeons and anesthetists to be aware of the anatomical variations that may occur in the region of the IOF, when surgical interventions at the level of the middle third of the face or regional block of the infraorbital nerve are performed. Thus, the comprehensive knowledge of the facial foramina i.e. IOF is important to avoid accidental/ iatrogenic complications.
Aim nd Objectives 1. To examine the presence or absence of infraorbital foramen bilaterally
2. To examine the multiplicity of foramen.
3. Locating the infraorbital foramen from different bony landmarks.
Materials and Methods
The morphometric study on the IOFs was conducted on dry human skulls. The number of foramina were noted. The distances from the foramen to the infraorbital margin (IOM), anterior nasal spine (ANS) was noted. The measurements were made with the aid of digital calipers with precision to 0.01 mm. Presence of foramina and their multiplicity was also observed. The center of the foramen was taken as the reference point, and distances were measured from this point. In cases of multiple foramina, the reference point was taken to be on the largest foramen.
Results
All the skulls had foramen bilaterally.
SMean distance from foramen to IOM right side 6.622 mm, left side 7.124 mm.
Mean distance from foramen to ANS right side 31.112 mm, left side 34.878 mm.
conclusion
Many studies show the evidence that, there is racial variation in the position of the infraorbital foramen (IOF). Therefore, detailed knowledge of the population specific data on biometric features of (IOF) will help the clinicians, operating surgeons in the therapeutic, diagnostic and also surgical procedures to be done more precisely in the vicinity of the maxillofacial region.

AMORPHOLOGICAL STUDY AND INCIDENCE OF FORAMEN MENINGO – ORBITALE IN DRY HUMAN SKULL

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Abstract

Introduction

Themeningo-orbital foramen is situatedin the greater wing of the sphenoid bone close tothe superior orbital fissure.This structure isnot invariably present in human skulls, hence not mentioned in "NominaAnatomica". It communicates with the interior of the orbit with the middlecranial fossa. The foramen represents the remnant of an embryonic conduit for the supraorbital division of the middle meningeal artery en route to the orbit and the developing ophthalmic artery.

Aim and Objectives

The knowledge of this foramen and structure related with it, is of great significance for ophthalmologist and neurosurgeons for surgeriesin the orbit and cranial fossa. With this background in mind the present study was conducted.

Material & Methods

48 dry human skull of adult age from department of Anatomy and department of Forensic medicine, SVMCHRC, Ariyur were observed for the presence or absence of foramen. The patency of foramen was confirmed by passing a probe. The foramen distance from the supra orbital foramen or notch, and from the fronto- zygomatic suture were measured using digital Vernier'scaliper.

Results

We studied 48 (96 orbits) dry human skulls of adult age in which meningo-orbital foramen were observed in 38 orbits (40%). In 14 (15%)orbits this foramen were bilateral and unilateral meningo-orbital foramen were found in 24 orbits (25%). The distance of foramen from supraorbital notch or foramen was 34.00mm 927-42mm), and distance from the fronto-zygomatic suture was 24.4mm (20.3-34.2mm).

Conclusion

The presence of foramen and the anastomosis branch with middle meningial artery and ophthalmic artery passing through it, is of valuable information for surgeons reconstructing the base of skull, and in excision of meningiomas.

Key Words: Meningo-orbital foramen, Middle menengial artery

EFFECT OF ANTI-DEPRESSANTS ON SERUM ELECTROLYTES AMONGTHE PSYCHIATRIC PATIENTS IN A TERTIARY CARE HOSPITAL

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Abstract

Objective

To evaluate the effect of anti-depressants on serum electrolytes and to ascertain its class specificity.

Methodology

This hospital based cross sectional study was conducted among psychiatric patients treated with anti-depressants in our hospital, from November 2017 to October 2018. The study consists of 2 groups (n=80). Control group consists of age and sex matched healthy individuals selected after biochemical test, complete physical and psychiatric examination. Test group consists of psychiatric patients treated with anti-depressants for a duration of >3 months. Serum electrolytes were analyzed in both groups. Data was analyzed using SPSS 21.0 software and expressed in descriptive statistics.

Results

The mean age of the study population was 41.5 years, out of which 45% were males and 55% were females. The mean BMI was 27.8 ± 4.5(overweight). The mean duration of treatment was 5.5 months. The commonly prescribed anti-depressants were Mirtazapine (30%), Sertraline (25%) and Escitalopram (20%), followed by 5% of Amitriptyline, Duloxetine, Citalopram, Fluoxetine& Venlafaxine each. The mean serum levels of sodium were 132.5 ± 1.7 in the test group when compared to control subjects 141.2 ± 3.1 ($p<0.001$). The potassium and chloride levels were (4.08 ± 0.2, 4.08 ± 0.3) and (102.6± 3.7, 100.4 ± 2.2)in test and control groups respectively.

Conclusion

Our study elicits a statistically significant reduction in the serum sodium levels among the patients treated with anti-depressants, when compared with healthy controls.

KEYWORDS: Anti-depressants, serum electrolytes, psychiatric disorders

A RETROSPECTIVE STUDY ON CLINICAL PROFILE OF RTPCR NEGATIVE COVID 19 PNEUMONIA PATIENTS IN A TERTIARY CARE HOSPITAL

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Abstract

Introduction

The ongoing COVID-19 pandemic, with its clinical unknowns and a lack of standardized quantitative assays for Severe Acute Respiratory Syndrome Corona virus 2 (SARS-CoV-2), has brought on an unprecedented level of interest in the clinical utility RTPCR status and CORADS scoring. The proposed disease pathogenesis of COVID-19 indicates two overlapping disease states: an initial viremic phase where signs and symptoms are attributable to viral replication and a subsequent inflammatory phase where the severity and critical illness is attributable to a "cytokine storm." While these two phases have different management implications from a treatment choice perspective – antiviral/antibody therapy versus anti-inflammatory therapy – a clear prognostic approach is still missing. The ability to predict prognosis and infectiousness of patients during the viremic phase could fundamentally impact triage and management of patients and, potentially, of their contacts.

Aims and objectives

To study the clinical profile of RTPCR swab negative with HRCT features suggestive of pneumonia in covid suspect patients admitted in a tertiary care hospital in Pondicherr

Methodology

A retrospective study of 30 patients RTPCR negative CORADS 4 and CORADS 5 patients are identified and their clinical profile compared with 30 patients of RTPCR-positive Covid pneumonia. Clinical profile like age, sex, day of positivity ,comorbities ,days of Oxygen requirements ,need of CPAP/invasive ventilation were compared.

Results

The results of the study will be dissucussed in the conference.

Implication

The outcome of the study is , could RTPCR status in covid pneumonia patients be a prognostic marker of early recovery and less morbidity.

NON-RESPONDERS TO HEPATITIS B VACCINE IN A GROUP OF HEALTH CARE WORKERS

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Abstract

Introduction

Hepatitis B infection is one of the major public health problems globally and is the tenth leading cause of death. Worldwide, more than two billion of the population have evidence of past or recent HBV infection and there are more than 350 million chronic carriers of this infection. Occupational exposure to hepatitis B virus is a well- recognized risk for healthcare workers (HCWs), and it is dependent on the frequency of percutaneous and permucosal exposure to blood or body fluid containing blood, which commonly occurs due to needle stick or other sharp device injuries (NSIs). Throughout the world, millions of healthcare professionals work in health institutions and it is estimated that 600,000 to 800,000 cut and puncture injuries occur among them per year, of which approximately 50% are not registered. The seroconversion rate differs among individuals following vaccination against HBV. Information on HBV vaccination coverage, the practice of universal precautions among HCWs, the assessment of protective levels of anti-HBs among vaccine recipient and the need for booster vaccination is scanty, but this information is needed to rationalize strategies for better HBV infections controls.

Aim

To analyse non responders to hepatitis B vaccine in health care workers.Anti-HBs level was tested in HCWs (Nursing staffs and lab technicians) following HBsAg vaccination as seroconversion rates differ after vaccination against HBV.

Methodology
This is an analytical study to measure the anti-HBs levels and HBsAg using ELISA (Bio Rad (EVOLIS) Twin Plus) machine, (DiaproHBsAb assay kit)in a immunised group of HCWs.300 Health care workers were included in this study.3ml of blood were collected from the HCWs in person explaining them the exact protocol of this study.
Results
The study population comprised 258 Females and 42 Males of the total participants 300.204 (68%) had received all three doses of vaccination and the rest 79(26.4%) had received two doses only.17(5.6%) participants had taken the boosters dose in addition to their three dose regimen vaccination,Overall 300, 58(19.4 %) of the vaccines did not develop a sufficient anti-HBs<10IUml.44 (14.6%) had an anti-HBs titer between 10 to 100 lU/ml and the rest 198 (66%) had an anti-HBs titer of >100 lU/ml. Detection of HBsAg was done in the 58(19.4%) non responders. All the non-responders were negative for HBsAg.
Conclusion
HCWs who have taken vaccine tend to be careless about following universal precaution, as there is a general assumption of complete protection,this study clearly under mines that assumption.
Keywords: Health care workers, Non responders to hepatitis B vaccine, HBsAg, ELSIA.

MEDICAL SCIENCES-POSTER

A RARE CASE OF TORSION OVARY DURING 3RD TRIMESTER OF PREGNANCY

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Abstract
Introduction: Torsion of ovary is total or partial rotation of the adnexa around its vascular axis or pedicle, most commonly seen are dermoid and serous cystadenoma. Risk of ovarian torsion rises by 5 folds during pregnancy. Incidence is 5 per 10000 pregnancies. Ovarian torsion occurs most commonly in first trimester, occasionally in second trimester and rarely in third trimester.
Case History: 29 years G3 P1 L1 A1at 33 weeks of gestation presented to casualty with sudden onset of right lower quadrant pain associated with 3 episodes of vomiting for 1 day. She gives history of ovarian cyst prior to pregnancy but records unavailable. No bleeding/ leaking PV and able to perceive fetal movements well.
On examination: afebrile, vitals stable.
P/A: uterus corresponds to 32 weeks. Single fetus in longitudinal lie in cephalic presentation. Fetal heart rate was good and regular. Severe tenderness in right iliac fossa.
P/V: cervix posterior, 50% effaced, parous os , cephalic.
Pelvic USG: Right ovary enlarged 10* 4.3 cm. a dermoid cyst of 6.5 * 6.4 cm with few cystic lesions with no visible arterial or venous flow in right ovary suggestive of ovarian torsion.
After obtaining informed consent patient underwent emergency laparotomy with right salphingo-oopherectomy.
Intra OP Findings: right ovarian dermoid cyst of size 8*5 cms, gangrenous.
Gravid uterus of 32 weeks size.
HPE: benign serous cystadenoma.
Discussion: ovarian cyst <6cm and benign on usg are treated conservatively as they undergo spontaneous resolution, > 10 cms are resected surgically. Ovarian cyst in pregnancy can be managed conservatively when diagnosed in first trimester. Optimal time of surgical intervention is 16-28 weeks. Irrespective of Gestational age, surgery may be warranted in case of ovarian torsion or rupture ovarian cyst or malignancy. Pregnancy loss seems to be very rare despite surgical interventions in these cases.
Conclusion: Ovarian torsion is a surgical emergency. Surgery must be considered irrespective of gestational age during pregnancy in these cases.

SCAR SITE ENDOMETRIOSIS

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Abstract

Introduction: Endometriosis is presence of functional and morphological endometrial glands or stromal structures outside the uterus. It generally occurs in the ovaries, posterior cul-de-sac, uterine ligaments, pelvic peritoneum, bowel, and rectovaginal septum. Extrapelvic endometriosis can be found in unusual places like in the nervous system, thorax, urinary tract, gastrointestinal tract, and in cutaneous tissue.

Case Report: 26years P2L2 with previous 2LSCS came with complaints of pain, itching and dragging sensation over scar site during menses for past 1 year, inspiteof medical management with Leuprolidepatient complained persisting pain during menses.

Investigations: MRI PELVIS- Two fairly defined lesions /irregular nodules in the anterior abdominal wall appears isointense to muscle onT1w image/T2w image suggestive of scar endometriosis. No evidence of hemorrhagic foci noted.

Intraoperative Findings: Endometrioma excision with dilatation and curettage with LNG IUD insertion done. A 3x3cm endometriotic nodule on the right side of the scar, in subcutaneous plane above recurs sheath, A2x2cm endometriotic nodule on the left side of the scar in subcutaneous plane above rectus sheath removed and sent for **HPE.**

Discussion: Scar site endometriosis commonly present with subcutaneous mass typically accompanied by cyclicalor constant pain. Menstruation usually aggravates the disease. The most evident risk factor for presence of endometriosis in scar tissue is a previous history of obstetric surgical procedures.Treatment of choice is wide excision if medical management fails.

Conclusion: Scar endometriosis is a rare entity. HPE is the gold standard for diagnosis of scar endometriosis. Scar endometriosis is one of the differential diagnoses when a woman present with painful swelling in the abdominal scar specially with history of LSCS. Since, scar endometriosis is a rare and often elusive diagnosis that can lead to both patient and physician frustration. Through physical and clinical examination is very important.

VERNEUIL'S DISEASE – THE PATIENTS BURDENSOME!

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Abstract

Introduction: It is a modified chronic painful inflammatory skin disease.The follicular unit is plugged and distended by retained keratin due to exogenous and endogenous reproductive hormones.Characterized by inflammatory reaction mediated by innate immune system.More common in females; peaks in third,fourth decade of life; occurs in single/multiple areas like axillary,inguinal,inframammary,genital regions.Clinically presents as inflamed deep seated acneiform nodules – abscesses - chronic draining sinuses- scarring-life altering disability.

Case report: A 36 years old male came with multiple swellingswith discharge in bilateral axillary,groin,scrotum,perineal region for 2 years. Multiple acneiform nodules with pus discharging sinuses,surrounding signs of inflammation in bilateral axillary, groin, scrotum, perineal region.On investigations, total counts were raised. Treated with IV antibiotics.Wide local excision done. HPEproved hidradenitis suppurativa. Patient is on regular follow up for dressing and being planned for rotational flap cover for complete curative measure.

Discussion: The diagnosis of hidradenitis suppurativa is clinical & biopsy is rarely needed.

Diagnostic criteria:

Typical lesions: Deep seated painful nodules(blind boils) in early primary lesions or abscesses, draining sinuses, bridged scars, and tombstone open comedonesin secondary lesions.

Typical topography: Axillae,groin,genitals,perineal,perianal,buttocks,infra and intermammary areas.

Chronicity and recurrence.

Treatment Modalities:

Medical: Topical clindamycin, oral tetracyclines, steroids, Immunomodulators- adalimumab, eternacept.

Surgical :CO2 Ablation, deroofing,excision,excision and reconstructive surgeries.

Conclusion:

This poster is for rare, extensive, multiple representation of Hidradenitis suppurativa.

IMMUNE MEDIATED NEUROMYOPATHY – A RARE CASE REPORT

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Abstract

Introduction: Immune mediated inflammation of both peripheral nerves and muscle fibres is a rare phenomenon. It is rarely seen in dermatomyositis or polymyositis. The exact pathogenesis of this involvement is unclear. In our patient, we describe the biopsy proven inflammatory changes in both nerve and muscle fibres.

Case Report: Mr. Pandiyan, a 31 year old male presented with decreased sensation in both foot for 3 months associated with difficulty in walking for 20 days. No significant past history. No family history of any neurological disorders. His lower limb power was 4+/5 with absent knee and ankle jerk. He had graded sensory loss below knee level in both sides. Vibration sensation over Medial Malleoli was reduced. His proprioception over Great Toe is lost. His nerve biopsy showed axonal neuropathy and muscle biopsy showed inflammatory myopathy. He was treated with intravenous immunoglobulin. Patient's sensory symptoms improved and but had persistent muscle weakness and was on follow up.

Discussion: Inflammatory myopathy is a broad term given to immune mediated inflammation of muscle fibres and nerves. The involvement of peripheral nerves in inflammatory myopathy is very rare. The recovery depends on amount of axonal damage involving peripheral nerves. Though antibody workup and electrodiagnostic studies can provide details, only muscle and nerve biopsies confirm the diagnosis. There are no proposed treatment guidelines. We treated this patient with intravenous immunoglobulin

Conclusion: Immune mediated neuromyopathy is a rare phenomenon. The treatment options include intravenous immunoglobulin or a trail of steroids. The earlier the treatment, prognosis is better. The motor recovery is delayed in this patient due to axonal involvement.

NEUTROPHIL LYMHOCYTE RATIO - A MARKER OF COVID-19 PNEUMONIA SEVERITY

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Absrtact

Introduction: In late December 2019, an outbreak of a mysterious pneumonia characterized by fever, dry cough, and fatigue, and occasional gastrointestinal symptoms. The pathogen of the outbreak was later identified as a novel beta-coronavirus, named 2019 novel coronavirus (2019-nCoV) and recalled to our mind the terrible memory of the severe acute respiratory syndrome (SARS-2003, caused by another beta-coronavirus)

Aim and Objectives: India is one among the highest number of coronavirus disease 2019 (COVID-19) cases worldwide. The aim of the study to determine the efficacy of neutrophil / lymphocyte ratio (NLR) as a marker of the severity of COVID-19 pneumonia in the South Indian population.

Materials and Methods: It is a retrospective cohort study done by observation of 70 case sheets who have admitted in COVID ward during the time period of March 2021 to July 2022. All case sheets with age more than 18yrs with both gender were included in the study. RT PCR negative and lack of documents in case sheets were excluded from the study. Collected data were statistically analysed.

Results: A total of 70 patients were included. Among the different haematological parameters, the WBC, neutrophil and lymphocyte count were significantly associated ($p<0.05$) with severity of COVID 19 infections. Higher WBC, neutrophil count and lower lymphocyte count were significantly associated with severe COVID19. Higher Neutrophil / Lymphocyte ratio (NLR) was significantly associated ($p<0.05$) with bad outcome (Death).

Conclusion: Neutrophil lymphocyte on admission is a strong predictor of in-hospital mortality in patients with COVID-19. Hence we should focus on neutrophil lymphocyte ratio through complete blood count a basic clinical investigation based on that early admission in ICU with early aggressive treatment before the worsening of clinical condition will reduce the mortality.

A STUDY ON PREVALENCE OF MULTIDRUG RESISTANT PATHOGENS AMONG DIABETIC CASES WITH CHRONIC OSTEOMYELITIS

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Abstract

Introduction: Osteomyelitis is one of the most common complication of diabetic foot infection. Osteomyelitis is frequently missed and under diagnosed in patients with diabetic foot. This problem is present approximately in 10%-15% of moderate and in 50% of severe infections. Approximately 60% of diabetic foot ulcers are complicated by infection. However, the simultaneous presence of peripheral arterial disease and infection increases the risk of non- healing ulcers leading to amputation. Early diagnosis of chronic osteomyelitis with effective treatment can reduce the risk of minor and major amputation. Present study is done to detect the bacterial agents causing chronic osteomyelitis in diabetics and their antibiotic susceptibility pattern.

Materials and Methods: 100 wound swabs were collected aseptically from patients with chronic osteomyelitis were subjected to bacteriological study over a period of 1 year in the department of microbiology, SVMCH & RC, Ariyur. Culture isolates were identified by a seriesof standard methods. Antibiotic susceptibility testing was performed and interpreted as per CLSI guidelines.

Results: Study group comprised 76 males and 24 females. Majority of the patients were in the age group of 11 –60 years. Out of 100 cases with osteomyelitis analyzed, diabetic patients with vascular insufficiency constitutes about (19%), Positive cultures were (90%) and negative cultures (10%). The commonest organisms isolated were *Staphylococcus aureus* (51%) and *Pseudomonas aeruginosa* (16%) followed by *Staphylococcus epidermidis* (13%), *Escherichia coli* (11%), *Klebsiella pneumoniae* (9%), *Enterobacter* (5%) and *Proteus mirabilis* (4%). Antibiotic sensitivity testing of Gram positive organisms showed maximum sensitivity to Amikacin, Linezolid and Vancomycin with less sensitivity to Pencillin. Gram negative organisms showed maximum sensitivity to Amikacin & Imipenem with less sensitivity to Ampicillin and Ciprofloxacin. Prevalence of MRSA among gram positive organisms being 51%, prevalence of ESBL 64.4% and MBL 22.2% among gram negative organisms.

Conclusion: Present study showed that commenst organism isolated was *Staphylococcus aureus* followed by *Pseudomonas aeruginosa* with prevalence of MRSA among S.aureus and ESBL, MBL producers among gram negative organisms being high. Hence diabetes and its complications are increasing at an alarming level in India. Early clinical detection of diabetic foot infection and microbiological characterization along with routine antibiotic sensitivity testing of organisms testing for MRSA, ESBL and MBL production will help the clinicians for proper management of patients also helps in preventing antibiotic resistance.

Association of Albumin-creatinine ratio with bilirubin levels in patients with chronic type 2 Diabetes mellitus

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Abstract

Background: Diabetes mellitus is a chronic disease condition with hyperglycemia. Around the globe, it has been observed that 8.2% has diabetes. It is critical for clinicians to understand the relationship between diabetes and vascular disease with the increase in the prevalence of diabetes all over the world. Till date, conflict exist in the effect of bilirubin on the diabetic microvascuar complications.

Objective: To assess the associations of serum direct and indirect bilirubin with albumin-creatinine ratio among type 2 diabetic adults.

Material and methods: This cross-sectional study including 25 patients diagnosed of diabetes. The biochemical lab parameters like total bilirubin, direct bilirubin and albumin creatinine ratio (ACR) were assessed. Bilirubin level was assessed using Vitros 5600 dry chemistry analyser. The urine albumin was assayed by pyrogallol method and urine creatinine by Jaffe's method using Erba semi auto analyser. Then, ACR was calculated. **Results:** Of the 25 diabetic patients, 16 (64%) were male and 9 (36%) were female. Mean age of the patients were 56.40 ± 9.49. The median [interquartile range] of ACR was found to be 88.44 mg/g [35.75 - 440.65]. Prevalence of microalbuminuria indicated by high ACR ratio of more than 30 mg/g was observed in 20 out of 25 patients. Serum total bilirubin was found to be 0.49 mg/dL [0.29-0.95], direct bilirubin was observed to be 0.11 mg/dL [0.10-0.25], and indirect bilirubin was 0.35 mg/dL

[0.17-0.56] which were all found to be within normal range. Of all the patients, ACR was not found to be correlated with total, direct and indirect bilirubin levels ($p>0.05$).
Conclusion: Diabetic patients with higher ACR were not significantly correlated with serum bilirubin levels. Further investigations with higher sample size are warranted.
Key words: Albumin-creatinine ratio, bilirubin, microvascular complications, diabetes

ASEPTIC MENINGITIS-SCRUB TYPHUS FEVER: AN EMERGING INFECTIOUS THREAT

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Abstract
Introduction: Aseptic meningitis is an illness characterized by serious inflammation of the linings of the brain (i,e., meninges), usually with an accompanying mononuclear pleocytosis.Clinical manifestations may vary, with headache and fever predominating. The illness is usually mild and runs its course without treatment; however, some cases can be severe and life threatening.Aeptic meningitis syndrome is not caused by pyogenic bacteria. Although it is usually caused by certain virus, it has a number of other etiologies as well as both infectious and non infectious. Hence, the term aseptic meningitis is no longer synonymous with viral meningitis although the two are still often used interchangeably.Central nervous system (CNS) involvement in scrub typhus is seen in up to a quarter of patients. However, the literature on cerebrospinal fluid (CSF) analysis and outcome in meningitis due to scrub typhus is scant.
Case Report: A 45 -year –old female patient with complaints of fever for 7 days , altered sensorium and decreased food intake for 3 days, it was associated with pain in the back of her neck, but no headache , vomiting , dizziness, vision difficulty , altered mentation, cough, chestpain, abdominal pain .
Early diagnosis and prompt institution of doxycycline therapy lead to early cure of scrub typhus meningitis.
Discussion-Many cases of scrub typhus associated with meningitis or meningoecephalitis have been reported. To the best of our knowledge, this is the rare case describing the details of Presence of scrub meningitis.
Conclusion: Scrub typhus should be considered one of the differential diagnoses of aseptic meningitis.

A VERY RARE CASE OF PAPILLARY TYPE OF INVASIVE DUCTAL CARCINOMA OF BREAST MIMICKING AS BENIGN BREAST CYST

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Abstract
Introduction: Papillary Breast Carcinoma is a very rare type of invasive ductal carcinoma accounts for fewer than 1 % of all Invasive Breast Cancer. Papillary breast carcinoma is histologically classified into intraductal and intracystic papillary carcinoma. SolidInvasive papillary carcinoma are rare and accounts for 0.5% of all Invasive breast carcinoma. Most of the cases are seen in postmenopausal women and has a good prognosis. Bloody nipple discharge is a relatively common presenting sign occurring in 22-34% of cases or may present as a palpable mass. Though the frequency of axillary node metastasis is low, treatment often involves mastectomy and axillary node dissection.
Case report: A 77 years old female post-menopausal women came with complaints of painless progressive lump in the left breast upper outer quadrant of 2 years duration. On examination a 10 x 7 x 5 cm lump present over the upper outer quadrant, smooth, soft to firm in consistency, mobile with the breast tissue, not fixed to pectoralis major muscle or skin. Radiologically and cytologically it was reported as benign Breast cyst following which excision biopsy was done and it reported as solid papillary invasive carcinoma with cystic degeneration and it ER, PR was positive and HER-2 was negative. Later patient underwent modified radical mastectomy.
Conclusion: Regardless of its invasive nature, solid papillary invasive carcinoma carries an excellent prognosis. This is to emphasize that in any woman who present with discrete lump, malignancy should be ruled out first even if clinically or cytologically presenting as a benign cyst.

A RARE CASE OF CUTANEOUS ZYGOMYCOSIS - ANTERIOR ABDOMINAL WALL

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Abstract

Introduction: Primary cutaneouszygomycosis is a rare infection caused by the class zygomycetes, which occurs most often in diabetics, thermal burns andimmunocompromised patients. The primary cutaneous form has a better prognosis than the more deeply invasive form, but is still associated with long-term morbidity if not diagnosed early. We present a rare zygomycosis case which initially presented with an infected sebaceous cyst.

Case report: A 45-year-old male patient, presented with an infected sebaceous cyst in the anterior abdominal wall for which excision was done under local anesthesia. After a week patient presented with wound dehiscence and discharge which was managed conservatively.In spite of continued local care of wound with proper antibiotic cover, the wound showed no signs of healing and there was persistent pus discharge with features of spreading ulcer. Suspecting a fungal infection,wide local excision was done under anesthesia; the excised specimen was sent for histopathological examination and KOH mount, which turned out to be cutaneous zygomycosis. Postoperatively, proper wound care was given and started on Itraconazole. The wound started healing and granulation tissue appeared and defect was closed with transpositional flap cover.

Conclusion: Though rare and what looked like a small outpatient problem finally turned out to be a significant disease with a long hospital stay; it is important to spread awareness of this disease so that timely treatment can be started and a good outcome can be achieved

BILATERAL SUPERNUMERARY INVERTED TEETH PRESENTING AS NASAL MASSES AND RECURRENT RHINITIS

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Abstract

Introduction

Supernumerary tooth (ST) also known as hyperdontia is a tooth or odontogenic structure that is formed in normal dentition other than any region of the dental arch. Their presence in the nasal cavity is a very rare clinical entity. The exact pathophysiology is not clear and most of the time the patient presents with non-specific symptoms only.

Case Presentation

We encountered a 51-year-old male patient with symptoms of bilateral nasal obstruction and nasal discharge for 10 years duration. Anterior rhinoscopy and diagnostic nasal endoscopy revealed a grayish white mass covered with mucopurulent discharge over the floor of the left nasal cavity and a mucosa covered bulge was noted in the right nasal cavity floor. CT showed 2 hyperintense lesions in the maxilla reaching the floor of both nasal cavities. Diagnosis of supernumerary teeth was made and treatment accordingly.

Discussion

Although the presence of tooth has been reported from ovaries, maxilla, maxillary sinus, mandibular condyle, mediastinum, their presence in the nasal cavity is a very rare entity. The radiological investigations such as orthopantomogram and CT helps in the diagnosing the condition. The treatment option is the extraction of the tooth to alleviate the symptomsHowever, if there is no retention of permanent teeth or pathological conditions are present, observation with regular radiographic control is advised

Conclusion

Supernumerary intranasal teeth are rare and the supernumerary tooth presenting to both the nasal cavity has never been reported before. So, the Ear nose throat specialist and oromaxillo facial surgeons should always be aware of this when dealing with a patient with nasal masses.

A CASE OF ERUPTIVE XANTHOMA AS THE FIRST PRESENTATION OF UNDIAGNOSED DIABETES MELLITUS IN YOUNG.

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Introduction : Eruptive xanthomas are benign skin lesions that are caused by localized deposition of lipids in the dermis, which are typically caused by elevated levels of serum triglycerides and uncontrolled diabetes.

Case History: A 25 years old gentleman presented with 5 months history of an evolving papules ,initially appeared on the extensor surface of the knee bilaterally and progressed to bilateral extensor aspect of the forearm. This lesion was not associated with fever, pain, pruritis or any other symptoms. These lesions were diffuse erythematous crops of yellowish papules visible over the knees and forearms .The patient was initially diagnosed with folliculitis and treated with oral antibiotics with no clinical improvement observed. This necessitated the need to find an alternative explanation for this presentation.

Investigations:

Weight- 75 kg ,Height-165cm . BMI-27.5 kg/m2

BLOOD SUGAR (mg/dl)	LIPID PROFILE (mg/dl)
Fasting-243	Total cholesterol-213
Postprandial- 384	Triglyceride-1199
	HDL -23

Skin Biopsy : Reticular dermis shows foamy histiocytes in groups and focally showing lace like eosinophilic material between the collagen bundles-suggestive of ERUPTIVE XANTHOMA

Discussion

Eruptive xanthoma is a characteristic manifestation of extreme hypertriglyceridemia. The hyperlipidemia responsible for xanthomas may be the result of a primary genetic defect that yields defective apoproteins or may be secondary to an underlying systemic disorder such as diabetes mellitus, hypothyroidism, or nephrotic syndrome. On initial workup he was diagnosed to have diabetes mellitus and negative for other secondary causes. No relavantfamily history of dyslipidemia.Hewas started on fenofibrate (statin) and insulin.His lesions began to resolve within one month of treatment.

Conclusion

Prompt recognition of eruptive xanthomas and awareness of its association with hypertriglyceridemia, newly diagnosed or decompensated diabetes mellitus can help to prevent complications such as coronary artery disease and acute pancreatitis. Before and after treatment with fenofibrate and insulin.

Punch biopsy-Reticular dermis shows foamy histiocytes in groups and focally showing lace like eosinophilic material between the collagen bundles.

A RARE CASE OF RETROPERITONEAL MASS

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Introduction

Paraganglioma is rare type of catecholamine secreting neuroendocrine neoplasm with

Hypertension and sympathetic hyperactivity as clinical presentation but can present asymptomatic. We report a case of retroperitoneal paraganglioma which was asymptomatic at presentation but had a dramatic course during resection.

Case History
A 39 year female presented with an incidentally detected retroperitoneal mass in ultrasound. On CECT abdomen it was a well definedhypodense lesion of size 6*5 cm in right hypochondrium towards lateral aspect of second part of duodenum probably neuroendocrine tumour or GIST. Endoscopic ultrasound showed a heteroechoic mass lesion below the hilum of liver abutting the wall of IVC & D2 and being highly vascular, guided biopsy was deferred. PET CT was suggestive of Paraganglioma. Urinary metanephrines was negative but Chromogranin A was elevated. On Laparatomy the mass was of size 6*5 cm well encapsulated tumour with numerous feeding vessels posterolateral to duodenum abutting IVC, resection was done which was very much eventful during handling with initial malignant hypertension, ventricular tachycardia and later hypotension. HPE confirmed the tumor was a Paraganglioma. Patient recovered well postoperatively.
Conclusion
This Paraganglioma was nonsecreting, asymptomatic on presentation but the intra operative period was of an unpredictable acute hypertensive crisis and arrythymias which has to manage accordingly
Keywords: Neuroendocrine Tumour, Paragangliom

SEROPREVALANCE OF HBcAb AMONG HBsAg NEGATIVE BLOODDONORS AND HEMODIALYSIS PATIENTS

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Abstract
Introduction
Inspite of implementation of mandatory screening for HBsAg(Hepatitis B surface antigen) detection by ELISA(enzyme linked immunosorbent assay) method, still Transfusionassociated hepatitis B viral infection remains to occur.
Objective
To monitor the seroprevalence of anti-HBc among the HBsAg negative blood donorsand hemodialysis patients.
Materials and Methods
HBsAg negative serum sample from blood donors and hemodialysis patients wascollected. All the samples were tested for anti-HBc by ELISA method.
Results
Out of Eight one cases of HBsAg ELISA negative 61 were blood donors and 20 werehemodialysis patients. Of these, 5 cases were positive for anti-HBc by ELISA method.
Among the 5 cases, 2 were blood donors and 3 were hemodialysis patients.
Conclusion
Anti-HBc seroprevalence was 6.17%. It has been demonstrated that in some HBsAg negative individuals Hepatitis B virus(HBV) continue to replicate. Testing for HBsAg alone may not be sufficient to rule out presence of HBV infection. Thus screening for Anti-HBc mayprevent Transfusion associated hepatitis B viral infection.

FUNGAL AETIOLOGICAL AGENTS IN PATIENTS WITH VULVOVAGINITIS- A HOSPITAL BASED STUDY IN PUDUCHEERY''

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Abstract
Introduction: Vulvovaginal candidasis is a common health problem of adult women mostly caused by candida species[1] Hence laboratorybased diagnosis helps us to identify the species[6], which is essential for correct treatment & preventing the resistance to antifungal drugs[5]
Aim&Objectives: To determine the fungal aetiological agents causing vulvovaginitis & to identify its species
Materials& methods: Vaginal swabs received in the Microbiology Lab (SVMC&RC) for a period of 6 months were included in this study. Initially gram stain performed in all samples in this gram positive budding yeast cells is processed further &inoculated into chromagar for species identification
Results: During the study period,out of 50 clinically suspected cases of vulvovagnitis samples processed for culture 8(16%) candida species were isolated. Out of which the most common isolates were 3c.albicans(6%) , 3were c.krusei,(6

%), followed by 1 c.parapsilosis (2%)& 1 c.tropicalis (2%)species were identified.All confirmed cases of candida albicans were germ tube test positive.
Conclusion: This study help us to understand the magnitude of the problem & to implement necessary treatment modalities. Culture positive results will be correlated clinically with definite diagnosis of VVC.

LATE PRESENTATION,ACCURATE DIAGNOSIS AND SUCCESSFUL TREATMENT OUTCOME IN AN ADOLESCENT PRESENTING WITH MALROTATION OF GUT

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Abstract
Introduction: Malrotation of the gut refers to abnormal positioning ofthe intestine within the peritoneal cavity and this mayinvolve the small intestine or the large intestine.The incidence of the condition is said to be about1 in 6000 births and 85% of cases present in the first2 weeks of life,but in minority of cases, the patient becomes symptomatic only in adolescence or adulthood.

Case report:
A 11 year old male child who became symptomatic prior to admission with history of repeated episodes of abdominal pain presented with abdominal pain associated with bilious vomiting. On examination he was found mildly dehydrated, abdomen was soft with epigastric distension and with gastric succussion splash. Later he presented with bouts of bilious vomiting whichlead to the suspicion of upper gastro intestinal obstruction. Xray erect abdomen showed double bubble sign. CECT abdomen revealed features of D2 obstruction secondary to malrotation of gut. All the above findings were in favor of Malrotation of Gut without volvulus. He was taken up for emergency laparotomy and exploration on which constricting LADD'S bands were present over c loop of duodenum with caecum in midline. Ladd's procedure was performed and the obstruction was relieved. Post operativelypatient improved well and started on diet from POD 3.
Conclusion:
This case illustrates the accurate diagnosis of malrotation of Gut which had a late presentation in adolescence and its successful treatment outcome. It also explains the necessity of suspicion to diagnose Malrotation even in adolescent age group.

AN INTERESTING CASE OF NEPHROTIC SYNDROME

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Abstract
Introduction
Nephrotic syndrome is a rare manifestation of IgA nephropathy. It is reported to occur only in 5% of IgA nephropathy patients.Episodic hematuria and proteinuria <3g/day are the classic manifestations. It affects young males under 30 years and is uncommon in the elderly.
Case report
A 45 year old female, not a known case of diabetes and hypertension, presented with anasarca for 10 days.No recent h/o of fever/ sorethroat / urinary tract infection or any skin infection. There was no rash or joint swelling.Patient had bilateral pitting pedal edema. BP was 110/70mmhg. Systemic examination was unremarkable.
On further evaluation, there was proteinuria (24 hr urine protein 5g/day) and hypoalbuminemia (2 g/dl). WBC (9800), Hb 11g/dl, Platelet (2,80,000), Serum creatinine (0.8mg/dl), $S.Na^+$ (130mEq/l), $S.K^+$ (3.5mmol/L) and Liver enzymes were normal. Urine analysis showed no RBC/ cast. Cardiac 2D-ECHO was normal. Chest X ray showed minimal right sided pleural effusion. USG abdomen showed normal sized kidney with normal echoes and well maintained CMD.C3, C4 were normal, ANA was negative and ASO titre was unremarkable. HBsAg, Hepatits C virus antibody and HIV were negative.
Patient was evaluated for 'Acute Onset Nephrotic Range Proteinuria'. Renal biopsy was performed. Light microscopic and immunofluorescence features were consistent with IgA Nephropathy (MEST – C Score: $M_0E_0S_0T_0C_0$. Ig A and C3 deposition along the mesangium. No IgM/IgG/C1q deposits).'IgA Nephropathy with Nephrotic Syndrome'was diagnosed.Patient was started on steroid therapy (prednisone 1mg/kg). After 8 weeks of full dose steroid therapy, patient attained partial remission (>50% decrease in proteinuria) and steroid was gradually tapered. Patient's general condition improved.

Discussion

IgA nephropathy is characterized by predominant IgA deposition in the glomerular mesangium. It is one of the most common glomerulonephritis worldwide. The inciting cause is unknown. It can be a primary (renal limited) disease or secondary to hepatic cirrhosis,celiac disease, seronegative arthropathy and infection such as HIV and cytomegalovirus. Susceptibility to IgA nephropathy is inheritable.There are no serologic test that aid in the diagnosis. Serum complements are normal.MEST – C score from biopsyprovides earlier risk prediction for IgA nephropathy. Patients with ($M_1E_1S_1T_{1or2}C_{1or2}$) have worse outcomes. Our patient had mesangial hypercellularity in <50% of glomeruli(M_0), absence of hypercellularity within glomerular capillary lumina(E_0), absence of segmental glomerosclerosis(S_0), 2-25% of cortical area showing glomerular atrophy(T_0), no crescents(C_0).Treatment approach is tailored to the risk of progression of IgA nephropthy. Patients with no hypertension, normal GFR, minimal proteinuria have low risk for disease progression and can be monitored annually while Patients proteinuria >1g/day, decreased GFR, or hypertension have high risk of disease progression and should be treated with ACEI/ARB. Corticosteroid therapy has showed positive outcomes. Trial of steroid can be given in patients with proteinuria >1g/day despite being on 6 months of conservative management and preserved GFR >50ml/min and in patients with nephrotic syndrome, RPGN and high E score in biopsy. Approximately one third of patients experience spontaneous clinical remission, one third have a stable course and one third progress to CKD.

Conclusion

Nephroticrange proteinuria a rare manifestation of IgA nepropthy can be seen early in the disease course as well as in patients with advanced disease. The degree of proteinuria in IgA nephropathy predicts poor prognosis. This case is reported for its uncommon manifestation.

A RARE CASE OF TUBERCULOUS MESENTRIC LYMPHADENITIS ENCASING SUPERIOR MESENTRIC ARTERY

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Abstract

Introduction: Atypical extrapulmonary presentation of tuberculosis may significantly delay its diagnosis and management.tuberculous lymphadenitis is an extrapulmonary manifestation of a mycobacterium tuberculosis infection.it is characterized by necrotizing mycobacterial infection of the lymph nodes

Case presentation

A 21yr old male came to surgery opd with the c/opain around the umbilicus for the past 6month.CECT abdomen taken shows aill defined mildly lobulated mesentric mass like lesion with calcifications. The lesion is found at the level of l4 vertebral body/just above the bifurcation of the aorta into the common iliac artery in the midline. The lesion shows mild to moderate enhancement with encasement of the sma vessels.No areas of necrosis could be made out. Few mesentric lymph nodes adjacent the lesion largest on the left with almost similar pattern of enhancement to the mesentric lesion. Planned for exploratory laparotomy and proceed. Intraoperatively two omental band crossing and attached to the mesentric mass and another one to rif near appendix.3 large lymph nodes maximum size 5x4x3 cm near superior mesentric artery origin.two lymph node were excised and sent for HPE. Necrosed material was found in lymph node. Another lymph node enasing the sma were found was not removed. There were no peritoneal deposit and no other lymph node enlarged.post operative period was uneventful.HPE shows mesenteric mass and lymph node-caseating granulomatous lymphadenitis of tuberculous etiology.Patient was referred to pulmonary medicine for ATT.

Conclusion

Incision biopsy of tuberculous mesentric lymphadenitis near superior mesentric artery done except the one mesentric lymphadenitis encasing the superior mesentric artery

A RARE CASE OF LARGE SOLID OVARIAN BENIGN MASS

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Abstract

Introduction

Solid mass arising from ovaries usually tend to be a malignant tumour, exception is aOvarian fibroma which represents 4% of all ovarian neoplasms. Mostly it occurs in peri- and post-menopausal age group, median age being 52 years. It tends to be

asymptomatic.An ovarian fibroma is a benign sex-cord tumour.On gross pathology, it is firm and white or tan. Microscopically, there are intersecting bundles of spindle cells producing collagen. We report a case of large solid ovarian fibroma with unique radiological presentation and normal serum CA 125.

Case report

A case of 70year old female presented with complaints of lower abdominal pain and abdominal distension, was evaluated who had CA 125 in normal limits. Clinical examination revealed mass palpable per abdomen about 12 weeks size. CT revealed a 72x72x50 mm dense lesion present in the anterior wall of the uterus suggestive of pedunculated myoma.Diagnostic laparoscopy showed a large solid ovarian mass. Excision and Laparoscopicmorcellation were done. Histopathological examination revealed features of aOvarian fibroma.

Conclusion

This poster reveals the unique clinical and radiological presentation of a large solid ovarian mass, which can be a benign ovarian fibroma rather than the usual malignancy.

Keywords: Ovarian fibroma, radiographic features, spindle cell tumour, CA-125

RARE CASE OF RUPTURED OVARIAN ECTOPIC PREGNANCY

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Abstract

Introduction

Ovarian ectopic pregnancy is a condition where the gestational sac implants in the ovary.this is rare but its incidence is rising due to the increasing use of iucd,art and ovulation induction drugs.incidence is 1:2000 to 1:60000(3% of all ectopic).here we report a rare and interesting case of ruptured ovarian ectopic pregnancy managed by laprotomy and oopherectomy

Case history

25yrs old p2l2 with previous 2nvd not sterlized came with complaints of abdominal pain for past 2days with minimal spotting on her last menstural period(lmp-2/8/21).no other complaints.on examination her vitals were unstable(pr-124b/m and bp-100/60mmhg).investigation

Her urine pregnancy test was positivehb-7.8

Tvs-usg done revealed uterus normal size, et-8mm, free fluid present in pouch of douglas, left adenexal mass present, right ovarian cyst of size 3x3cms noted.Intra-operative findings:Patient was taken up for emergency laparotomy

Intraoperatively 1litre of haemoperitoneum noted,uterus appeared normal size ,bilateral tubes apears normal, left ovarian ruptured ectopic pregnancy noted and right ovarian cyst of size 3x3cm noted.Left oopherectomy done, right ovarian cyst aspiration done and Ruptured ovarian ectopic sent for hpe which revealed presence of trophoblastic tissue in ovary.

Discussion

Ovarian pregnancy is relatively uncommon which can be primary ovarian pregnancy or secondary to ruptured tubal pregnancy.Ovulation induction,art,pelvic inflammatory disease,intrauterine device,endometriosis are the associated risk factors

Diagnosis can be made on the basis of history,clinical features,serum b-hcg level and pelvic ultrasonography.Management of ovarian ectopic pregnancy is surgical like oopherectomy by laparotomy or laproscopy.minimal access surgery is now treatment of choice removal of gestational product by enucleation or wedge resection .alternative treatment for unruptured ovarian pregnancy is methotrexate

Our case presented with pain abdomen,upt-positive and ultrasound findings suggestive of ovarian ectopic pregnancy and hemodynamicaly unstable so medical management is not possible and taken up for emergency laparotomy

Conclusion

Though ovarian ectopic pregnancy is a rare event but it is expected to rise as many patients seek for infertility therapy.inspite of current medical advances preoperative diagnosis of ovarian ectopic pregnancy still remains a challenge.

However histopathological examination is always mandatory and confirmatory.

A COMPARATIVE STUDY OF CARDIOVASCULAR RESPONSE TO ISOMETRIC HAND GRIP EXERCISE TEST IN OFFSPRING OF HYPERTENSIVE AND NORMOTENSIVE PARENTS

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Abstract

Introduction:Essential hypertension is a multifaceted disease and it is a major risk factor for the development of cardiovascular disease. Offspringof hypertensive parents have 4 times the risk of becoming hypertensive in their later stage of life.Nearly 75% of the hypertensives have 1st degree family history of hypertension indicating a strong familial aggregation. Sympathetic overactivity has been implicated in the development of the condition in the offspring of hypertensive individuals. They also have an exaggerated stress-induced cardiovascular response at an younger age which could be detected by various physical stress tests. Hence this present study has been designed to compare the sympathetic response between offspringof hypertensive and normotensive parents using isometric hand grip exercise test.

Aim and Objectives: The aim of this study is to compare the blood pressure response to isometric handgrip exercise test between offspring of normotensive and hypertensive parents.

Material and Methods: This comparativestudy was conducted on 60 medical students between the age group of 18 – 22 years who were in normal BMI. After recording basal blood pressure and pulse rate, the participants performed the isometric handgrip exercise test. The participants were asked to hold the handgrip dynamometer in the right (or dominant) hand to get a full grip of it. The handles of the dynamometer was compressed by the participant by putting in maximum effort for few seconds. The whole procedure was repeated thrice with rests in between, to prevent fatigue. The average of the three readings was referred as the maximal isometric tension(Tmax).

Then, the participants were asked to hold the tension at 30% of Tmax for 2 minutes.Blood pressure and pulse rate was recorded from the non-exercising arm during the test , 2 minutes and 5 minutes after completion of the test .

Results: It was observed that the groups differed significantly withΔDBP_{IHG} (difference between maximum rise in diastolic BP during isometric handgrip exercise test and basal diastolic BP)and basal systolic blood pressure was significantly higher in the study group when compared to control groupthough their values were in normotensive range. There was no significant difference in basal diastolic blood pressure and pulse rate between the groups.

Conclusion: Hence from this study it could be inferred that offspring of hypertensive parents had exaggerated sympathetic response to stress. Thus isometric handgrip exercise test can serve as a vital tool in early detection ofvulnerable population and early intervention with lifestyle modification can be advised to postpone the onset of the disease.

Keywords: Isometric hand grip; Normotensive offspring of hypertensive parents;Systolic blood pressure.

A RARE CASE OF MALROTATION OF GUT PRESENTING IN A 55 YEAR OLD ADULT

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Abstract

Introduction

Intestinal malrotation is a congenital disorder resulting from partial to complete failure of the 270-degree counterclockwise rotation of the midgut around the superior mesenteric vessels associated with Ladd's bands which are fibrous stalks of peritoneal tissue that attach the cecum to the retroperitoneum in the right lower quadrant. Usually it is diagnosed in young age group incidentally, but presenting in elderly with obstruction features is rare, we present such a case.

Case report

A 55 years old male, presented with features of gastric outlet obstruction for which he was evaluated and was diagnosed to have malrotation of gut with duodenal(D3) obstruction in CECT Abdomen; the proximal part of the duodenum was hugely dilated (>7 cm) with Caecum on the left side. He was planned for diagnostic laparoscopy and proceed. Intraoperatively above findings were confirmed and Ladd's band was identified forming dense adhesions from Caecum and ascending colon onto the duodenal third part, compressing almost whole of the lumen of the 3rd part of the duodenum. Ladd's procedure was done. Post operatively patient improved dramatically.

Conclusion

Intestinal malrotation is very ccommon in childhood but can present in adults also. It is very rare in adults above the age of 50 years. Ladd's procedure either laparoscopically or via laparotomy can provide good resolution of symptoms.

A RARE CASE OFMASSIVE SIMPLE LIVER CYST

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Abstract

Introduction

Hepatic cyst is a term that usually refers to solitary non- parasitic cyst of the liver. Simple hepatic cysts are more often asymptomatic and are discovered incidentally on abdominal imaging scans. Most of the simple cysts do not need any treatment, only large symptomatic cysts need surgical intervention. Here we are reporting a case of massive liver cyst with abdominal discomfort, underwent laparoscopic deroofing.

Case report

A 59 year old female, came with complaints of heaviness of upper abdomen for 6 months duration. On examination, patient had abdominal distension with the liver border palpable 10 cm from the right costal margin in the right mid clavicular line. USG abdomen showed an anechoic cystic lesion of size 13 x 16 x 16.9 cm(2 litres), occupying the entire right lobe with wall thickness measuring 7 mm suggestive of simple liver cyst. CT scan showed large (17.4 x 16.1 x 14.5 cm) well defined non enhancing cystic lesionin the right lobe of liver. Patient underwent laparoscopic wide deroofing, the histopathology of the excised wall showed solitary unilocular biliary cyst. Post-operative ultrasound showed complete collapse of the cyst. Patient was followed weekly and no evidence of recurrence of the cyst was noted.

Conclusion

Though simple liver cysts as such does not cause any symptoms by itself and does not require any intervention, in this patient we had to intervene due to its huge size which caused abdominal discomfort and in future may become more symptomatic due to compression on adjacent structures.

A CROSS SECTIONAL STUDY OF ASSESSMENT OF BODY MASS INDEX AND OBESITY IN SECOND YEAR MBBS STUDENTS IN A TERTIARY CARE HOSPITAL, PUDUCHERRY.

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Abstract

Background: Diabetes Mellitus is a metabolic disorder with hyperglycemia. Dyslipiemia associated with DM patient and contributing to atherovascular events and increases the likelihood of CVD in future. Measurement of inflammatory marker like hs-CRP will improve the prediction of the risk of these events. This study it is aimed to assess the Cardiac risk indices and determine their association with hs-CRP in diabetic patients

Aim and objectives

To estimate the prevalence of overweight and obesity among Second year MBBS Students in SVMCH & RC.
To Find the association between Family History with BMI among the students.

Methods: This study included 150 MBBS students' BMI survey from SVMCHRC, Puducherry The height and weight of the students will be measured and BMI will be calculated by using formula. Students parents health status data are collected and find the association of BMI and family history.

Results: Based on student BMI details we found overweigh was 25.33% and obese was 8.67% Student parents health details obtained and studied possible future risk for obese student.

Conclusions: Students who are overweight-obese, that particular students family history were collected. They are susceptible to future development of cardiovascular disease, type II diabetes, hypertension, osteoarthritis, anesthesia risks, menstrual abnormalities as well as some types of cancers including those of colon and breast. So that students Necessary health promotional activities are suggested to adopt healthy life style including regular physical exercises, yoga and good dietary habits are advised for healthy life style.

PREVALENCE OF HUMAN PAPILLOMA VIRUS GENOTYPESIN WOMEN OF REPRODUCTIVE AGE GROUP (18 TO 40 YEARS)

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Abstract
Background: Human papillomavirus (HPV) infection is considered as the major risk factor for the development of cervical cancer, second most frequent cancer in India. However, the magnitude of the problem and the associated factors remain unrevealed in the Puducherry region.
Aim:This study aimed to determine the prevalence of HPV infection in reproductive age group women.
Methods:Prospective study was done among study population of 80 patients aged between 18 to 40 years . Study duration was 2 months. Both PCR and cervical cytology study was done.
Results: Among the 80 patientscytology was positive in 2 patients and negative in 78 patients. Polymerase chain reaction: Negative in all the patients including those who had a positive cytology. The two cases tested positive with cytology represent false positive cases.The sensitivity of cytology in our study was high compared to PCR and the specificity was high in PCR.
Conclusion: Cervical screening awareness and early detection of HPV infection are important aspects in prevention of cervical cancer.Standardized cervical cancer screening programs worldwide use pap smear as the accepted screening tool although its sensitivity is low compared to PCR. The low sensitivity of PCR in our study could be due to low prevalence in our region, small study population and small study period. A standardized national level cervical cancer screening programme is not in practice currently.A standardized cost effective national level cervical cancer screening programme should be implemented in India.

EVALUATION OF ANTI-ANXIETY AND ANTI-DEPRESSANT EFFECT OF PALONOSETRON IN SWISS ALBINO MICE

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Abstract
Background: Depression and anxiety remain serious problems in the given unexpected circumstances like Covid-19. Serotonin is one of the most important neurotransmitters influencing mental health and is a potential target for pharmacological treatments. $5HT_3$ receptor antagonists like ondansetron which is mainly used for management of acute & delayed cancer chemotherapy induced emesis, has also shown anxiolytic and antidepressant effect in various studies. Another $5HT_3$ antagonist Palonosetron which is approved for delayed cancer chemotherapy induced emesis, has yet not been studied for anxiolytic and antidepressant effects.
Material & Methods: Light-dark compartment model was used for anti-anxiety effect evaluation and Swim despair test was used for evaluation of anti-depressant effect. Swiss albino mice were used and were randomly divided in 5 groups of 6 each to receive following 5 treatments: Group 1(n=6) Vehicle i.e normal saline (0.1 ml/10 g of body weight i.p.), Group 2: Diazepam (1mg/kg i.p), Group 3: Fluoxetine (18mg/kg i.p), Group 4: Palonosetron (0.025 mg/kg i.p), Group 5: Palonosetron (0.05mg/kg i.p) in each animal model. In light-dark compartment model efficacy was assessed at the end of 15, 30, 60 and 120, 180 & 300mins by observing time spent in light compartment and number of transitions made between both compartments whereas in Swim despair test efficacy was assessed by recording of immobility period after 1 hr. of drug administration and then after 24 hrs.
Results: Time spent in the light compartment at the end of 30, 60 and 120 min with palonosetron (0.05mg/kg) was statistically significant more as compared to control & fluoxetine. No of transitions made between both compartments also increased with palonosetron (0.05mg/kg) at the end of 15, 30, 60 & 120 mins as compared to control and fluoxetine groups.
In Swim despair test palonosetron (0.05mg/kg) showed statistically significant antidepressant activity as compared to control and fluoxetine group at 1 hr & 24 hrs respectively.
Conclusion: Palonosetron (0.05mg/Kg) possesses significant antidepressant & anti-anxiety activity in animal models.

THYROID DERMOPATHY -MANIFESTATION OF AUTOIMMUNE THYROID DISEASE

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Abstract
Introduction: Thyroid dermopathy is an infrequent manifestation of autoimmune thyroid disease characterized by localized thickening of the skin commonly seen in the pretibial area. It is almost always associated with ophthalmopathy (96%) and sign and symptoms of hyperthyroidism. The diagnosis of thyroid dermopathy is based on clinical sign and symptoms, serological thyroid hormone abnormalities supported by skin pathology. Isolated dermopathy is a rare manifestation of hyperthyroidism..
Case Report: A 25-year-old female presented with 7 months history of asymptomatic, multiple skin colored tumorous growths over anterior aspect of both legs along with the presence of a gradually progressive reddish raised lesion on his left lower leg.
Discussion: Thyroid dermopathy or localized myxedema is characterized by localized thickening of the skin and is a late and rare manifestation of autoimmune thyroiditis, particularly of Graves' disease. . It most commonly affects middle-aged females with female to male ratio of approximately 4: 1. It is commonly localized in the pretibial area and is therefore often referred to as pretibial myxedema
Conclusion: Isolated lesions of the thyroid dermopathy in the absence ofophthalmopathy or other evidence of hyperthyroidism are a rare presentation and represents a diagnostic challenge.
Key words: thyroid dermopathy , cutaneous manifestation.
Abstract for Poster presentation.

A CASE OF THYROTOXIC PERIODIC PARALYSISAND SUBTLE SIGNS & SYMPTOMS OF THYROTOXICOSIS

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Abstract
Introduction
Thyrotoxic periodic paralysis (TPP) is a rare complication of hyperthyroidism, characterized by the abrupt onset of hypokalemia along with severe limb weakness. In Asia, it's estimated prevalence 2% of thyrotoxic patients.
Methods : Case Report
An 36-year-old male hailing from Villupuram , presented to the emergency department(ER) with acute onset bilateral lower extremity weakness and upper extremity weakness. Associated symptoms included Fever cough palpitations and nausea. The patient denied prior similar episodes. Upon presentation, his vital signs were for BP: 110/60 mmHg HR 180/min and RR 25/min Temp : 101 F. He did not have exophthalmos or lid lag or thyroid glanden largement. His neurological exam was significant for quadriplegia weakness 0/5 proximal 2/5 distal and upper extremity weakness 0/5 2/5 distal ;. Laboratory workup was significant for hypokalemia(1.6mmol/L), suppressed TSH(0.01 u IU/ml). Burch-Wartofsky score upon presentation was 40, consistent with the impending storm. Upon arrival to the ER 80mEq of potassium chloride was given per the hospital electrolyte replacement protocol. The patient was started on propylthiouracil, propranolol, and dexamethasone. Serum potassium was closely monitored. The patient developed mild hyperkalemia(5.8mmol/L) ;potassium replacement was held as the diagnosis of TPP was considered.
Discussion :
Patients with TPP can present with subtle symptoms of thyrotoxicosis and prominent neurologic symptoms. Hypokalemia results from the shift of potassium into the intracellular space due to the effect of excess thyroid hormone on the Na-K ATPase channel and Kir2.6 mutation. Treatment of hyperthyroidism helps normalize potassium levels at the extracellular space..
Conclusion : The main purpose of this abstract is to highlight and make clinicians to be aware of TTP diagnosis and treatment in asian patients.Aggressive potassium replacement is thus not recommended as it can result in rebound hyperkalemia.

SAFETY AND EFFICACY OF LNG-IUS IN PATIENTS WITH ABNORMAL UTERINE BLEEDING WITH ASSOCIATED MEDICAL DISORDERS

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Abstract

Introduction

AUB affects 17.9% of reproductive age group women in India.(1)About 80% of all hysterectomy are carried out for non oncological reason and AUB is one of the most common indication for hysterectomy in perimenopausal age group.Of all the alternative treatment for AUB , LNG-IUS shows a major effect on the endometrium and reduces the need of hysterectomy.

Case 1 :

45year old female, P2L2 came with complaints of heavy menstrual bleeding for 3 years , 10/30 days cycle, associated with clots and dysmenorrhea.K/C/O Ischemic heart disease for past 1 years on treatment. K/C/O systemic hypertension and diabetes for past 2 month on regular treatment.INVESTIGATION:USG: Uterus normal size, B/L adnexa normal,2D Echo : Mild concentric LVH, EF: 65%, no LV dysfunction, COURSE OF Stay in Hospital**:** Patient planned for FC with LNG IUS , and the same done under spinal anesthesia, patient condition improved hence discharged.HPE report: Proliferative endometrium.Patient followed up for 3 months , patient condition improved.

Case2

40 years old P2L2 came with complaints of heavy menstrual bleeding for past 2 years , 10-15/30 days cycle , associated with clots and dysmenorrhea.K/C/O hypertension for 10 years on regular treatment, K/C/O CKD on dialysis (twice weekly) for past 10 years.INVESTIGATION:Urea: 76mg/dl , Creatinine : 12mg/dl .USG : Uterus normal size, ET : 15mm , B/L adnexa normal.COURSE OF STAY IN HOSPITAL: Patient planned for FC with LNG IUS insertion and the same done under GA , patient condition improved hence discharged .HPE report : Proliferative Endometrium.Patient followed up for 3 months , patient condition improved.

Discussion

Nearly 30% of all hysterectomies are performed to alleviate the problem of heavy menstrual bleeding.(2,3)On comparing the effectiveness of LNG IUS over other treatment modalities , LNG IUS has most safety, cost effectiveness, health related quality of life, and fertility sparing effects.

Conclusion

LNG-IUS is having a high success rate in controlling menstrual symptoms, thereby improving the quality of life avoiding hysterectomy in women with AUB.Thus LNG-IUS can be used as a substitute for hysterectomy in AUB-PALM a well as in non structural causes i.e. AUB-COEIN.

Key Words: Abnormal uterine bleeding, QOI, Hysterectomy

EMPYEMA THORAXNECESSITANS AS A RARE LONG TERM COMPLICATION OF THORACIC TRAUMA

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Abstract

Introduction

Empyema thorax necessitans is a rare long-term complication of poorly or uncontrolled empyema thoracis characterized by the dissection of pus through a weak part of the chest wall, forming a superficial abscess and then even a fistula between the pleural cavity and the skin.The usual causes are usually infective conditions of the lungs, but it is very uncommon for a thoracic trauma to complicate and present as Empyema thorax necessitans. Treatment of this condition would include thoracotomy, decortication with intercostals tube drainage, and also drainage of the superficial abscess with proper antibiotic coverage.We present a rare case of thoracic trauma which complicated as Empyema thorax necessitans.

Case report

45 year old Mr. Dhandapani presented with purulent discharge from boggy swelling over a four months old ICD insertion site for two days. Examination of the respiratory system showed reduced air entry in the right lower zone and dull note on percussion.CECT thorax showed split pleura sign with loculated collection of size 8.2x3.1x5cm with fistulous tract.He underwent Right posterolateral thoracotomy and decortication. Intraoperatively, 50ml of pus was drained and pleural rind in posteroinferior aspect of right lung excised. Two intercostals drains were placed on the right side. The superficial abscess was drained and covered with dressing. Anitibiotics were given according to the culture and sensitivity. Postoperative xray showed resolution and reexpansion of the lung. Patient improved symptomatically and was discharged.

Conclusion: This case emphasizes that Empyema thorax necessitans can also be a rare complication of incompletely managed thoracic trauma.

EFFECT OF ANGIOGENIC AND ANTI-ANGIOGENIC MARKER IN SYSTEMIC CIRCULATION OF PREECLAMPSIA PATIENTS

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Abstract
Background: Preeclampsia is a pregnancy-related systemic condition characterized by hypertension and proteinuria. The anti-angiogenic protein soluble fms-like tyrosine kinase-1(sFLT-1) and pro-angiogenic protein placental growth factor (PLGF) is crucial to its pathogenesis.
Aim &Objective: To estimate the sFLT-1, PLGF and Endothelin (ET-1) in pregnant women with and without preeclampsia attending Tertiary care center at Puducherry.
Methodology: A case control study was performed to recruit antenatal women who were aged between 18 – 40 years based on ACOG guidelines (2013). Blood pressure, serum ET-1, sFlt-1 levels, PlGF levels and the sFlt-1: PlGF ratio was compared and APGAR score at 1 and 5 mins were collected in both the group. The concentrations of ET-1, sFlt-1 and PlGF were measured with commercially available ELISA kits. SPSS ver. 16.0 and JASP 0.16 was used to analyze the data.
Results: Total of 60 pregnant women were recruited and they were grouped into two with thirty each. There was no significant difference in age or gestational age in either study group. The concentration of ET-1, sFlt-1 and PLGF were highly significant in women with preeclampsia (p-value 0.000). Likewise, systolic and diastolic blood pressure was significantly associated (p-value <0.001).Significantly higher sFlt-1: PLGF ratio (91.77 ± 52.14 versus 2.66 ± 1.12) was found in preeclampsia subjects than normotensive pregnant women. APGAR score at 1 and 5 mins showed significant results between the groups.
Conclusion: Screening of ET-1, sFlt-1 and PLGF along with urine albumin can be used as aaccurate biomarker in the prediction or diagnosis of pre-eclampsia.
Keywords: Preeclampsia, Soluble fms-like tyrosine kinase − 1 (sFlt-1), Placental growth factor (PlGF), Endothelin-1
Abbreviations:
sFlt-1 – Soluble fms like tyrosine kinase-1
ET-1 – Endothelin 1
PLGF – Placental growth factor
ELISA – Enzyme linked immunosorbent assay
Declaration:
Hereby, we the authors declaring that our original article has not been published anywhere.

ATYPICAL CORNEAL CLOUDING IN MUCOPOLYSACCHARIDOSIS

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Abstract:
Introduction:
Mucopolysaccharidoses are rare storage disorders characterised by accumulation of glycosaminoglycans in different tissues resulting in multisystem dysfunction. It is associated with other ocular features like pigmentary retinopathy, refractive error, cherry red spot, cataract and multiple systemic association especially skeletal abnormality and dysmorphic facies. This is a report of a young girl with Scheie's syndrome who presented with bilateral corneal clouding, clear central zone with good visual acuity along with skeletal and facial features.
Case history
A young female patient presented with complaints of painless diminution of vision in both eyes since early childhood. There was positive history of second degree consanguinity. No family history was noted. Characteristic features noted were frontal bossing, hypertelorism, short stature, low set ears, macroglossia, bowed legs, short stubby fingers and hoarseness of voice.
Ophthalmological examination showed visual acuity in both eyes of 6/60 with correction of +4.50 D Spherical power to right eye and +4.00 D Spherical power to left eye, Visual acuity improved to 6/9 in both eyes and IOP was normal in both eyes. Slit lamp examination showed corneal clouding in both eyes with central corneal clear zone of 3mm, no vascularisation or degenerative changes observed. Lens and fundus appeared normal.
Multidisciplinary approach was sought. Baseline investigations were normal and urine Toludine blue test turned positive, X-ray imaging showed short bones with thickened metaphysis and abdominal scans revealed mild hepatosplenomegaly.

Enzyme activity assays for various MPS has been carried out and iduronidase activity was on lower side indicating possible MPS I S (Scheie's syndrome)

Discussion

Accumulation of GAG occur in tissues including tendons, cornea, liver, spleen, heart. Corneal involvement is often microscopic in most syndromes but characteristically cloudy cornea is seen in Hurler and Scheie's syndrome. Systemic features typically include abnormal facial characteristics and skeletal deformities. Urine analysis for the GAG metabolites with reduced leukocyte enzyme activity also helps in diagnosis.

Conclusion

Storage disorders are multisystem disease often diagnosed clinically and corneal clouding gives a clue towards it. However, MPS presenting with dense corneal clouding and clear central zone is very rare and enzyme analysis helps in making the diagnosis.

LASER – APACIFISTIC SWORD IN PERIODONTAL THERAPY

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Abstract

Introduction

Periodontitis is a chronic inflammatory multifactorial disease. It is the second most common oral disease. Periodontitis is defined as an inflammatory disease of supporting tissues of teeth caused by specific microorganisms resulting in progressive destruction of periodontal ligament and alveolar bone. The major problem in clinical periodontics is patient acceptance for treatment since the Conventional method includes need for anesthesia, suturing, surgical instruments which may cause bleeding, swelling and scar tissue formation.Soan alternative treatment procedure, Laser was introduced which was more of painless and bloodless procedure when compared to the conventional technique.

Case Presentation-. In this case series, we present the procedures done in Department of Periodontology, Sri Venkateshwaraa Dental College using Laser.

Discussion-Laser is a device that transforms light of various set of frequencies into an extremely intense, small, and nearly non-divergent beam of monochromatic radiation which is capable of mobilizing immense heat and power when focused at close range. The laser beam has various modes of emission and has different wavelength which helps in precise surgical cutting and better hemostasis and coagulation. Laser has immense advantages over conventional technique as it reduces the need for anesthesia, periodontal dressing, better wound healing and has minimal wound contraction, swelling, scarring. Lasers are used in various periodontal procedures such as pocket debridement,subgingival calculus removal, scaling and root planing, treatment of hypersensitivity, degranulation during flap surgeries, soft tissue surgeries, depigmentation, gingivectomy, crown lengthening and peri-implantitis.

Conclusion- The application of lasers has been recognized as an alternative approach in soft tissue surgeries and considered superior with easy ablation, decontamination and hemostasis with less postoperative pain.

THE GAME CHANGER IN REGENERATIVE PERIODONTICS- THE ONE AND ONLY PLATELET RICH FIBRIN

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Abstract

Introduction: Periodontitis is a chronic inflammatory multifactorial disease. It is the second most common oral disease whichis characterized by periodontal destruction resulting in alveolar bone and tooth loss.One of the most important and currently unsolved problem in clinical periodontics is prognosis and success of mucogingival deformity management.The goal of periodontal therapy includesnot only to arrest periodontal disease progression but also regeneration of structures lost in response to the disease. Though many regenerative materials introduced in the field of dentistry, not a single membrane is considered as gold standard in treating periodontal defects. Recently, Platelet Rich Fibrin has gained importance in the field of periodontal regeneration.

Case Presentation: This case series highlights the procedures done using Platelet Rich Fibrin in Department of Periodontology, Sri Venkateshwaraa Dental College, Puducherry.

Discussion: Platelet rich Fibrin is a rich concentrate of various growth factors which exhibit properties such as cell migration, cell attachment,cell proliferation, and cell differentiation.These properties allow for the enhancement of the

regenerative process and hence the use of the PRF has been increasing in the field of dentistry.The crux of PRF synthesis lies in the attempt to accumulate platelets and cytokines in a fibrin clot. It is a simple and cost-effective method at attempting enhanced healing and recovery of tissues. Treatment of mucogingival deformity is a now a major estheticconcern.

Conclusion: Based on the clinical outcome in these cases, platelet rich fibrin can be suggested as viable treatment option for the mucogingival deformity management.

TORQUE CONTROLLING DEVICES TO AVOID SCREW BREAKAGE / DISTORTION

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Abstract

Introduction

In recent days various methods for fracture reduction are being used. Out of which Open Reduction and Internal Fixation (ORIF) is commonly used for reduction of Maxillofacial bone fractures followed with implants fixation to stabilize the fracture site.

Discussion

During ORIF, Screw distoration is most frequently encountered scenario while fixation of miniplates. To avoid thiscircumstance Torque Controlling Screw Drivers are introduced to minimize the screw breakages / distoration.

Conclusion

In this presentation, various torque controlling devices and innovative design model of Novel Torque Controlling screw driver is briefly explained.

SURGICAL MANAGEMENT OF MUCORMYCOSIS

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Abstract

Introduction

Mucormycosis is a fungal infection caused by microorganisms belonging to phylum glomeromycotan. earlier they were categorized under rare fungal disease but at present they are an emerging pathogen in current pandemic situation.

Discussion

The disease starts with inhalation of the fungus into paranasal sinuses. This may further invade palate, sphenoid sinus, cavernous sinus, orbits or cranially to invade the brain.this condition may usually present with pain swelling oral ulceration and finally leads to tissue necrosis can result in palatal perforation. Early diagnosis and proper treatment plan still remain cornerstone in treating mucormycosis management.

Conclusion

Recent studies on this condition emphasizes more on need for combined management with surgical debriment of the necrotic and damaged tissues. early surgical intervention will improve the survival rate.This poster explains about surgical management of mucormycosis.

VITAL TOOTH BLEACHING

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Abstract

Introduction

White smile has been a symbol of beauty, health and vitality for hundreds of years. Discolouration, especially when the front teeth are affected, means a significant disturbance of aesthetics and can decrease a patient's self esteem. Public demand for aesthetic dentistry, including tooth whitening, has increased in recent years. Whitening, also referred to as bleaching, is the

most conservative treatment for discolored teeth.The pigments oxidation is responsible for tooth bleaching and can be carried out with two different products; carbamide peroxide and hydrogen peroxide

Objectives

This case report describes the remarkable change of tooth color by bleaching

Case Report

A 17 year old male patient reported to the Department of Conservative Dentistry and Endodontics with the chief complaint of discolouration of teeth. In the case of this patient, he had no history of any tooth sensitivity.

Discussion

Tooth bleaching is one of the most conservative and cost- effective dental treatments to enhance a person's smile. Bleaching of vital teeth in the dental office involves the application of bleaching agents, usually 30 to 35% hydrogen peroxide often in combination with heat and light. Hydrogen peroxide is an oxidizing agent that, as it diffuses into the tooth, dissociates to produce unstable free radicals which will attack organic pigmented molecules in the spaces between the inorganic salts in tooth enamel by attacking double bonds of chromophore molecules within tooth tissues.

Conclusion

Vital tooth bleaching is an effective treatment modality that can significantly change the appearance of teeth. Dental bleaching can be predictable, comfortable and safe with satisfying results provided it is performed correctly.

RECENT PANDEMICS – ITS EVOLUTION, PROGRESSION AND OUTCOME

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Abstract

Introduction

A pandemic is an epidemic that's spread over multiple countries or continents. We all have been affected by current COVID-19 pandemic. However, the impact of the pandemic and its consequences are felt differently depending on our status as individuals and as members of society. COVID-19 is killing people on a large scale.

Discussion

Many infectious disease leading to pandemics are caused by zoonotic pathogens that are transmitted to humans due to increased contact with animals through breeding, hunting and global trade activities. All human virus have animal origins namely natural hosts. Bats may be the natural hosts and the major natural reservoirs of alpha and beta corona virus. Furthermore rodents are the major cause of the outbreak.

The types of COVID-19 that have been labelled as variants of concern are: Beta, Delta, Gamma, and Omicron.

In future, there will be three possibilities – Immunization by vaccination, Immunization by infection and death due to infection.

Conclusion

The final result in which pandemic moves to endemic, where by increasing population immunity leads to seasonal peaks and depends on social behavior and by increasing the vaccine demand.Older persons and those with underlying medical conditions are at a higher risk of serious illness and death from COVID-19. Multiple and intersecting forms of discrimination experienced by older persons are exacerbated during the pandemic and aggravate their vulnerabilities. Training of all employees to follow infection control protocols, restricted travel for domestic and international related sectors.

APPLICATION OF CBCT IN ORAL AND MAXILLOFACIAL SURGERY

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Abstract

Introduction

Prior to the introduction of CBCT, panoramic radiography (OPG) was the most common imaging tool in private oral and maxillofacial surgery field.Cone Beam Computed Tomography (CBCT) is a most valuable imaging technique used in oral and maxillofacialsurgery.InCBCT the clinician has 3dimensional and multi-planar views for a more accuratediagnosis and treatment without the financial burden and radiation exposure of conventional computed tomography (CT) scan.

Discussion

CBCT images are not always able to replace other imaging modalities,dependingonthe field of view, CBCT scans show a large area of thefacial skeleton beyond the limits of a panoramicradiograph or a small area of focused clinical interestwhich helps for accurate diagnosis, treatment planning and postoperative outcomes compared to conventional 2D images.

Radiation exposure to the patient is very low inCBCT. Within the last decade, the technology and design of CBCT scanning machines has made the placement of the machines both physically and financially possible.

Conclusion

In this poster the clinical application, advantages and limitations of CBCT in oral and maxillofacial surgery are showcased.

MANAGEMENT OF SEPARATED INSTRUMENT IN ROOT CANAL

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Abstract

Introduction: Endodontic procedures may result in various mishaps. Among them, the most common complication is the fracture of endodontic instruments during root canal treatment. This mainly occurs either due to overuse or incorrect use of the instrument. The fractured segment may hinder cleaning and shaping procedures which have a potential impact on prognosis of treatment. It is necessary to manage this complication by the removal of separated instrument. Several methods are introduced that led to successful retrieval of the separated instruments.

Case Presentation: In this report, we present two cases of fractured instrument in root canalwhich was successfully retrieved with the combination of endosonic and IRS kit.Case 1 presented with fractured rotary file on mesiobuccal root of maxillary first molar. Case 2 presented with fractured stainless-steel K-file on calcified root canal of maxillary central incisor.

Discussion: Instrument separation during endodontic therapy is a frequent accident with a rotary-instruments being more likely to separate than manual ones. The treatment of cases with a separated instrument can either conservative or surgical. In our case report, the retrieval of instruments done under conservative approach. The Instrument Removal System (IRS) is one such a device for orthograde retrieval of separated instrument in root canal.

Conclusion: In the above case report, the separated instruments have been retrieved successfully with the combination of endosonic and IRS kit which is safe and conservativemethod. The separated instrument was retrieved with minimal dentinal removal.

POSTEXTRACTION MUCORMYCOSIS IN IMMUNOCOMPROMISED PATIENT

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Abstract

Introduction

Mucormycosis is a rare opportunistic fungal infection, that can produce widespread orofacial tissue necrosis. This primarily affects immunocompromised individuals. It is the deadliest and most rapidly progressing type of human-affecting fungal infection.

Discussion

Mucormycosisis characterized as per the target organ as follows: rhinocerebral, pulmonary, gastrointestinal,cutaneous, disseminated. The most commonly reported form of the disease is rhinocerebralmucormycosis, which is characterised by progressive fungal invasion of the hard palate, paranasal sinuses, orbit, and brain. Patients with rhinocerebralmucormycosis usually present with malaise, headache, facial pain, swelling and low grade fever.

Conclusion

This e-posterpresents cases of mucormycosis diagnosed after dental extractions in a compromised.

NANOTECHNOLOGY IN ENDODONTICS

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Abstract

Introduction

The era of Nanomaterials has a long lasting impression in the field of medical science. It's excellent use in medicine has led to its application in dental science. Serious concerns regarding the eradication of microbial biofilms from the root canal system still exists in the field of endodontics. Nanoparticles have proven to be much more efficient with good bonding

capabilities and surface chemistry as compared to the conventional materials. The practical applications of nanotechnology in endodontics has led to future prospects in research in this field.

Discussion

Nanoparticles in endodontics have shown promising results. The various nanoaprticles like graphene, silver nanoparticles, chitosan, hydroxyapaptite nanoparticles, Iron compound, zirconia, Poly (lactic) co-glycolic acid, bioactive glass, mesoporous calcium silicate, titanium dioxide nanoparticles, Magnesium, Calcium oxide and Copper oxide have been discussed. These nanoparticles have fetched and shown great results in various application in endodontics like incorporation of nanoparticles in sealers, obturating materials, irrigation, and intracanal medicament.

Conclusion

The application of nanoparticles from natural and synthetic materials is rapidly evolving in dentistry. These biomaterials have helped in treatment of oral diseases, in eradication of smear layer and biofilms, have been incorporated in various dental materials for their antimicrobial effects. Combining all their beneficial aspects, these nanoparticles will provide new paradigm shift in dentistry. This review on nanoparticles will provide the reader with the latest knowledge of these materials, their mechanism of action and its implications in endodontics

ARTIFICIAL INTTELLIGENCE IN DENTISTRY

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Abstract

Introduction

Artificial Intelligence refers to the idea of designing machines that are capable of performing tasks that are normally done by humans. In dentistry, it has witnessed some of the exceptional achievements. Hence, this situation demands every dentist to get associate with this technology as the future of dentistry is abutting the implementation of its applications.

Discussion

Artificial intelligence (AI) is the emerging technology that utilizes machine to mimic human behavior which are used in our day-to-day life. Major role of AI in healthcare is assistive and supplementary to medical and dental professionals. Dentronics is the wide field of modern dental technologies, such as medical robot systems and specialized artificial intelligence including hardware, software, human-machine interaction. Since autonomous robots in medical field are still beyond reach, the virtual component of AI, known as software-type algorithms is the main component used in dentistry. The present review outlines the progress and potential dental applications of AI in medical-aided diagnosis, treatment and disease prediction and discusses their data limitations, interpretability, computing power and ethical considerations. In the future, the AI-based comprehensive care system is expected to establish high-quality patient care and innovative research and development, facilitating advanced decision support tools.

Conclusion

The included studies describe that AI is a reliable tool to make dental care smooth, better, time-saving and economical for practitioners. They can also help to predict failures in clinical scenarios despite, the fact that application of AI in healthcare as a promising role but challenges both in technical and ethical aspects exists. While in no way, AI can replace the role of a dentist, it is of prime importance to be aware of the possibilities to integrate this technology in the future of a gratifying and successful practice.

BIOFOULING AND ANTIFOULING IN MEDICAL DEVICES

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Abstract

Introduction

Biofouling of medical devices involves the formation of biofilm by bacteria. The infecting bacteria produces extensive exopolysaccharides to form a confluent biofilm. Biofilms can develop on solid surfaces under conditions which facilitate microbial growth thus biofilms are ubiquitous in nature. Attachment and colonization of microorganisms on biomaterial surface poses a risk of local and systemic infections in patients.

Discussion

Biofilms cause a wide range of problems in both the health and industrial sector. Over 45% of nosocomial infections are device related. Bacteria within a biofilm can be 1000 times more resistant to antimicrobials and less susceptible to host

immune system. The urinary catheter is the most commonly used medical device as well as the second most common cause of hospital acquired infection which are associated with a relatively high rate of colonization and infection. Dental unit water systemcommonly used to irrigate the oral cavity during dental treatment is another medical device which is prone to biofouling

Anti-fouling technologies help to overcome biofouling in medical devices. The uncoated, polymer coated and anti-microbial coated urinary catheters shows reduction in catheter associated urinary tract infection. Several, either internally and externally coated antimicrobials in central venous cathetersare less likely to produce catheter related blood stream infection and less likely to be colonized. Anti-fouling of dental unit water line includes a plethora of automated flushing systems, filters, water disinfectants, independent bottle water systems and even fully detachable autoclavable DUWL. Chemicals may be introduced either intermittently or continuously to treat the waterlines

Conclusion

The suboptimal efficacy of antimicrobial agents against biofilm–embedded organisms may be attributed to a relatively slow bacterial growth within the biofilm, inhibition of activity by exopolysaccharide material and poor penetration of microbial agents. Anti-fouling systems can be defined as the coating, paint and surface treatment used to control or prevent attachment of unwanted organisms.

VACCINE COLD CHAIN MANAGEMENT AND ITS CHALLENGES IN THE VACCINATION PROGRAM-POSTER

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Abstract

Introduction

In cold chain the goal is to keep a specific temperature range throughout the entire manufacturing process until the product is delivered. Such a strong chain is crucial for the delivery of temperature-controlled life-saving vaccine to different parts of the world. The World Health Organization estimates that up to 50% of vaccines are wasted globally every year; a large part because of lack of temperature control and the logistics to support an unbroken cold chain. Considering today's world at the scale of COVID-19, the importance of cold chain in the distribution of vaccines for immunization processes, which must be presented to the consumer in same shape and form to achieve a desirable outcome With the help of Internet of Things platform and temperature monitoring devices, cold chain monitoring plays a major role in maintaining vaccine quality.

Discussion:

The objective of this poster to provide an overview of the cold chain in vaccine manufacturing, storage, and delivery and discuss how cold chain monitoring and management makes it an efficient process in the first section. Subsequently how some pharmaceutical companies are utilizing cold chain technology for their vaccine delivery systems, finally a summary and an outline on the challenges encountered during the process and effective ways to overcome it.

Primary cold chain requirements to fulfill its purpose are extra sensors, smart analytics and incident response plan. This ultimately results in

1. Prevent people's health and safety from risk
2. Reduce Spoilage rate of vaccines
3. Prevent potential damage of company's brand name and wastage of investment

Conclusion

Utilizing Internet of Things and Temperature Monitoring Devices in the cold chain, vaccine quality can be maintained for its efficacy and prevent vaccines from spoiling and losing their brand names.

THE DENTURE CAN MAKE YOU SMILE AND PROMOTES BETTER HEALTH A CASE REPORT OF NEUTRAL ZONE TECHNIQUE.

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Despite the growing trend of implant treatment and its proposal as the standard of care for the edentulous population, conventional completedenture therapy remains a substantial and more affordable treatment option for the majority of elderly edentulous patients, especially those with low socioeconomic status. However, the neutral zone technique is also considered to be an important alternative approach to patients complaining of unstable dentures, particularly when implant therapy is not feasible. This technique is by no means new but is not widely practised due to the lack of experience of dentists. This E-POSTERpresents the neutral zone technique in a functional approach to overcome the problem of instability of lower dentures caused in patients by a more potential musculature or in a patient who has dimensional or altered neuromuscular control.

MANAGEMENT OF SEPARATED INSTRUMENT IN ROOT CANAL – A CASE REPORT

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The fracture of endodontic instruments during root canal treatment is a complication for every endodontist must have to deal with it. The fractured segment may hinder cleaning and shaping procedures with potential impact on prognosis of treatment. Fracture of endodontic instrument often results from incorrect use or overuse. If breakage occurs clinically, the patient should be informed of the incident and consideration should be given whether to remove the fragment or not. When managed properly, the presence of a broken fragment per se may not adversely affect the outcome of root canal treatment. Various devices and techniques have been introduced in endodontics for retrieval of the fractured instruments, but none are consistently successful. This case report describes retrieval of fractured instrument from the root canal with the combination of endosonic and IRS kit.

MANAGENMENTN OF MYOFASCIAL PAIN DYSFUNCTION SYNDROME WITH SOFT OCCULUSAL SPLINT-A CASE REPORT

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Laser Diagnotic criteria- Four cardinal signs - Unilateral pain - Muscle tenderness - Clicking or propping noise in the TMJ - Limitation of jaw movement Negative characteristics - No radiographic evidence - No tenderness in TMJ area on palpation via the external auditory meatus

Investigations Radiological investigation, Advanced – CT,MRI,CBCT, Electromyogram, Sonography Vibration Analysis Thermography and Mandibuar tracking device

THEME III ENGINEERING SCIENCES

EXTRACTIVE TEXT SUMMARIZATION USING BERTSUM

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Abstract

In the Natural Language Processing field, text summarization is getting more popular day by day. With the increased reachability of the Internet, the amount of textual data is also increasing. These textual data are different in nature depending upon their originality and context. With the huge data available, it is not possible for humans to go through them all simultaneously. So, a need for a proper summarization system is imminent. Till now, even most of the state-of-the-art summarization models are unidirectional. As a result, they do not have a full understanding of the context. With the creation of the BERT model, it outperformed all the existing language models. BERT is bidirectional in nature. It is pre-trained with masked words, and it also has next sentence prediction capability. The beauty of the BERT model is that it can be fine-tuned to many downstream tasks. Among them, extractive text summarization was implemented with the BERTSUM model. The BERTSUM model showed promising results in terms of ROUGE scores. In this work, we further fine-tuned the BERTSUM model through DistilBERT. The DistilBERT model is a lightweight version of the BERT model created with knowledge distillation from the original BERT. It retains 95% of BERT's language understanding capabilities with 40% fewer parameters than the original BERT model and runs 60% faster. We fine-tuned the DistilBERT model with the parameters of BERTSUM on the CNN/DailyMail dataset. It performs almost the same as the BERTSUM model with ROUGE (1, 2, L) scores of 43.05, 20.08, and 39.47 respectively.

Keywords:*Extractive summarization, BERT, BERTSUM, DistilBERT*

Introduction

As the era of internet has already begun, the number of textual data is increasing day by day. People are getting more computer oriented than paper oriented and everything including from newspapers to medical records, educational documents to blogposts, the amount of textual data from multiple sources are increasing rapidly. To get the most important sentences, a proper automatic extractive text summarization system is much needed. The idea of extractive text summarization comes from the concept of extracting the important sentences or context from a given paragraph or multiple sentences of a specific topic. Also, the length of the summary should not exceed half of the length of the original text(s). The summarizer will extract the most important sentences by using predefined methods or through supervised or unsupervised model training. The importance of the sentences will be given accordingly to the topic, keywords, position of the sentences and also with its contextual meaning. Till now, there are many automatic extractive text summarization approaches available. With the use of neural networks, some of the models achieved highRecall-Oriented Understudy for Gisting Evaluation(ROUGE)(Lin, 2004) scores and were able to produce proper summaries. One of the state-of the-art summarizer called SUMMARUNNER (Nallapati et al., 2017) used a GRU-RNN based sequence classifier trained on the CNN/DailyMail dataset and DUC 2002 single document summarization dataset. The REFRESH(Narayan et al., 2018) summarizer model which considers extractive text summarization as a sentence ranking task and used reinforcementlearning. It consists of a CNN based encoder and a RNN based sentence extractor. It achieved noticeable ROUGE scores with the CNN/DailyMail dataset. The Structured Summarization Model (SUMO) (Liu, et al., 2019) considered the tree-induction problem and generated a multi-root dependency tree while predicting the summary. It uses a sentence level encoder as a baseline transformer (Vaswani, et al., 2017) model. CNN/DailyMail and the New York Times Annotated Corpus were used for training. BanditSum proposed by (Dong, et al., 2018) was trained on the CNN/DailyMail dataset. The summarization problem was considered as a contextual bandit problem where the context is the document to be summarized and the action is the selected sequence of sentences as output. A policy gradient reinforcement learning algorithm with Bidirectional Recurrent Neural Networks (BiRNN) as encoders were used to train the model. As these are the state-of-the-art models with high ROUGE scores, they did not use a proper language model. Currently, the mostly used language models are BERT, ELMo and GPT. Among them, BERT (Devlin, et al., 2019) has the upper hand with the bidirectionality and fine-tuning feature. Many natural language processing related tasks such as sentiment analysis, question answering, named entity recognition can be done with the pre-trained BERT model. Extractive text summarization with BERT was introduced by (Miller, 2019) where text embeddings generated by the BERT model was clustered using k-means clustering and sentences closest to the clusters were used as the output summary. This approach was further improved by (Srikanth et al., 2020) where the pre-trained BERT model was trained on the CNN/DailyMail dataset and dynamic clustering was used to extract the most important sentences from the BERT embeddings. The BERT model was fine-tuned for extractive text summarization by BERTSUM (Liu & Lapata, 2019) where the [CLS] token of the BERT model was added at the beginning of every sentence and the sentences with the highest [CLS] token value was selected for summarization. This method achieved the state-of-the-art

performance in terms of ROUGE scores.The CNN/DailyMail and the XSum dataset was used here. The BERTSUM model was with a transformer summarization layer is called BERTSUMEXT.

Materials and methods

The BERTSUM model used the BERT-base model pre-trained on the Toronto Book Corpus and English Wikipedia consisting of 110 million parameters. The BERT-base model is pre-trained with maskedwords and next sentence prediction task.It has token embeddings, interval segment embedding and position embeddings. With its huge parameters, it is expensive to train and requires a lot of resources to run. To overcome this problem, in our work, a lightweight model of BERT called DistilBERT (Sanh, et al., 2020) is fine-tuned in with the parameters of BERTSUM and summary of English document was generated.

DistilBERT

DistilBERT was introduced by the researchers of theHugging Face community and follows a teacher-student architecture.The DistilBERT model is trained with 66M (40% less) parameter where the teacher BERT-base model contains 110M parameters. But the hidden state dimension is same as the original teacher BERT with number of layers reduced to 6. Also, the token-type embedding of the original BERT model is removed. The token type embedding is used for predicting next sentences. As a result, the student model 60% faster than the original BERT with 97% accuracy of the original BERT model.The pre-trained DistilBERT model is trained on the BooksCorpus dataset and English Wikipedia, same as the BERT-base model.

CNNDailyMail Dataset

The CNN/DailyMail dataset is the most used English language dataset in area of text summarization. The dataset consists over 300k news article from the CNN and DailyMail website. Summaries are human generated as bullet points from the CNN and DailyMail stories and they were non-anonymized. There are 90,266 train docs, 1,220 validation docs and 1,093 testing docs in the CNN data. Simillarly, there are 196,961 train docs, 12,148 validate docs and 10,397 testing docs in the DailyMail data. The split was done by standard splits of (Hermann, et al., 2015). The time-period of the CNN articles was between April 2007 to April 2015 and for DailyMail it was June 2010 to April 2015. ROUGE score is used to validate the dataset.The dataset is first tokenized with the CoreNLP toolkit and then the sentence splitting was done. The splitted sentences were converted into JSON and later to PyTorch files. Here, for model training, the non-anonymized pre-processed CNN/DailyMail version 3 data from the Hugging Face community was used.

Model Fine-Tuning and Training

We train the pre-trained DistlBERT model with PyTorch on the CNN/DailyMail dataset with the parameters of BERTSUM with transformer summarization layer. The DistilBERT model do not have token type ids which is used for the next sentence prediction task. As for summarization, according to the BERTSUM model the Next Sentence Prediction (NSP) task is not needed. The segment embeddings and the position embeddings generate their embeddings and they are summed up and fed to the DistilBERT model. The fine-tuned DistilBERT model trained on the CNNDailyMail dataset then generates the word embeddings. Then the generated word embeddings are fed to the transformer summarization layer which selects the most important sentences for summarization. The model was trained on a standard Kaggle GPU for almost 5 hours. It was trained for 50000 steps and the model checkpointswere saved every 10000 steps.

ROUGE Score

The Recall-Oriented Understudy for Gisting Evaluation (ROUGE) (Lin, 2004) is widely usedfor evaluating automatic summarization systems. It evaluates the human generated summaries with automated summaries. TheROUGE-N calculates the overlapping of n-grams between the candidate and reference summary. There are two types of ROUGE-N matrices. ROUGE-1 and ROUGE-2 calculates the overlapping of unigrams and bigrams between the generated and reference summaries respectively. Lastly, the ROUGE-L calculates the Longest Common Sequence (LCS) between the summaries and the sequence matches are considered. The ROUGE 1.5.5 was used in this work.

RESULTSANDDISCUSSION

The trained model achieves ROUGE score of 43.05 as ROUGE-1, 20.08 as ROUGE-2 and 39.47 as ROUGE-L. The ROUGE score was compared with existing state-of-the-art summarization models. The ROUGE (Table 1) score comparison shows that the trained model outperforms manystate-of-the-art summarization models such as SUMMARUNNER, REFRESH, SUMO and BANDITSUM. It also performs almost same as the original BERT model. Despite being having 40% less parameters, it even performed very close to the BERT-large model. It must be considered that the model is also 60% fasterthan the original BERT model.

Table 1. ROUGE Score Comparison

Model	ROUGE-1	ROUGE-2	ROUGE-L
SUMMARUNNER	39.60	16.20	35.30
REFRESH	40.00	18.20	36.60
SUMO	41.00	18.40	37.20
BANDITSUM	41.50	18.70	37.60
BERT-base (110M parameters)	43.23	20.24	39.63
BERT-large (340M parameters)	43.85	20.34	39.90
DistilBERT	43.05	20.08	39.47

Fig. 1. Performance Analysis with 3 Sentence Summary

Input Text	Summarized Text
Super Typhoon Rai, known locally as Odette, slammed into the eastern coast of the Philippines on Thursday afternoon, bringing torrential rain and the threat of widespread flooding across the archipelago. The storm intensified rapidly as it approached the coast, strengthening from a Category 1 to a Category 5 storm in just 24 hours. By the time it made landfall on Siargao Island, a popular tourist and surfing destination on the central east coast, the storm had reached sustained winds of 260 kilometers per hour (160 miles per hour) with gusts over 300 kilometers per hour (185 miles per hour). Around 198,000 people have already evacuated from their homes to government shelters, the country's National Disaster Risk Reduction and Management Council (NDRRMC) said on Thursday. Many preemptive evacuations and storm preparations began earlier in the week as the country began seeing heavy rain. In central Misamis Oriental province, the Agay-ayan River overflowed on Tuesday, flooding streets and homes with muddy brown water. A rescue worker helps a girl wade through flooding caused by Typhoon Rai in Cagayan de Oro City, the Philippines, on December 16. The human-induced climate crisis is making typhoons, hurricanes and cyclones more intense and destructive, and the Philippines is one of the world's most climate-vulnerable nations. The super typhoon is expected to travel through the country's central and southern regions. Some of the worst conditions are expected in Surigao Province, which lies on the northern tip of Mindanao, one of the country's major islands. The storm is also expected to hit a number of provinces in the country's Visayas region, a central group of islands. More than 20 million people live in the Visayas, according to 2020 official figures. In Surigao Province, more than 2,600 people have been evacuated as of Wednesday evening, according to the state-owned Philippine News Agency. Photos from Surigao show one sports complex turned into an evacuation center, with plastic tents set up in a large hall and families asleep on rugs and tarps on the floor. Meanwhile in Eastern Visayas, more than 45,000 people have evacuated to government shelters in the Eastern Visayas region, according to the National Disaster Risk Reduction and Management Council on Thursday. "We are getting pounded already by strong wind and rain," said Governor Ben Evardone of Samar Province, located in Eastern Visayas.	Super Typhoon Rai, known locally as Odette, slammed into the eastern coast of the Philippines on Thursday afternoon, bringing torrential rain and the threat of widespread flooding across the archipelago. The storm intensified rapidly as it approached the coast, strengthening from a Category 1 to a Category 5 storm in just 24 hours. Meanwhile in Eastern Visayas, more than 45,000 people have evacuated to government shelters in the Eastern Visayas region, according to the National Disaster Risk Reduction and Management Council on Thursday.

Fig. 2. Performance Analysis with 5 Sentence Summary

Input Text	Summarized Text
The United Kingdom's Covid-19 response was under pressure on Tuesday, with the National Health Service (NHS) website crashing due to demand for booster appointments, lateral flow test kits no longer available online and long queues at vaccination walk-in centers. In the run-up to a holiday season that the British government promised would return to normality after last year's heavily restricted affair, a weary nation is instead being wracked by a new crisis: Omicron. The scramble for booster shots and tests comes just days after UK Prime Minister Boris Johnson announced a slew of new Covid-19 measures in the face of an incoming "tidal wave" of infections from the Omicron variant. Omicron has left one person dead in the UK and prompted warnings it could surpass the Delta variant to become dominant in the country by Christmas. Johnson has told people to "set aside" the idea that the variant is mild. Britain's beleaguered leader faced a major test of his authority on Tuesday, with members of his own Conservative party rebelling against measures on working from home, Covid passports and mask-wearing in a vote in Parliament. He was forced to rely on support from the opposition Labour Party to pass the new restrictions, which are a significant departure from the government's pandemic response in recent months. Johnson lifted all Covid-19 rules on "freedom day" in July and has until now resisted the more robust mitigation measures imposed in parts of Europe, like vaccine passports and mask mandates. The Prime Minister is also embroiled in a scandal over reports that Downing Street held a number of staff holiday parties last winter, when the rest of the UK was living under strict rules banning social mixing. He has been forced to deny that he fast-tracked "Plan B" Covid rules in order to distract from his political woes. Opening the debate in Parliament on the new Covid regulations Tuesday, UK Health Secretary Sajid Javid said that though the measures were not ones he would like to put in place, the situation demanded them. "As we look ahead to a winter with Omicron in the midst, the measures before the House [of Commons] today will fortify our natural defenses and guard the gains that we've all made against this deadly virus," Javid said. Scientists have never seen a variant that can spread so quickly and the growth in cases of Omicron in the UK is now mirroring the rapid increase in South Africa, Javid said, warning that the observed doubling time was two days. Until recently, ministers had said that cases were doubling every two to three days.	The scramble for booster shots and tests comes just days after UK Prime Minister Boris Johnson announced a slew of new Covid-19 measures in the face of an incoming "tidal wave" of infections from the Omicron variant. Omicron has left one person dead in the UK and prompted warnings it could surpass the Delta variant to become dominant in the country by Christmas. The United Kingdom's Covid-19 response was under pressure on Tuesday, with the National Health Service (NHS) website crashing due to demand for booster appointments, lateral flow test kits no longer available online and long queues at vaccination walk-in centers. Opening the debate in Parliament on the new Covid regulations Tuesday, UK Health Secretary Sajid Javid said that though the measures were not ones he would like to put in place, the situation demanded them. Johnson has told people to "set aside" the idea that the variant is mild.

The Fig. 1 and Fig. 2 shows the generated summary for 3 and 5 sentences. In the Fig. 2 it can be noticed that the sentence order of the generated summary differs from the input sentence order. It happens due to thevalue of the [CLS] token of the sentences. The model can identify the significance of the sentence and the sentences are stored with their proper order. The summary compared with BERTSUM model differs in the last sentence.

Fig. 3.Performance Analysis with 3 Sentence BERTSUM Summary

BERTSUM	DistilBERT
Super Typhoon Rai, known locally as Odette, slammed into the eastern coast of the Philippines on Thursday afternoon, bringing torrential rain and the threat of widespread flooding across the archipelago. The storm intensified rapidly as it approached the coast, strengthening from a Category 1 to a Category 5 storm in just 24 hours. By the time it made landfall on Siargao Island, a popular tourist and surfing destination on the central east coast, the storm had reached sustained winds of 260 kilometers per hour (160 miles per hour) with gusts over 300 kilometers per hour (185 miles per hour).	Super Typhoon Rai, known locally as Odette, slammed into the eastern coast of the Philippines on Thursday afternoon, bringing torrential rain and the threat of widespread flooding across the archipelago. The storm intensified rapidly as it approached the coast, strengthening from a Category 1 to a Category 5 storm in just 24 hours. Meanwhile in Eastern Visayas, more than 45,000 people have evacuated to government shelters in the Eastern Visayas region, according to the National Disaster Risk Reduction and Management Council on Thursday.

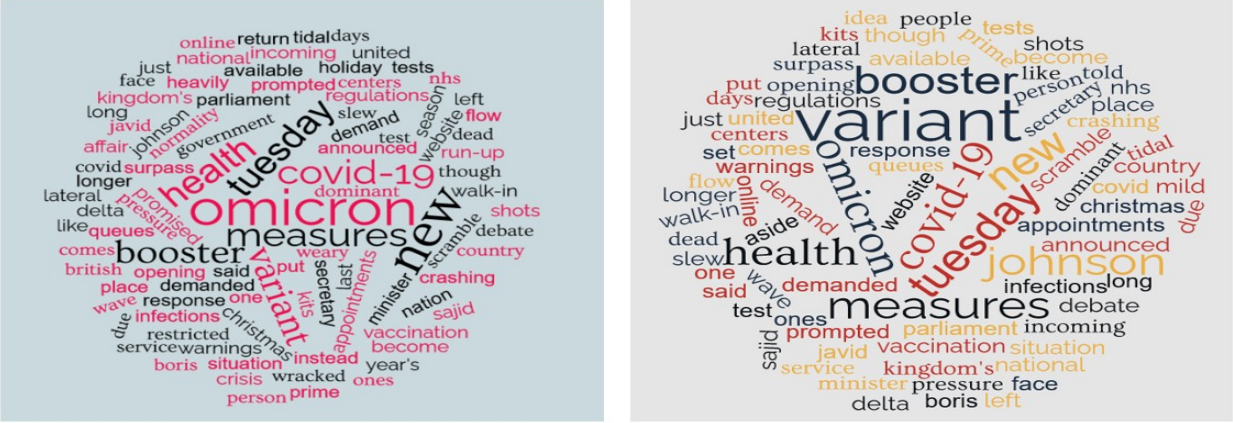
We compare our model's output with the BERTSUM model also. For generating 3 sentence summary (Fig. 3), it can be seen that, the first two sentences are same but the last sentence is different. But, if we check the significance of the last sentence generated by the trained model, it has also selected sentence of almost same significance. While

Fig. 4.Performance Analysis with 5Sentence BERTSUM Summary

BERTSUM	DistilBERT
The scramble for booster shots and tests comes just days after UK Prime Minister Boris Johnson announced a slew of new Covid-19 measures in the face of an incoming "tidal wave" of infections from the Omicron variant. The United Kingdom's Covid-19 response was under pressure on Tuesday, with the National Health Service (NHS) website crashing due to demand for booster appointments, lateral flow test kits no longer available online and long queues at vaccination walk-in centers. Omicron has left one person dead in the UK and prompted warnings it could surpass the Delta variant to become dominant in the country by Christmas. Opening the debate in Parliament on the new Covid regulations Tuesday, UK Health Secretary Sajid Javid said that though the measures were not ones he would like to put in place, the situation demanded them. In the run-up to a holiday season that the British government promised would return to normality after last year's heavily restricted affair, a weary nation is instead being wracked by a new crisis: Omicron.	The scramble for booster shots and tests comes just days after UK Prime Minister Boris Johnson announced a slew of new Covid-19 measures in the face of an incoming "tidal wave" of infections from the Omicron variant. Omicron has left one person dead in the UK and prompted warnings it could surpass the Delta variant to become dominant in the country by Christmas. The United Kingdom's Covid-19 response was under pressure on Tuesday, with the National Health Service (NHS) website crashing due to demand for booster appointments, lateral flow test kits no longer available online and long queues at vaccination walk-in centers. Opening the debate in Parliament on the new Covid regulations Tuesday, UK Health Secretary Sajid Javid said that though the measures were not ones he would like to put in place, the situation demanded them. Johnson has told people to "set aside" the idea that the variant is mild.

Generating 5 sentence summary, it can be seen that the first four sentences are same but the last sentence differs in both. But, context wise, both of the sentence is significant. Also, the change in the sentence order of the summary can also be seen. It indicates the sentence understanding capabilities of the trained DistilBERT model. But, after going through the passage context, it is understandable that both of them are acceptable.

Fig.5. Word Cloud Analysis



The word cloud shows that both model has that both model contains almost same words in them. The left is for the BERTSUM and the right is for the DistilBERT model.

Conclusion

In this work, the trained model can understand the topic context and generate extractive summaries from a given document. Future work includes training the model in a multi-lingual aspect for native languages such as Bengali, Tamil, Telegu etc.

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PREDICTION OF CLUBFOOT TREATMENT OUTCOME FOR AN INFANT IN A VIRTUAL ENVIRONMENT

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Abstract
Clubfoot is a congenital talipes equinovarus (CTEV) that twists the infant's foot inwards or outwards. In India, approximately 33,000 children are born with clubfoot every year. However, only 13% have access to treatment. With a yearly backlog of 28,000 untreated children, most born with clubfoot are at the risk of living with this disability for life. Clubfoot is generally corrected by non-surgical treatment by Ponseti method, in which the foot is twisted and stretched with gentle manipulations. A plaster cast is applied on the clubfoot for every manipulation and the cast has to be removed and replaced every week based on the corrections obtained. The main drawback in this method is, the infant's leg is fully constrained and unable to move it as the cast is heavy. As a remedial treatment, an orthosis (externally fitted shoe) can be used for treating the clubfoot. A three-dimensional finite element model of the clubfoot of an infant and the orthosis assembly is developed. Finite Element Analysis (FEA) is performed on the clubfoot-orthosis assembly, on applying the necessary loading and boundary conditions, to predict the effect of orthosis in the treatment of clubfoot.

Keywords: Clubfoot, orthosis, Finite Element Analysis (FEA), Treatment

Introduction

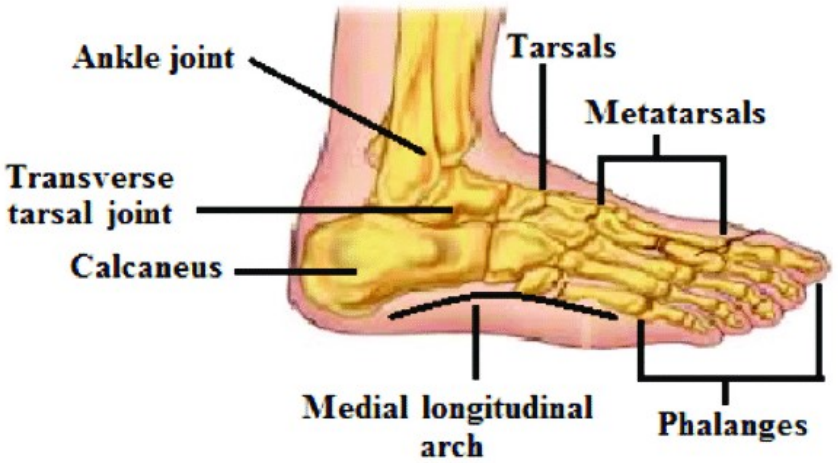
Foot is an anatomical structure found in most of the animals and it is the terminal portion of the limb in most cases. The main function of the foot is to support the weight of the body. During locomotion, the foot acts as a lever for propelling the body forward, thereby catering the needs of the vertebrates. Each species has unique foot geometry and functionality that supports their lifestyle. The foot anatomy clearly shows the presence multiple segments of the bone, including the claws or nails at the terminal portion.

The human foot is well suited to walk for long distances, whereas lion's feet can assist them to move faster, while kangaroo's feet is anatomically designed to damp the shocks during crawl-walking. The bird's foot assists them during its flight and catching the prey and provides necessary support while resting.

In this study the human foot is taken into consideration and the geometry and function of the human foot will be discussed in the following sections.

The human foot is the complex analytical structure in the lower limb consisting of twenty six bones, thirty three joints, over hundred ligaments, nineteen muscles along with the soft tissues. The anatomical structure of the foot with all bones is shown in Figure 1.

Fig. 1. Anatomy of human foot



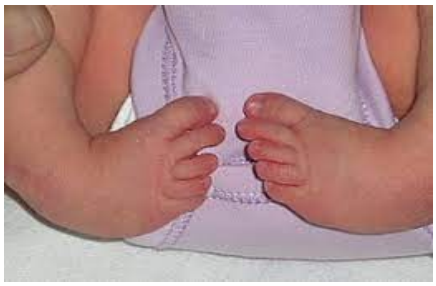
The foot complex with all elements lets one to stand upright and support in performing day to day activities like walking, running, jumping etc. The human foot has three different sections namely fore foot, mid foot and the hind foot.

1. The phalanges (five toes) along with the five long metatarsal bones forms the fore foot.

2. The cuboid, navicular and the cuneiform bones form a pyramid-like collection of bones providing necessary foot arches that together forms into the mid foot.
3. The hind foot, forming the ankle, consists of talus which supports the tibia and fibula (bones of the leg), and the heel bone (calcaneus), which is the largest foot bone.
4. The complex movements in the foot which helps in locomotion and maintaining the body equilibrium are assisted by the ligaments, tendons and muscles present in the foot. The Achilles tendon connecting the calf muscle and the calcaneus supports in performing the different activities of the foot.

Congenital Talipes EquinoVarus (CTEV), commonly referred as clubfoot, has the foot twisted and rotated inwards at the ankle allowing the subject to move on the lateral surface of the foot, as shown in Figure 2. It's a congenital deformity (present from birth) which can be rectified when identified and treated at the right time, preferably during the first week of birth.

Fig. 2 Child with clubfoot



Clubfoot Treatments

The objective for any treatment of clubfoot is to provide fully functional foot that generates no pain while performing the day-to-day activities like standing, walking and running.

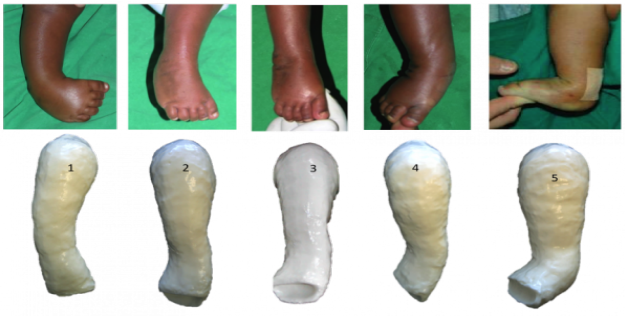
Ponseti method

It is a manipulative technique developed in the 1950s at the University of Iowa Hospitals and Clinics, USA by Dr. Ignacio V. Ponseti [1-3]. It is a standard treatment for clubfoot which involves serial casting. Considering the elasticity of the tissues forming the ligaments, joint capsules and tendons, it is preferred to start the treatment in the first week of birth. The different stages of Ponseti method are as follows:

Manipulation and casting

In the infant's clubfoot the muscles, ligaments and tendons are contracted which can be stretched by serial manipulation followed with a plaster cast for the entire leg from the toes to the thigh. The entire process of casting and stretching is repeated every week until, the foot is corrected, as shown in Figure 3. In general, about six to eight weeks of manipulation and casting required for correcting the clubfoot in infants.

Fig. 3. Ponseti treatment for clubfoot correction



Clubfoot also coined as 'Congenital Talipes Equinovarus' (CTEV) is one of the birth deformities with obscure etiology in foot caused by inward and downward twisting of the foot at the ankle associated with underdeveloped bones, muscles and ligaments within the womb. The clubfoot will be shorter than a normal foot and feels 'fixed' as it cannot regain itself a normal profile. Dimeglio et al., [6] and Dobbs et al., [7] report that about 150,000 – 200,000 children are born every year around the world with this congenital deformity. David et al., [8]

claimed that boys are predominantly affected than girls and, most of the cases are bilateral in nature. The methods of treatment available currently for a clubfoot are non-surgical as well as surgical. Generally non-surgical is preferred for obvious reasons.

The biomechanics of clubfoot and its pathoanatomy are essential to attain in-depth understanding of clubfoot. During 1800, Antonio Scarpa described the anatomy of clubfoot and stated that clubfoot is caused due to the dislocation of talocalcaneonavicular (TCN) joints. Also, Goldstein claims the outward rotation of the talus in the ankle mortise as the primary cause for clubfoot [9]. In infants the biomechanical and functional restrictions are absent in the normal foot but will appear in the plantigrade position when children start to stand and walk. The ossification center of the navicular and three cuneiforms are not present at birth. At the same time, the hindfoot bones (calcaneus and talus) are ossified at birth [10]. Anand and Sala [9] states clubfoot is characterized with a firm equinus position of the hindfoot, retraction of the gastrosoleus muscles, and calcaneus in the varus position (inverted position). In varus deformity, the hind foot is rotated inwards, especially at the talocalcaneonavicular joint.

At the same time, adductus deformity occurs at the subtalar and talonavicular joints. The medial side of the forefoot faces in an upward direction in relation to the hind foot. The cavus deformity is involved with forefoot plantar flexion along with contribution to equinus position. Jeevan et al., [56] defined the medial displacement and inverted position in relation to the talus, which is seen in the clubfoot due to misalignment of the calcaneus, cuboid, and navicular bone. Sometimes, the neck of the talus bone is shorter or absent. With palpation examination, the talus bone is found to be near the medial malleolus.

The calcaneus (a hind foot bone) is rotated medially (horizontal plane), inverted and adducted under the talar head in the equinus [10-12]. Pirani et al., [13], Riegger [14] and Maranho [15] identified that in clubfoot the calcaneal and talus bone are in a planter flexed position, which causes the varus deformity in the hindfoot.

Maranho and Volpon [15] states that the cuboid moves towards the direction of the medial side of the foot and in relation to the calcaneus. The medial displacement of the tarsometatarsal joints causes the adduction of the forefoot and metatarsal diaphysis. In addition, the tarsometatarsal joints and metatarsal diaphysis are medialized and pronated in relation to the hindfoot. The hindfoot is more supinated than the forefoot, thus causing cavus deformity and posterior skin creases.

Apart from the surgical procedures, Ponseti technique [3, 4] developed by Ignacio V Ponseti is a common nonsurgical procedure for infant clubfoot which has wider benefits over surgical procedures. It involves serial manipulation and immobilization.

Ramanathan developed IMAR clubfoot scale to assess clinical and biomechanical clubfoot data. Balasankar Ganesan evaluated the severity in clubfoot by developing a three-dimensional (3D) assessment method based on thermophysiological changes in the clubfoot.

All living things exhibit a special feature of moving its entire or part of its body for locomotion from one place to other. In case of human beings, the general movements include: crawling, walking, running, jumping, swimming and so on. The foot plays a vital role in performing these actions in humans. Any deformity in the foot affects locomotion which in turn may have severe effects on their lifestyle. The World Health Organization reports the disability prevalence across the countries varying from under 1% to over 30 % [16] and 7.9 million children approximating to 6 % of total births have severe birth defects [17, 18].

The common foot deformities include Congenital Talipes Equinovarus (CTEV) also termed as clubfoot, Larsen syndrome, Talipes calcaneovalgus, Metatarsus adductus, Metatarsus varus, Pes planus (flat feet). One of the most prevalent congenital physical disabilities worldwide, is clubfoot which occur in 1 to 3 of every 1,000 births. In India, every year about 50,000 children are born with clubfoot which alarms the medical, social and research community. Each of them is working to find ways and means to either eradicate the clubfoot or rectify the clubfoot through surgical procedures or to live with the clubfoot through support devices that adjusts or compromises their lifestyle to make locomotion less difficult and reasonably comfortable. The success rate is high if the rectification of clubfoot is done at early stages of child's growth, say within six months. In order to regulate the growth pattern of the clubfoot to a normal profile, one of the methods would be to provide a mechanical device, generally called Orthosis, which can be fitted / worn by the child. This device constrains the growth in the right directions to achieve normal foot profile.

Biomechanics of clubfoot deformity

The clubfoot is generally due to the improper alignment of bones, where the calcaneus and cuboid bones are medially shifted and inverted with reference to the talus. Researchers in their study reported that the major irregularity in clubfoot arises at the head of the talus. Also in addition to the skeletal deformity the irregularity in the muscles and tendons also exists in clubfoot. As stated by Ponseti, the clubfoot is often characterized by:

Adduction of fore foot due to cavus (high arch) at the mid foot.

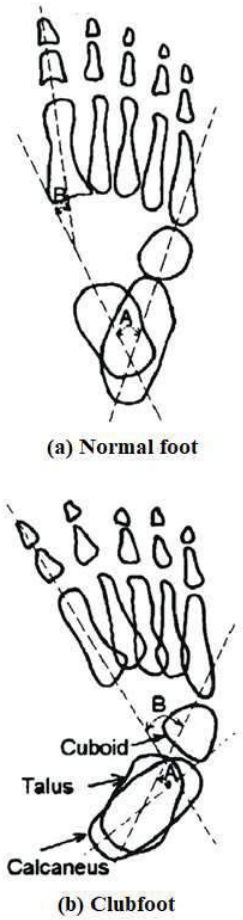
Equines of forefoot as the toes point downwards.

Tightening of Achilles tendon which draws the heel upwards resulting in difficulty while placing the foot flat on the floor.
Inward twisting of the heel towards the other foot resulting in varus of heel.
Smaller size of the foot and leg compared to the normal foot of the same age group.
Smaller calf muscles.
Stiffer foot that lacks motion.

Jain stated that the tendons like extensor digitorum longus, extensor hallucis longus and tibialise anterior at the ankle of the clubfoot are medially displaced, whereas tibialise posterior tendon is gradually widens until its insertion. In all the clubfoot, the deltoid ligaments are shorter making the foot to be twisted inwards.

In the presence of ample fibrous tissues, the ligaments connecting the tibia, talus, fibula and the calcaneus are shorter and thicker, thereby entangling with it. The normal and clubfoot's skeletal arrangement is represented in Fig. 4.

Fig. 4. Skeletal arrangement of normal foot and clubfoot



(a) Normal foot

(b) Clubfoot

The talocalcaneal angle and talonavicular angle are reprented as angle A (∠A) and angle B (∠B) respectively. For a normal foot, the talocalcaneal angle in the top view can vary from 20º to 40º and the abnormality occurs when this angle is less than 20º. Similarly, in the lateral view the normal variation can be between 25º to 50º and the abnormality occurs when this angle is less than 25º.

Clubfoot treatment, as stated by Ponseti [3], involves foot manipulation and serial casting to maintain the foot in the same position, followed by bracing. If the Ponseti method is unsuccessful due to severe structural deformity in clubfoot, then Achilles tenotomy, a surgical procedure is generally followed in recent times.

Finite element modelling of clubfoot

The same methodology adopted for developing the finite element model of normal adult foot is followed here. The Institutional Ethical Committee provided necessary ethical clearance (refer Appendix 1) for subject selection. Also, the parents are informed about this work and with their approval the subject (6 months old infant),

affected with clubfoot (right foot, cavus) is considered for this study. Computed Tomography (CT) scan is the preferred tool for image capturing of the clubfoot because of the following reasons:

As an infant¬'s movement cannot be restricted during scan procedures, CT scan is preferred over MRI as CT is less sensitive to subject's movement.

There will be no residual radiation after the CT scan¬

High precision when compared to the other methods¬

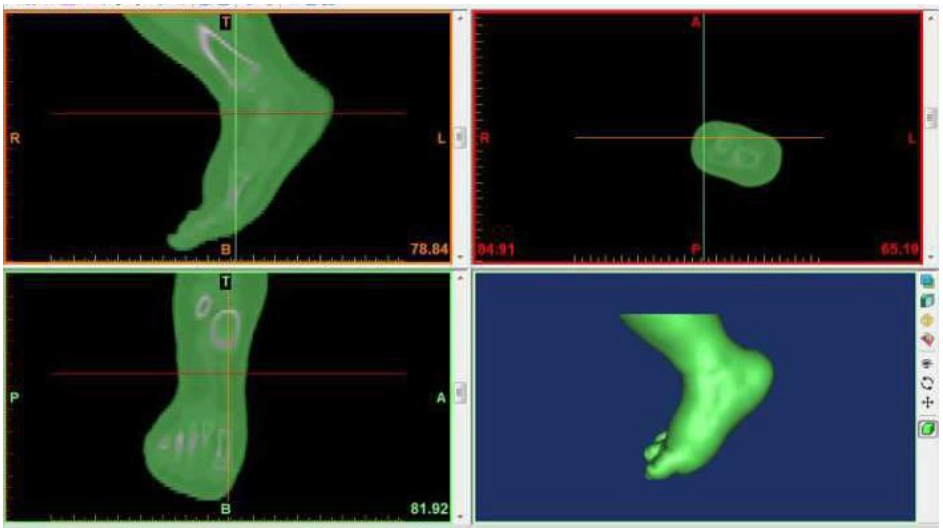
Cost effective and easy availability¬

The Computed Tomography (CT) of the affected clubfoot is captured at SRM Medical College and Research Centre, Kattankulathur, Tamil Nadu, India using SIEMENS Somatom Spirit scanner. During scanning, the subject is placed in supine lying position with the foot and the ankle joint in the biomechanical neutral alignment.

The 2D CT images taken with 1 mm slice thickness in the DICOM format are taken as the input for the surface and volume rendering. These Two-dimensional CT images in DICOM format is exported to Materialise Interactive Medical Image Control System (MIMICS 14.0), an image processing software for surface generation, which involves (i) segmentation (ii) Thresholding (iii) Region growing. Following the segmentation process the soft tissues (region of interest) are separated from other entities by thresholding. The three-dimensional clubfoot model of the selected subject is developed by varying the threshold frequency, ranging from to 225 (soft surface like skin) to (minus) -700 (hard surface like bone). After thresholding, the masked surface is divided into a finite number of objects by Region Growing in which unwanted pixels (noise) are removed.

The Computed Tomography images of the foot in Digital Imaging and Communications in Medicine (DICOM) format captured in Somatom Spirit scanner is then imported to Medical Imaging and Segmenting software called Materialise Interactive Medical Image Control System (MIMICS 14.0). The MIMICS screen has four main views representing the front view (Coronal view), top (or) bottom view (axial view), the side view (sagittal view) and the three-dimensional pane to visualize the three-dimensional model represented in Figure 5.

Fig, 5. Image reconstruction in MIMICS



The segmentation, thresholding, region growing is repeated for further refinement and the 2D information is transformed into a 3-D model as shown in Figure 6.

Fig. 6 Finite element model of the clubfoot

It is very obvious that the clubfoot of the 6-month old infant is not going to be functional till the age of walking, say 12 to 18 months. The study of stress induced during mobility or any discomfort arising out of the clubfoot has no significance.

ORTHOSIS FOR CLUBFOOT DEFORMITY

Orthotic devices are the externally fitted supporting devices envisioned to regulate the musculoskeletal functions. The primary objective of using orthotic treatment is to support the subjects to achieve normal gait patterns. Ankle Foot Orthosis (AFO) generally encompasses the ankle joint and few or entire portions of the foot. These AFOs corrects the deformity, or compensates for any weakness by controlling the position and motion of the ankle.

The Ankle Foot Orthosis (AFO) are custom made as per the instructions of the physician with cautious impression techniques to meet the objectives of non-invasive treatment methods and techniques.

The orthosis is modelled for clubfoot deformity, based on numerous features like (i) subject's age, (ii) deformity level, (iii) Ratio of width to length (bean ratio) and (iv) nature of deformity. The deformity of the foot can be minimized by using an orthosis that covers the significant regions of the clubfoot thereby regulating or restricting the growth of the foot to the required profile.

The clubfoot orthosis encompasses the ankle along with the entire foot. These orthoses are used for non-surgical treatment of clubfoot for infants. With reference to ISO 9999, the orthosis is a single unit made of leather with buckles to maintain continuous contact between the foot and the orthosis.

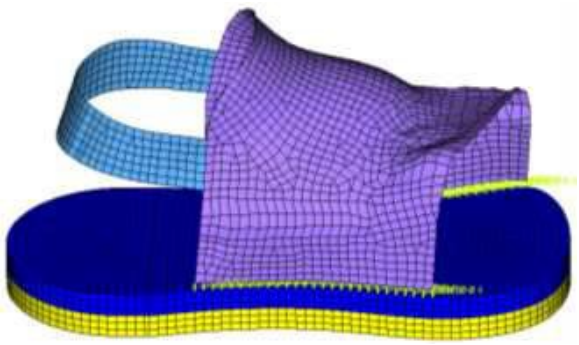
The computerized three-dimensional model of the clubfoot orthosis has been designed in SolidWorks. For the simulation studies the model is divided in to finite number of elements predominantly of rectangular and triangular shapes. The 'nodes' connect these elements together to support the finite element studies.

Considering the main factors contributing the design of orthosis like stiffness, geometrical shape and material types the design of orthosis is achieved. The materials properties of the various components of the orthosis are selected in accordance with the published literature.

Orthotics is a speciality within the medical field concerned with the design, manufacture and application of orthosis which is an externally applied device used to modify the structural and functional characteristics of the neuromuscular and skeletal system. Orthotics combines knowledge of anatomy, physiology, biomechanics and engineering. Foot orthosis comprise of a custom made insert or foot bed fitted into a shoe commonly referred to as "orthotics". These orthoses provide support for the foot by redistributing ground reaction forces as well as realigning foot joints while standing, walking or running. An ankle-foot orthosis (AFO) is an orthosis or brace that encumbers the ankle and foot. AFOs are externally applied and intended to control position and motion of the ankle, compensate for weakness, or correct deformities. AFOs can be used to support weak limbs, or to position a limb with contracted muscles into a normal position. They are also used to immobilize the ankle and lower leg in the presence of arthritis or fracture and to correct foot drop. The main problem with using the traditional orthosis is that it is difficult for both the child and the parents since both legs are constrained whether they both require treatment or not. Normal development like turning over, crawling and standing are hampered and the bar is cumbersome and awkward in daily

use. Sometimes the infants struggle to overcome the rigid constraints of the traditional orthosis which will result in skin breakage due to struggling.
The finite element model of the orthosis, customized to the subject of study, is developed, as shown in Fig. 7.

Fig. 7. Finite element model of the orthosis

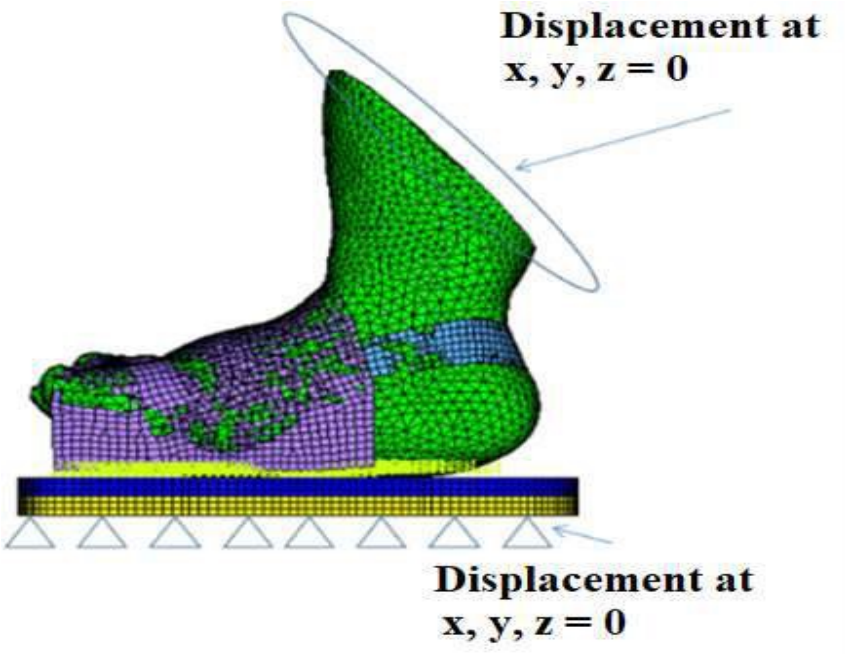


Finite element analysis of clubfoot with orthosis

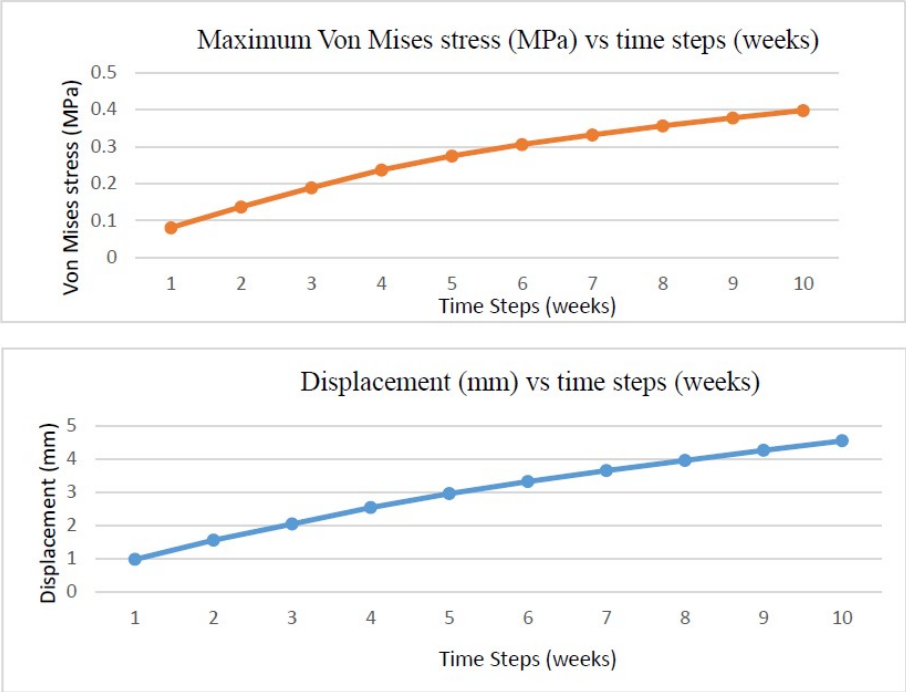
Many researchers had developed clubfoot orthosis but did not perform the finite element analysis of its effect on the clubfoot. An attempt has been made in this work to study the effect of Orthosis – clubfoot assembly using finite element model. To simulate the realistic behaviour of the infant's clubfoot (bones are not developed) hyperelastic material is assumed for the soft tissues. The interaction between the clubfoot and the orthosis is defined with the material properties taken from published literature

The effective load acting on the foot and orthosis assembly has not been reported in literatures. Few have developed the orthosis and not performed the finite element studies. To overcome this difficulty, the paediatricians and the orthopaedists are consulted to ascertain the quantum of load that an infant can withstand without much discomfort while using the orthosis. They were unanimous that a nominal load of not greater than 100 g could be applied tangentially along the strap covering the clubfoot. Accordingly, the loading conditions are applied to the FE model of the assembly to study the effect of orthosis on the corrections in deformity as a tool for non-surgical treatment. Necessary boundary and loading conditions on the Orthosis-clubfoot assembly are applied as shown in Fig. 8.

Fig.8. Boundary conditions applied on the clubfoot with orthosis



In order to simulate the constraining effect of orthosis against the growth of the clubfoot, the tangential load of 100 g when applied will be uniformly distributed over the surface of the foot covered by the strap. The meshed model of the clubfoot with orthosis is analysed for the maximum stress and displacement opposite to the deformity for a period of 10 weeks and the deformation and the maximum stress obtained in the clubfoot-orthosis assembly is shown in Fig. 9.



Result and conclusion

1. The finite element analysis on the clubfoot of the infant subject under study, when test loaded nominally, shows minimal stress. This is expected because, in real life environment there exists no load on the clubfoot of the infant (the balanced standing condition never exists).
2. The subject specific orthosis is designed for the non-surgical treatment of the infant's clubfoot and the clubfoot with orthosis is analysed to study the effect of orthosis on the clubfoot's growth pattern.
3. The treatment to rectify the clubfoot involves incremental loading every week by tightening the strap of the orthosis.
4. In order to simulate the constraining effect of orthosis against the growth of the foot for a time period of 10 weeks, uniformly distributed load is applied tangentially on the strap of the FE model of clubfoot with orthosis. The stress and the displacement are analysed for each time step.
5. The load is kept constant for every time step of 1 week, while appropriate corrections in the mesh are done to accommodate the displacement.
6. The constraint loading caused by heel strap, shifts the maximum compressive stress gradually from bottom of the heel to the achiles tendon. The flap covering the midfoot shifts the maximum compressive stress from midfoot to the ankle over the time period of 10 weeks, which provides the necessary displacement for clubfoot correction.
7. The cumulative displacement found in the clubfoot is increasing gradually for close to 7-week timestep. After that the displacement and stress take a flatter path. It is to be expected that the deformation will be permanent even after the removal of orthosis.

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PRODUCTIVITY IMPROVEMENT BY WORLD CLASS MANUFACTURING SYSTEM INSTEAD OF CONVENTIONAL METHOD

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To accomplish world-class status, organizations must change methods and ideas to enhance their procedures. This will prompt reproducing associations with providers, buyers, makers, and clients.

World Class Manufacturing is a process-driven approach that generally involves implementing the following philosophies and techniques:

- Make-to-order
- Streamlined flow
- Small lot sizes
- Families of parts
- Doing it right the first time
- Cellular manufacturing
- Total preventive maintenance
- Quick changeover
- Zero Defects
- Just-in-time production
- Variability reduction
- Employee involvement
- Cross-functional teams (quality control circles)
- Multi-skilled employees
- Visual signals
- Statistical process control

Important rules if you implement World Class Manufacturing system in your work:

- Voice of the customer is heard to the last level in the organization
- People are the driving force of change
- Motivating environment
- All faults are visible
- Continuous improvement through loss eradication
- No type of waste is accepted
- Methods for improvements are applied strictly

Seven keys to becoming a world-class manufacturer

1. Accelerate time-to-market
2. Reduce lead times
3. Simplify outsourcing processes
4. Manage the global enterprise
5. Business performance!
6. Overtop customer expectations
7. Cut operations costs

The organization, which executes WCM is as yet changing, to accomplish the status of a world pioneer, looks for open doors for steady enhancements in key regions for intensity.

World Class Manufacturing Strategy

- Develop People
- Training
- Coaching
- Empowering individuals for self-learn
- Develop Processes
- Standardize and adjust techniques and instruments
- Transfer WCM information sharing accepted procedures
- Develop Organization
- Promote WCM individuals joining
- Keep WCM people group alive

Key concepts:

1. Elimination of Parts on Floor
2. Improve 5S
3. Elimination of 3M (Muda, Muri and Mura)
4. Implementation of 3C Practices
5. Elimination of Breakage.

Implementation Methodology

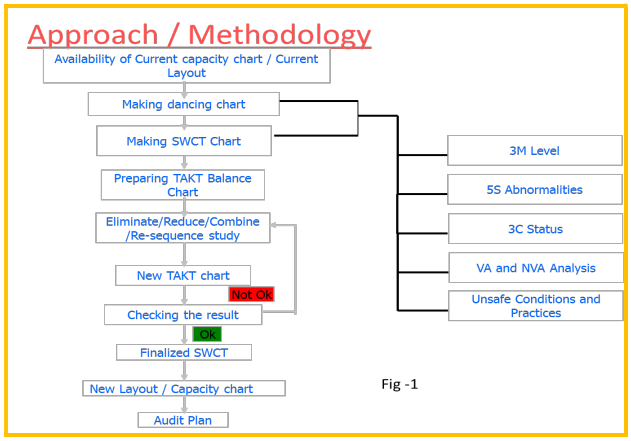


Fig -1

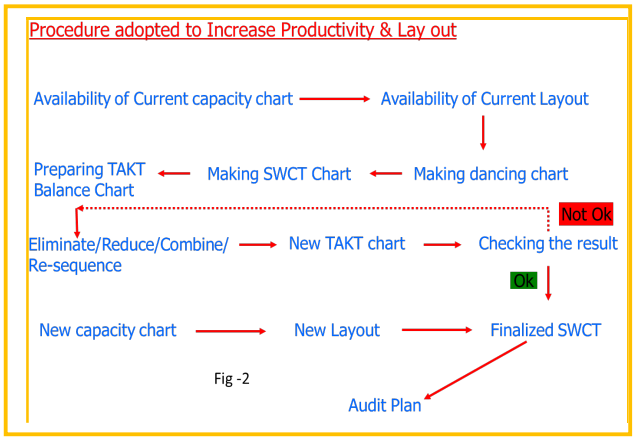
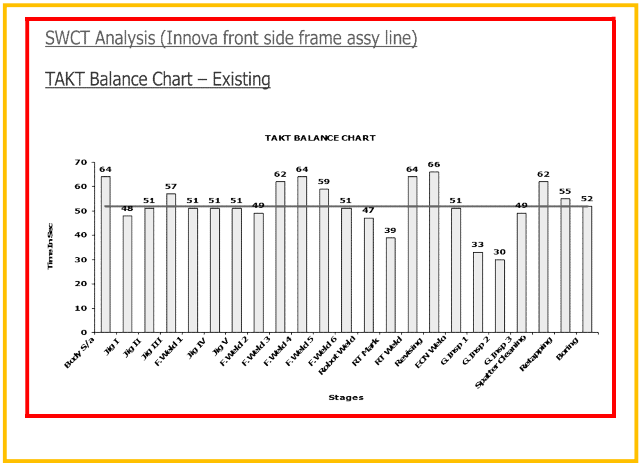
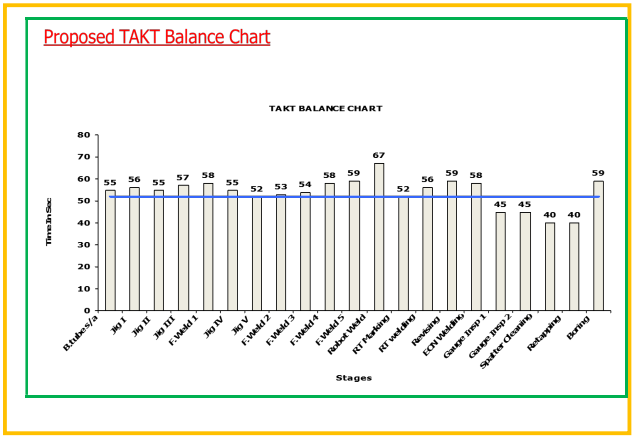


Fig -2

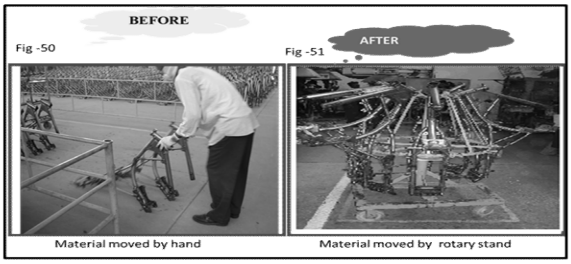


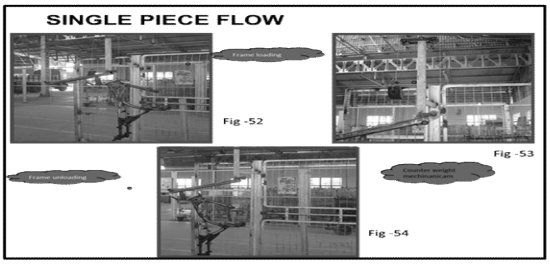


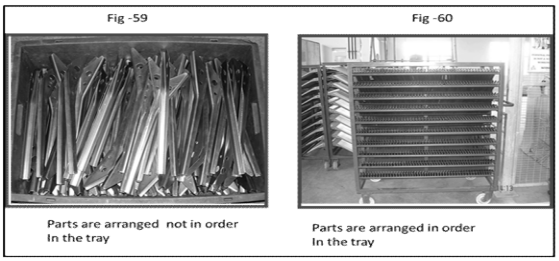
Proposal as per 19 Points Criteria

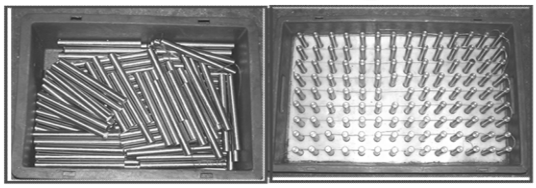
S.no	Description	UOM	Existing	Proposed	%
1	Saving of Space	Sq.mts	297.7	207	0.30%
2	Space Reduction / Part	Sq.mts	0.7	0.5	28.50%
3	Per person Space Reduction	Sq.mts	9	6	33,30%
4	Output Increase	Nos	425	425	-
5	Productivity Increase / Per man	Nos	12.9	17.7	27.10%
6	TAKT Balance chart (Difference between peak and low volume in terms of % of the TAKT Time)	%	50%	30%	40.00%
7	Vertical Space Utilization	Sq.mts	2.4	5	200%
8	Empty Space / Unutilized Space in the line	Sq.mts	161.7	90	45%
9	Material Flow – Cross Flow, Reverse Flow, Vertical Returns	Nos	23	0	100%
10	Reduction in Material Travel Distance	Mts	73.6	50	31%
11	No of people of people	Nos	33	24	27.2%
12	Reduction in Inventory	Nos	16580	9520	42%
13	Ease of Quality Control / QA Activities	A / NA	Yes	Yes	Yes
14	Ergonomics – Elimination of 3M	Sq.mts	20	10	50%
15	Complete Cell type with Cell balanced		-	Yes	
16	Flexibility of making people to handle more than one machine in case of low demand		0	Yes	
17	System of Identification and process of making the Bottleneck process / machine as the pacemaker of the line		Nil	Andon Board Display	
18	Material Handling and Movement between the Stations, From and to the line		Once in a Shift	Once in 2 Hour	
19	System of Material / RMC / Sub-assy / Major Assembly feeding throughout the line	Duration	Once in a shift	Once in 2 Hour	

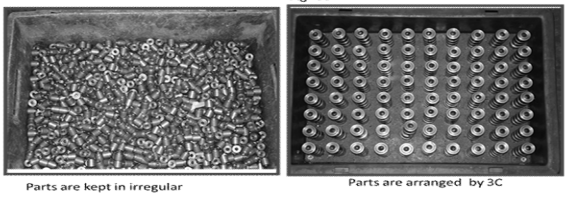
MATERAIL MOVEMENTCAUSES AND IMPROVMENT





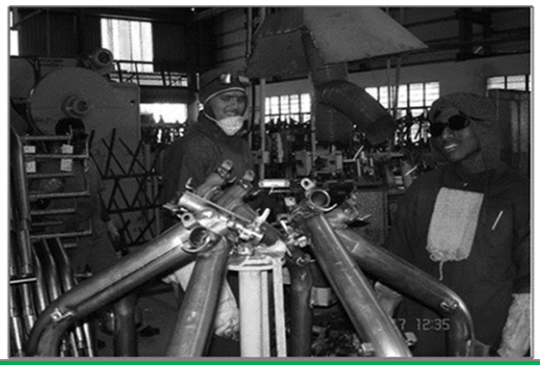






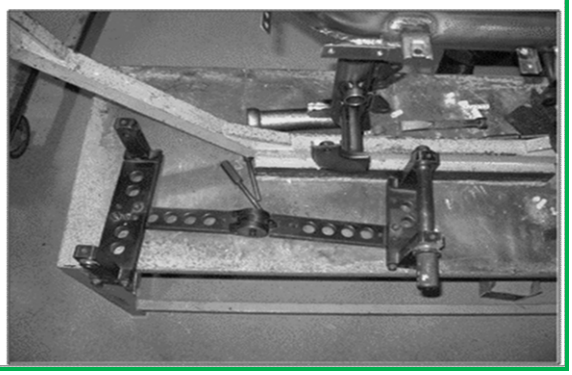
MURI

- Lot of Efforts of the Operator is being wasted in Handling the Product (MURI) – Pick & Place of the Frame – from Jig / Booth to WIP Stand (MUDA of WAITING). On an average, every Operator is handling around 7000 Kgs per shift.



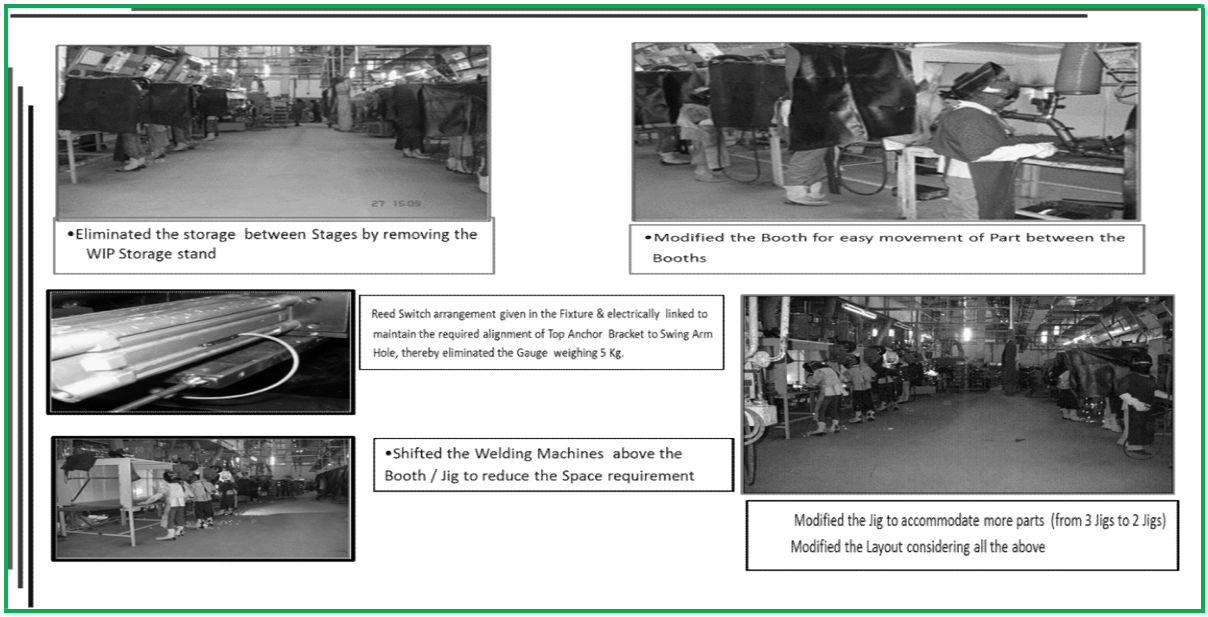
MUDA

- Hence, Muda of time in Man, Motion, Material Movement (Transportation) and Material waiting
- Gauges for Inspection too heavy (Top Anchor to Swing Arm Bush



Propose the Counter Measures

- Eliminate the temporary storage Stand between Stages
- Modify the Booth for easy movement of Part between the Booths
- Eliminate the Gauging Process by making Poka-Yoke in the Jig
- Shift the Welding Machines above the Booth / Jig to reduce the Space requirement
- Reduce No. of Jigs from 3 to 2 in Robot Line
- Modify the Layout considering all the above changes



Comparsion Between conventional type and WCM Methodlogy:

	Before	After	% Improvement
Production / Shift	450	480	6% ⬆
Man Power / Shift	33	23	30% ⬆
Per man Productivity	13	20	35 % ⬆
WIP in Nos.	176	40	77 Nos
Area in Sq. mtr	297	196	35 % ⬆
MLT in minutes	52	16	69% ⬆
MTD in mtrs	48	20	52% ⬆
No. of Welding M/cs	18	16	12% ⬆

Result :

All the above resulted in an Annual Savings of Rs17.2 Lacs

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EFFECT OF CRYOGENIC AND DEEP CRYOGENIC TREATMENT OF THE METALLURGICAL AND CORROSION BEHAVIOUR OF AZ91D MAGNESIUM ALLOY

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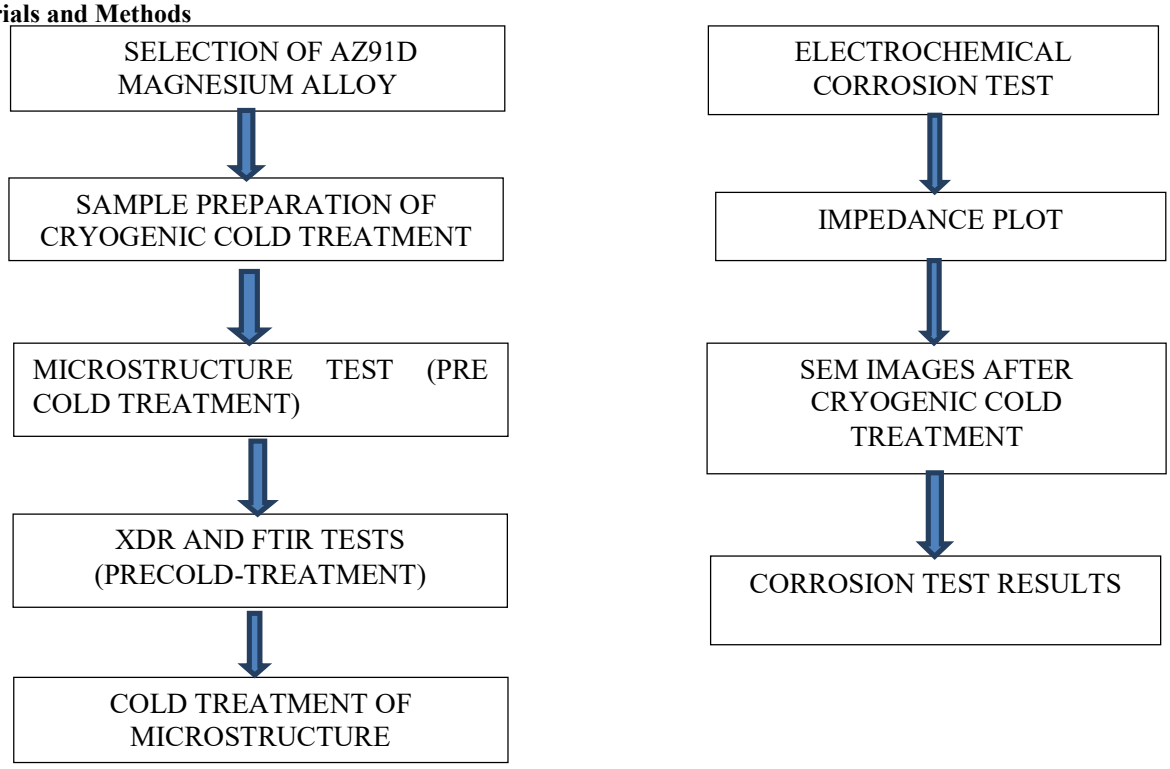
Abstract:
Magnesium alloys exhibit poor formability and possess only moderate strength compared to other alloys. One of the promising methods for increasing ductility and strength in Mg is through micro structural refinement. Commercial applicability of improved materials with novel crystallographic textures, microstructures, and compositions materials depends heavily on the development of economical and robust manufacturing methods. Due to the promise of excellent properties, such as super plasticity, high strength, good ductility, enhanced high cycle fatigue life, and good corrosion resistance interest has grown in nanostructure bulk materials. The removal of retained austenite combined with fine dispersed η-carbides precipitation has been widely observed and their effects on mechanical properties have been measured. In addition, some recent studies have pointed out a different mechanism for fatigue strengthening of stainless steels, which involves nano-martensite formation during the CT. The present paper summarizes the state of art about CT, focusing on methods, parameters, results and assumed microstructural mechanisms.

Key words: *Metallurgical and Corrosion Behaviour*,*AZ91D Magnesium Alloy.*

Introduction

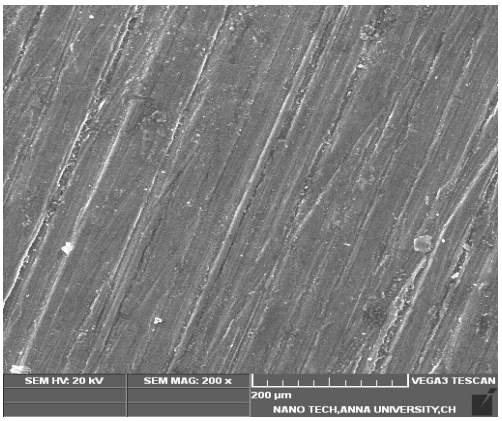
A combination of light weight, high specific strength, and good cast ability makes magnesium alloys a promising engineering material for the automotive and aerospace industries. Mg-Al-based alloys, especially the AZ and AM series, combine good room-temperature strength and ductility with satisfying salt-spray corrosion resistance and excellent cast ability. Vehicle weight reduction is one of the major means available to improve automotive fuel efficiency. High-strength steels, aluminum (Al), and polymers are already being used to reduce weight significantly, but substantial additional reductions could be achieved by greater use of low-density magnesium (Mg) and its alloys. Magnesium alloys are currently used in relatively small quantities for auto parts, generally limited to die castings. Magnesium - 33% lighter than Aluminum; 60% lighter than Titanium and 75% lighter than Steel. This material has the highest strength-to-weight ratio known to mankind. It's stronger per unit volume than other structural metals.The magnesium alloys absorbs 16 times more shock and vibrations, making it the ideal metal that gives competitive sports that extra edge.Besides its basic functions as a component in aluminum alloys, titanium, and steel production, magnesium can also be cast into various mechanical parts and replace aluminum alloys for virtually anything to be made lighter and stronger.

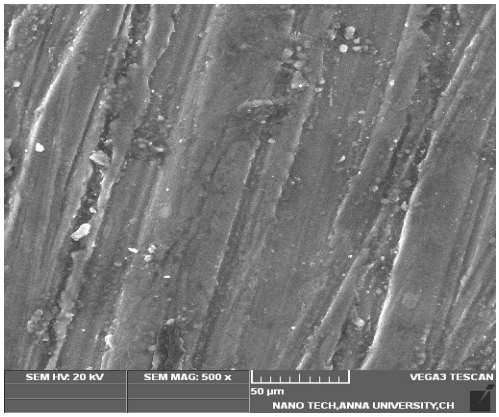
Materials and Methods



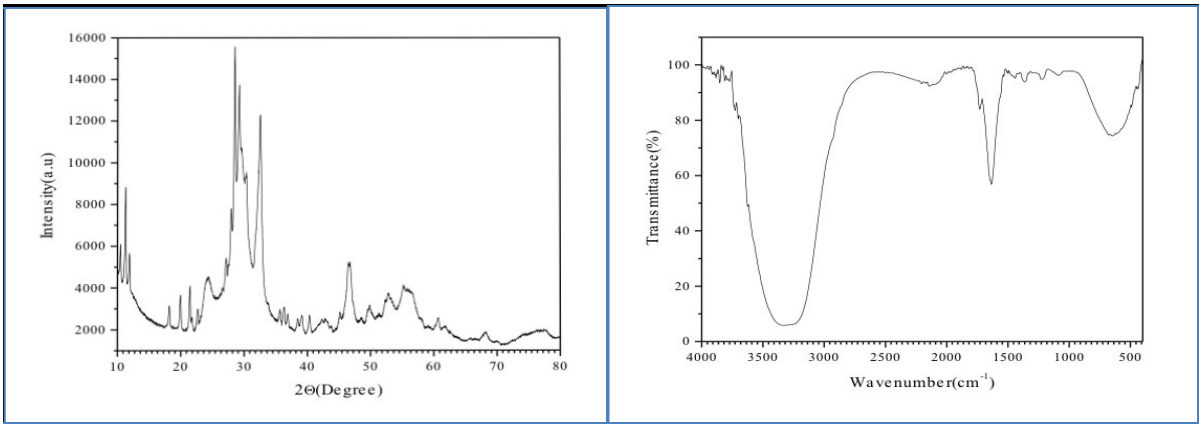
Results

Material confirmation test has been conducted through SEM images of AZ91D.Scanning Electron Microscopy (SEM) provided high-resolution imaging useful for evaluating various materials for surface fractures, flaws, contaminants or corrosion.



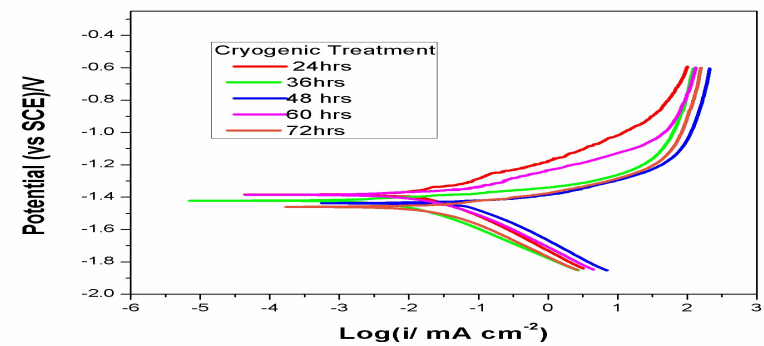


X-Ray Diffraction, frequently abbreviated as XRD, is a non-destructive test method used to analyze the structure of crystalline materials. XRD analysis, by way of the study of the crystal structure, is used to identify the crystalline phases present in a material and thereby reveal chemical. **Fourier Transform Infrared Spectroscopy**, also known as FTIR Analysis or FTIR Spectroscopy, is an analytical technique used to identify organic, polymeric, and, in some cases, inorganic materials. The FTIR analysis method uses infrared light to scan test samples and observe chemical properties.

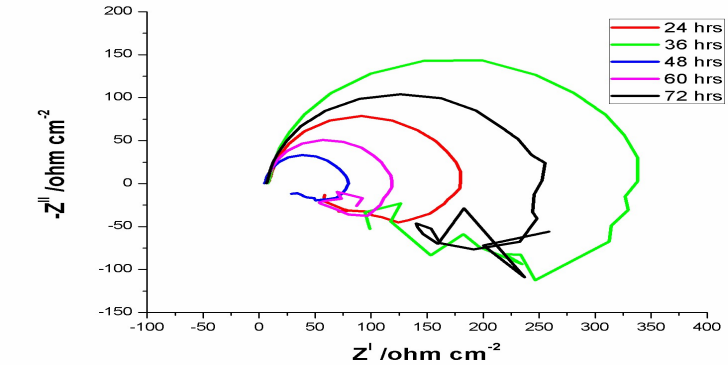


Before and after cryogenic treatments was carried out for various hours and analysed.

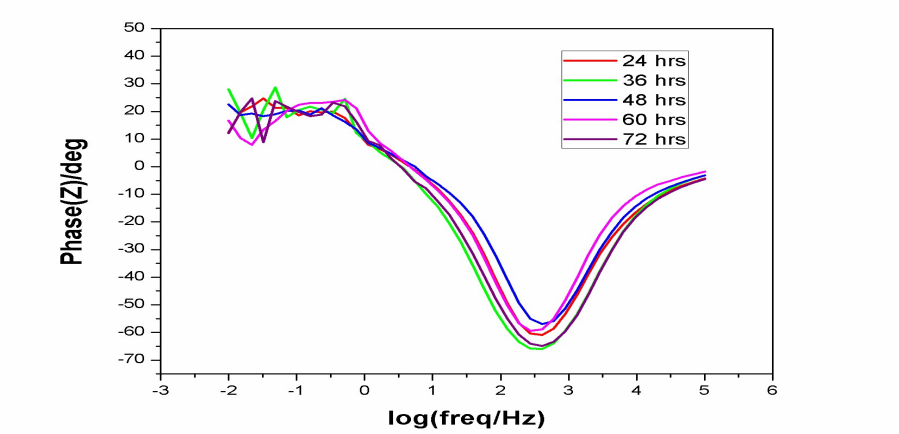
COMPARISION GRAPHs AFTER TREATMENT



Shows Polarisation Graph for Mg AZ91D- CRYOGENCIC TREATMENT



Shows Nyquist Impedance for Mg AZ91D- CRYOGENCIC TREATMENT



Shows Bode Impedance for Mg AZ91D- CRYOGENCIC TREATMENT

Corrosion result

S.No	PROCESS (Cryogenic treatment)	^{E}Corr (mV)	^{I}Corr (µA)	Corrosion Rate(mmpy)	^{R}p (ohm)
1.	24 Hours	-1383.302	15.157	0.58326	1965
2.	36 Hours	-1421.366	7.940	0.305541	1754
3.	48 Hours	-1437.589	69.818	2.68668	228
4.	60 Hours	-1385.817	14.541	0.559556	1231
5.	72 Hours	-1460.858	24.572	0.945561	786

Conclusion

The procedure was performed in order to find the varying corrosion rate of the material AZ91D. From the performance of the cryogenic treatment on the material with different timings followed with the corrosion rate 1) 24hrs:0.5832; 2) 36hrs:0.3055; 3) 48hrs:2.6866; 4) 60hrs:0.55955 and 5) 72hrs:0.9455. From the final conclusion it has been arrived that 36hrs =0.3055 is very effective in rate of corrosion resistance.

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INSECT PROTEIN – AN ALTERNATIVE SOURCE OF PROTEIN TO IMPROVE NUTRITIONAL AND FOOD SECURITY

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Introduction

Entomophagy is gaining importance in recent years due to its potential to use it as a food since insects are healthy, nutritious alternatives to mainstream staples such as chicken, pork, beef and even fish from ocean catch. Current status of world population shows that by 2050 the world population will be around 9 billion, Many studies showed that insect can be the food and feed for the future world. Traditionally, edible insects are consumed by around 2 billion people. Till now around 1900 insect's species are reported that can be used as food. Insects are alternative sustainable protein source for humans and animals. It is estimated that at least 113 countries consume around 2000 species of edible insects. Commonly consumed insects are flies (Diptera 2%), dragonflies (Odonata: 3%), termites (Isoptera: 3%), leafhoppers, plant hoppers, scale insects and true bugs (Hemiptera: 10%), crickets, locusts, grasshoppers (Orthoptera: 13%), ants, bees, wasps (Hymenoptera: 14%), caterpillars (Lepidoptera: 18%), beetles (Coleoptera: 31%) & others (5%). There is a scope to use edible insects as food and feed for human and animals.

Importance of Entomophagy

Food is the basic need of human's life. Food availability, food accessibility and food use are considered as three pillars of food security (WHO, 2013). Present data shows 821 million of population all over the world are suffering from undernourishment, facing food security problems. Growing world population results in climate change, economic crisis, agricultural problems, poverty, *etc.*, Plant and animal sources are not sufficient for the present and future growing population. Since there is high diversity in insect species and their metamorphological stages edible insects are good source of nutrients contributing high quality protein and fat required for human nutrition and it can be an alternative source of food for humans and animals. Nutritional quality of insects vary depending upon the developmental stages of insect (adult, pup, larvae, egg), feeding pattern and availability of food source. Insects are considered as the novel source of future food which can feed the world. Many insects are rich in minerals such as iron and zinc which are important for humans. Since, there is no regulatory norms framed for consumption of insects and recommended dietary allowances exists, there is a need to focus on the bioavailability of nutrients from insects to humans.

Status of Entomophagy

Consumption of silkworm pupae is traditionally practiced in South Korea, China, Malaysia and some parts of the European countries. In China, consumption of silkworm pupae is approved as novel food source by Ministry of Health of the People Republic of China (Zhou and Han, 2006a,b). In India, consumption of silkworm pupae is practiced by tribal from early days in north eastern parts of India. Silkworm pupae has significant role in nutritional composition. Silkworm consumption provides tremendous health benefits. Silkworm pupae oil is used in medicine and cosmetics industry.

India, rich in biodiversity since ancient times there is harmonious coexistence between tribal communities and natural resources. Chakravorty*et al*. reported 298 edible insects belonging to coleopteran (34%), orthoptera (24%), hemiptera (17%), hymenoptera (10%), odonata (8%), lepidoptera (4%), isoptera (2%) and ephemeroptera (1%). Insect diversity in Arunachal Pradesh (158 species), Manipur and Nagaland (41 species each), Assam (38 species), Meghalaya (16 species), Kerala (5 species) and Karnataka, Tamil Nadu, Odisha and Madhya Pradesh (1 species each). Currently, most of the edible insects are collected in nature and domesticated insects, particularly silkworms are considered for outdoor and indoor rearing on commercial scale. In North East region, silkworm larvae and pupae are consumed as food whereas in other places mulberry silkworms are destined for cocoon production. In this respect, some important points are elaborated below to perceive the current situation. In India, eating of honey bee comb with the brood (eggs and larvae) is common practice in certain areas. Interestingly, some Indian tribes eat the pupae of silkworm. FAO (Food and agriculture Organisation), 2013 reported insects as a viable replacement for meat in the event of a food shortage over the next century because of their high nutritional value

Table. 1. Comparison of average protein content among insects, reptiles, fish and animals

Animal group	Species and common name	Edible product	Protein content (g/100 g fresh weight)
Insects (raw)	Locusts and grasshopppers: *Locusta migratoria, Acridium melanorhodon, Ruspolia differens*	larva	14–18
	Locusts and grasshopppers: *Locusta migratoria, Acridium melanorhodon, Ruspolia differens*	Adult	13–28
	Sphenarium purpurascens (chapulines – Mexico)	Adult	35–48
	Silkworm (*Bombyx mori*)	Caterpillar	10–17
	Palmworm beetles: *Rhynchophorus palmarum, R. phoenicis, Callipogon barbatus*	Larva	7–36
	Yellow mealworm (*Tenebrio molitor*)	Larva	14–25
	Crickets	Adult	8–25
	Termites	Adult	13–28
Cattle		Beef (raw)	19–26
Reptiles (cooked)	Turtles: *Chelodina rugosa, Chelonia depressa*	Flesh	25–27
		Intestine	18
		Liver	11
		Heart	17–23
		Liver	12-27
Fish (raw)	Finfish	Tilapia	16–19
		Mackerel	16–28
		Catfish	17–28
	Crustaceans	Lobster	17–19
		Prawn (Malaysia)	16–19
		Shrimp	13–27
	Molluscs	Cuttlefish, squid	15–18

Source: FAO, 2012

Table. 2. List of major edible species documented from India

Method of processing	Insect species	Order: Family	Insect life stage(s)	State
Baked	*Apis cerana indica*	Hymenoptera: Apidae	Egg, larva, pupa	Assam[19]
	Antheraea assamensis	Lepidoptera: Saturnidae	Pupa	Assam[19]
	Oecophylla smaragdina	Hymenoptera: Formicidae	Egg, adult	Assam[19]
	Rhynchophorus phoenicis	Coleoptera: Curculionidae	Larva	Assam[19]
	Rhynchophorus ferrugineus	Coleoptera: Curculionidae	Larva	Assam[19]
Cooked	*Pentatomid* sp.	Hemiptera: Pentatomidae	Adult	Arunachal Pradesh[43]
	Locusta sp.	Orthoptera: Acrididae	Adult	Arunachal Pradesh[43]
Cooked + baked	*Polistes stigmata*	Hymenoptera: Vespidae	Egg, larva, pupa	Assam[19]
	Samia ricini	Lepidoptera: Saturnidae	Larva, pupa	Assam[19]
	Myrmica rubra	Hymenoptera: Formicidae	Larva, pupa	Assam[19]
Dry/deep fried	*Reticulitermes flavipes*	Isoptera: Rhinotermitidae	Adult	Assam[19]
	Dihammu scervinus	Coleoptera: Cerambycidae	Larva	Assam[19]
	Meligethes aeneus	Coleoptera: Nitidulidae	Larva	Assam[19]
	Batocera rufomaculata	Coleoptera: Cerambycidae	Larva	Assam[19]
	Okanagan sp.	Diptera: Asilidae	Adult	Assam[19]
Dry/deep fried + baked	*Megasoma elephas*	Coleoptera: Scarabaeidae	Larva	Assam[19]
	Apis dorsata	Hymenoptera: Apidae	Larva, pupa	Assam[19]
	Apis cerana indica	Hymenoptera: Apidae	Larva, pupa	Assam[19]
	Apis florea	Hymenoptera: Apidae	Larva, pupa	Assam[19]
Deep fried + roasted	*Mantis religiosa*	Orthoptera: Mantidae	Nymph, adult	Assam[19]
	Melanopus sp.	Orthoptera: Acrididae	Adult	Assam[19]
Raw/fresh	*Aeshna mixta*	Odonata: Aeshnidae	Nymph, adult	Assam[19]
	Neurothemis fluctuans	Odonata: Libellulidae	Nymph, adult	Assam[19]
	Apis dorsata	Hymenoptera: Apidae	Larva (hive)	Arunachal Pradesh[43]
	Apis cerana indica	Hymenoptera: Apidae	Larva (hive)	Arunachal Pradesh[43]
	Vespa mandarinia	Hymenoptera: Vespidae	Larva	Arunachal Pradesh[43]
Roasted	*Schizodactylus monstrosus*	Orthoptera: Gryllidae	Nymph, adult	Assam[19]
	Gryllus campestris	Orthoptera: Gryllidae	Nymph, adult	Assam[19]
	Gryllotalpa africana	Orthoptera: Gryllotalpidae	Nymph, adult	Assam[19]
	Odontolabis cuvera	Coleoptera: Lucanidae	Adult	Assam[19]
	Lucanus elaphus	Coleoptera: Lucanidae	Adult	Assam[19]
	Cyrtotrachelus buqueti	Coleoptera: Curculionidae	Larva	Arunachal Pradesh[43]
	Eurytrachelus titan	Coleoptera: Dynastidae	Adult	Assam[19]
	Libellula carolina	Odonata: Libellulidae	Adult	Assam[19]
	Schistocerca gregaria	Orthoptera: Acrididae	Nymph, adult	Assam[19]
	Belostoma indicus	Hemiptera: Belostomatidae	Adult	Arunachal Pradesh[43]
	Vespa tropica	Hymenoptera: Vespidae	Larva	Arunachal Pradesh[43]
	Vespa bicolor	Hymenoptera: Vespidae	Larva	Arunachal Pradesh[43]
	Polistes sp.	Hymenoptera: Vespidae	Larva	Arunachal Pradesh[43]

Nutritional composition of edible insects

Insects have the potential to improve the nutritional requirement and can provide high quality diet to populations who are at the risk of malnutrition. They are rich in most of the essential amino acids.Womeni et al. (2009) investigated thecontent and composition of oils extracted from several insects. Theiroils are rich in polyunsaturated fatty acids and frequently contain the essential linoleicand α-linolenic acids. The nutritional importance of these two essential fatty acids is wellrecognized, mainly for the healthy development of children and infants.

Greater attention has been paid to the potential deficient intake of these omega-3 andomega-6 fatty acids in recent times, and insects could play an important role.The nutritional values of edible insects are highly variable because of its richness in species. Insects are rich source of protein (40-60%) & minerals, act as an alternative source for meat. Commercially insects are sold as raw or whole meal powder in some of the countries like China, Korea, Thailand and some parts of India. Oils extracted from insects reported to have rich in omega-3 and omega-6 essential fatty acids and were used commercially for medicinal application.

Silkworm pupae consist of 38.13% of protein, the true protein content of crude protein was 81.02% (Niveditha.H et.al.,2020)

Silkworm pupae contain essential fatty acids such as omega-3 and *omega*-6 which was identified using the LCMS-MS method, plays an important role in curing cardiovascular diseases and diabetes (Patil et.al., 2019)The fatty acid composition of insects appears to be influenced by the plants on which theyfeed. The presence of unsaturated fatty acids may lead to rise to rapidoxidation of insect food products during processing, causing them to go rancid quickly

Feed of livestock

Poultry, beef, fish and livestock are the major source of protein, their production is not meeting up the requirement of the rapid growing population. Henceforth, edible insects fetched as a feed for insects and also as alternative source for consumption. It requires very less production cost and low technology based. It is also beneficial for the feed conversion efficiency where it is defined as the capacity of the animal to convert feed in to biomass where as in case of insects since they are poikilothermic, they do not use more energy to maintain body temperature.

Global warming Vs growing of animals & Insects

The major source for global warming is greenhouse gases as produced by several means like fossil fuel consumption, industrialization, farm animal manure and enteric fermentation. The study by Oonincx*et al*.,2012 revealed that the greenhouse gases from the three insect's mealworm larvae, crickets and locust produced less greenhouse gases and ammonia per Kg mass compared to hectare per Kg mass of greenhouse gas produced by pigs.

	 Insect	 Chicken	 Pig	 Cow
Greenhouse gases released per kg of live weight, g	2	NA	1,130	2,850
Feed required per kg of live weight, kg	1.7	2.5	5	10
Land required per g of protein, m²	18	51	63	254
Water required per g of protein, li	23	34	57	112

Insect as food has environmental benefits with respect to less special usage and H_2O requirement. For example, 150 g grasshopper requires less water (3.78 L) than same amount of cattle meat which requires 3290 litres and for beef 10 times more plant materials is needed to one kilogram of insect biomass.(Premalatha *et al.*, 2011)According to the FAO (Food and Agriculture Organization), nearly 2.5 billion humans regularly eat insects in the world. The majority of edible insects are gathered from forest habitats, but in many countries, innovation in mass-rearing systems has begun. Edible insects have always been a part of human diets. They contain high-quality protein, essential amino acids, and vitamins for humans.

Fortification Of Foods With Edible Insects

Kim et al., 2016 studied the incorporation of the pre-treated mealworm larvae andsilkworm pupae as a novel protein ingredient in preparation of emulsion sausages. This studyconcludes that the technological properties and

nutritional quality of meat emulsion preparedby the incorporation of pre-treated edible insects as a novel non-meat ingredient. Both insectswere freeze dried, powdered, defatting and acid-hydrolysed. By replacing 10% lean pork with insect flours along with 60% lean pork, 20% ice and 20% back fat control sausage wasformulated. The result concludes that defatting and acid hydrolysis process found to proteinpercentage in both flours. But in turn protein solubility was found to decreased by acidhydrolysis (P = 0.002). Incorporation of pre-treated insect flours did not show any impact onprotein solubility in emulsion sausages, but regardless of pre-treatment methods and types of insects, it was found that cooking yield and hardness were increased to a similar extent. The12study concludes that, further optimization of above insects as a novel protein ingredient foremulsified meat products can be achieved by the process of separation of mealworm larvaeand silkworm pupae and also without making compromise in nutritional and technologicalproperties of food products, protein source from edible insects can be practically utilized as anon-meat food ingredient in processed meat products as well as in other food applications.

Çabuk and Yılmaz, 2020 evaluated the proximate composition, nutritional quality,cooking properties and sensory characteristics of traditional egg pasta (erişte) fortified withedible insects and legume flours. Samples of egg pasta was produced by varying blends ofwheat flour, legume flour and wheat flour (lentil and white kidney bean) and with edibleinsect flour (mealworm and grasshopper). Cooking time was significantly increased with the fortification. In egg pasta fortified with edible insect flours resulted inreduced volume expansion compared to control (236.7 %) to grasshopper flour (215.6%) andmealworm flour (196.9%), respectively. But higher nutritional profile was also found insamples containing grasshopper flour and grasshopper flour. The results showed the influenceof fortification of pasta with kidney beans was proved positive on some of the productcharacteristics of product like smoothness of pasta, but it got weakened by the incorporationof grasshopper flour. From sensory evaluation, higher flavour and overall acceptability valueswere found in pasta fortified with lentil and white kidney bean flour compared to pastafortified with insects flours and control sample.

Conclusion

The concern to guaranty food security cannot be dissociated from food safety, and under the *Codex Alimentarius* principles of food hygiene, insects would be comparable to other types of foods of animal origin. The processing and storage of insects and their products should follow the same health and sanitation regulations as for any other traditional food or feed items in order to ensure food safety. Because of their biological composition microbial safety, toxicity, palatability, and the presence of inorganic compounds should be considered. Allergens if any present in the purified protein, methods for removal of the same before incorporating into the food and feed will be taken into consideration. The functional characteristics of edible insect protein may vary based on the insect. Foaming stability, foaming capacity, emulsion stability, emulsion capacity, gelation capacity, bulk density, tap density and water absorption capacity of the proteins needs to be studies. Also the safety aspects of traditional edible insects practiced by the people will be ensured for the future.

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INSECT GENOMICS IN THE 21ST CENTURY: PROSPECTS AND PROGRESS

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Abstract

Insects are the dominant animals in the world, with more than one million described species. Insects, not only produce direct damage to plants but also great medical and veterinary importance, mainly because they are vectors of diseases affecting humans, livestock, and companion animals. In recent years, next-generation sequencing (NGS) techniques have provided fascinating opportunities to understand the basic biology, biochemistry, and molecular biology of these intimate and intriguing relationships. The decrease in sequencing costs and extensive sequencing services from NGS providers has brought many scientists to be involved in genome sequencing of insects and their associated entomopathogens. By using high-throughput genomic technologies, scientists can elucidate the virulence, host adaptation and gene function of the particular entomopathogen including virus, fungi, bacteria and nematode. There are about 18 taxonomic group can be used as edible insects across the world. These insects are rich source of proteins vitamins and anti-microbial peptides (AMP's). These AMP's are used in developing anti-infective drugs against cancer, wound healing, anti-inflammatorydisorders, antibacterial drugs, and food preservatives.

Key words: Next-generation sequencing, Genomics, Insect pathology, Entomopathogenic organisms

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Introduction

Insects are the most successful group of animals on the planet and are ecologicallyand economically extremely important. Today's entomology field has gone beyondborders and is termed as a "super science." With its multidisciplinary approach,entomology explores new scientific frontiers. It has emerged to provide some of themost powerful tools in resolving fundamental biological questions and problemsusing genomic (genome sequencing, assigning functions to genes, determininggenome architecture) and proteomic (nature of proteins, 3D structure, posttranscriptional modifications) approaches.

Whole-genome sequence projects for insect model organisms (29 insect speciescompleted and many more under way) and the concurrent growth of sequence databasesprovide the biological sciences with invaluable sources of information. Thetwo volumes of this book are intended to share the efforts of major contributors withthe genomic and proteomic communities. This will pave the way toward the developmentof new and innovative approaches to improve public health and agricultureusing effective and ecologically sound pest management systems.

With rapid development of gene sequencing technologies, scientists have proposed many sequencing projects, eg the Genome 10 K project and the Bird 10 K project (Genome 10K Community of Scientists, 2009). Recently, the Earth BioGenome Project was proposed at the BioGenomics 2017 conference, with the goal of launching genome sequencing for all living organisms.

In 2011, Robinson and colleagues proposed the 'i5k' initiative to sequence the genomes of 5000 insects and other arthropods with important biological significance or economic value before 2017 (Robinson *et al*., 2011). Unfortunately, the goals of the 'i5k' initiative are still far from realized due to difficulty in assembling insect genomes and limited financial support. Nonetheless, this ambitious project generated a great deal of data for, and interest in, insect genomes. At the time of preparing this paper, 1219 insect genome-sequencing projects have been registered with the National Center for Biotechnology Information (NCBI): 401 insect species have complete genome assemblies with varied quality; the genome annotations of 155 insects have been publicly released; and over 100 insect genomes have been published in peer-reviewed journals. Building on these foundational resources, entomologists have generated copious functional-genomic data from insects, including transcriptomes, proteomes, and metabolomes (Fig.1).

Insect Genomics

Nowadays, the biggest challenge of insect genome sequencing is the difficulty of obtaining a high-quality genome assembly from the raw reads produced by second-generation sequencing techniques. Illumina HiSeq is the most widely used second-generation sequencing platform at present, and it is fast, cheap and highly accurate. However, it can only produce short reads (<250 bp). This brings a great challenge to genome assembly, because most insects, especially Lepidoptera, have high heterozygosity. The issue of heterozygosity is often compounded because insects are typically small, and it is necessary to pool together many individuals to obtain sufficient DNA for analysis. Furthermore, it is quite difficult to produce inbred strains for most insects. These facts can converge to create dilemmas for many insect genome-sequencing projects. Though some genomes have been successfully sequenced, such as that of *Plutellaxylostella*, other efforts have suffered substantially from the failure to get assembled scaffolds with high contig N50 lengths.

The use of the third-generation sequencing techniques, which can produce long reads (>10 kb), is a promising remedy to these challenges. PacBio is the first widely used third-generation sequencing technique. PacBio reads are even longer than many contigs in a number of insect genome assemblies. The genomes of several insects have been publicly reported using PacBio or the combination of Illumina and PacBio. However, in our opinion, the third-generation sequencing techniques are far from mature at present. One of the downsides for long-read technologies is the quantity of high-molecular-weight DNA required for the methods. In addition, these methods have high error rates in sequencing, although error correction can rely on using overlapping raw sub-reads to improve the base accuracy. Generally, PacBio requires more depth of coverage for better corrections.

In 2017, the *Aedes aegypti* genome was greatly improved to yield a chromosome-level genome assembly using the Hi-C technique (Dudchenko et al. 2017). Long-read assembly was also used to resolve large repetitive regions for a cell line (Whitfield et al. 2017), which were unable to be determined by previous methods. Recently, a better assembly of mosquito genomes was implemented by combining both the PacBio and Hi-C techniques (Matthews et al. 2018).

However, we are optimistic that these problems will be solved as improvements are made in these third-generation sequencing technologies. But at present, insect genome sequencing still faces many hurdles in obtaining highly accurate assemblies.

Edible Insects

The consumption of edible insects started nearly 7000 years ago (Ramos-Elorduy 2009). More than 2300 species of 18 orders have been reported as edible insects, of which 5 orders are with at least 100 records(Van Huis et al. 2013). These insects inhabit in both aquatic and terrestrial environments (Jongema 2017). The majority of them are harvested from nature though some species are farmed in a large scale.

Lepidopterans, Orthopterans, Isopterans and Hymenopterans are all regarded as common food sources in many areas. Culturally and religiously, entomophagy is particularly popular in tropical and subtropical regions due to the warm and moist climate (Jongema 2017). Tropical insects are generally large with stable life history, which can facilitate harvesting (Gaston &Chown 1999). The immature forms of insects (pupae and larvae) are preferred for their abundant amino acids and fatty acids, which not only ensure the nutritional value, but also provide a unique and splendid flavor.

The production of animal protein is under huge pressure as the world population is rapidly increasing (Gerland et al. 2014). Consequently, people are facing the enduring protein undernourishment and seeking alternative protein resources. Entomophagy is seen as one of the best choices. As it could provide large amounts of multiple nutrients rapidly, it might provide a solution to address famine (Van Huis 2013). Great attention have been paid to the utilization and production of edible insects. However, the industrial chain of edible insects, from fundamental research to marketing, still needs to be developed.

Edible insects also serve as a feeding source for livestock and aquaculture now. It is believed that fowls fed by insects, which can provide nice protein supplies, are more nutritional than those fed by grains (Józefiak et al. 2016). Using insects as fodders is particularly popular in areas where vegetable feeds is expensive (Krishnan et al. 2011). The cost is increasingly challenging for industries to feed farmed animals on traditional meals that are made of soy. Insect meal, however, can provide enough nutrition with cost that is distinctly low. Biomass could be recycled during the production of insects, which makes the protein sustainable. Moreover, pupae of Chironomidae and Muscidae are used as fishing baits and feeds (Awoniyi et al. 2004). Yellow mealworms have been widely used as the fodder for amphibious pets like lizards and salamanders (Liu et al. 2010).

Food additives can be extracted from insects, too. Carmine, a common natural colorant being used for hundreds of years, is obtained from Dactylopius coccus (Van Huis et al. 2013). It provides a bright red dye for clothes, cosmetics and of course, food. It is commonly used in stacks like jelly. In fact, the demand of the dye has been rapidly increasing as people are keen on natural dyes at present (Baskes 2000). Similarly, the lac insect (*Kerria lacca*) is a fabulous source of a water-soluble polyhydroxy-anthraquinones called lac dye. The pigment is originally bright red, but can be mordant from violet to red and brown. It was primarily used in coloring textile fibre, but it is now involved in beverage industries as well (Raman 2014). Lac resin secreted by the lac insect is commonly used in coating candies and fruits (Siddiqui 2004).

Beyond being eaten to allay the hunger or just for pleasure, insect extracts can be used as a source of medicine, healthcare and industrial products (Liu & Wei 2002). Industrial enzymes for biodiesel production have been successfully extracted from black soldier flies (*Hermeticaillucens*). The technique is seen as a solution to energy shortage (Su et al. 2019). The exoskeleton of adults is a rich source of chitin, which has been proven to enhance the immune system of different organisms (Van Dyken& Locksley 2018). More than 400 kinds of antibacterial substances have been extracted from insects. Antimicrobial peptides (AMPs)are increasingly popular recently with intensive research conducted. In fact, more than 170 defensins have been found in invertebrates and

most of them can be produced from insects (Józefiak et al. 2016). Houseflies (*Musca domestica*) have been used as a source of antimicrobials. Lac resin mentioned above is actually versatile in various industries. Besides coating food, it is widely used in insulated materials, printing and adhesive industries(Wang et al. 2006). Moreover, it is of great value in pharmaceutical industry with the potential in hepatoprotective and anti-obesity drugs (Iqbal & Khan 2019). White wax secreted from Chinese white wax scales (*Ericerus pela*) serves similar to lac resin and it is used in coating tablets (Yang et al. 2012). A special oil called um-buga, which is derived from melon bugs (*Coridiusvidutus*), contains a high number of antibacterial substances that control gram-positive bacteria (Mustafa et al. 2008). It is only utilized in some African areas, though. The famous fungus-insect complex of *Bombyx batryticatus* and *Beauveria bassiana* is a luxury traditional Chinese medicine. It has been proven to possess multi-pharmacologic functions including anti-convulsion, anticoagulation, hypnogenesis, anti-fugus, anticancer and hypolipidemic. The active constituents extracted from it, including polysaccharides, flavones and beauvericin have been developed into modern medicine targeting corresponding diseases (Hu et al. 2017; Wu et al. 2015). The medical potential of many other reared insects have been demonstrated, including several cases of antioxidants extracted from different groups of insects (Zielinska et al. 2017).

Anti-microbial peptides in Insects

Due to overuse of antibiotics or drugs, antibiotic resistance and multidrugresistance in pathogenic bacteria have been rising during the last two decades.Therefore, there is an urgent need for development of natural antibiotics in the formof antimicrobial peptides (AMPs). These are produced by nearly all organisms, frombacteria to plants and animals, and can protect against a broad array of pathogenicbacteria, fungi, parasites, viruses, and protozoa and thus may serve as alternatives tosynthetic (conventional) antibiotics. Apart from microbicidal properties of AMPs,they have been shown to act as immune-modulators with chemo-attractants, to exhibitsignaling activities, and to help in the management of beneficial endosymbionts. As a result, clinical programs on host defense AMPs have been established in theareas of cancer biology, infection, inflammation, and dermatology. Thus, understanding the modes of action of these AMPs will give insights into host-parasitecoevolution as well as enable design of next-generation antibiotics.

Insects have been remarkably successful in evolutiondue to their diversity, certainly enhanced by their ability to colonize new nichesand feed on nearly all plants and animals, use insects' cells for heterologous proteinproduction in a cost-effective way, and mount a high immune response when facedwith a constantly changing and diverse array of pathogens and parasites (Cornman et al. 2012). Tofight against infection or wounding, the insect's immune system employs both cellularand humoral immune systems, although they do not possess adaptive immunityas present in higher vertebrates. Cellular immunity is mainly mediated inhemocytes by executing processes such as phagocytosis, encapsulation, and nodulation (Lavine& Strand, 2002). Humoral reactions include synthesis of AMPs, which are secreted into thehemolymph and trigger proteolytic cascades leading to coagulation (Hoffmann, 1995), activationof a phenoloxidase cascade to produce the melanin and toxic intermediates againstinvading pathogens, and production of reactive oxygen species (Cerenius et al. 2008). Synthesis ofAMPs in insects occurs in special tissues such as fat bodies (similar to mammalianliver) as well as hemocytes upon microbial infection or septic injury. To date,more than 1750 AMPs have been identified from a variety of organisms; althoughmany are derived from vertebrates and plants, most are derived from invertebratespecies(Figure 2). The majority of these AMPs have antibacterial properties, followedby a few which are antifungal, anticancer, antiviral, or antiparasitic in nature (Gimenez et al. 2010).

Based on amino acid sequence features, AMPs are broadly classified into threecategories (Bulet et al. 1999): first, linear peptides such as cecropin having an α-helix and devoidof cysteine residues; second, peptides with a β-sheet globular structure stabilized by intramolecular disulfide bridges (e.g., heliomicin); and, third, peptides with an overrepresentationof proline or glycine residues (e.g., lebocin and moricin). AMPs areinvolved in many biological processes such as immune modulation, angiogenesis,and cytokine and histamine release (Brandenburg et al. 2012). Although AMPs are typically short andshow little similarity in sequence, they have some common features such as beinghighly cationic and tending to form an amphipathic α-helical structure that seemscrucial for their function as membrane-active agents or acting on the DNA or RNAinside the cell (Brandenburg et al. 2012). Compared to cationic AMPs, much less is known about theworking mechanism(s) of anionic AMPs (Harris et al. 2009).

Comparative studies of immunityrelatedgenes among lepidopterans and detailed comparative genomics with *Bombyx mori*have been hampered due to a lack of relevant genomic information, but the adventof the next-generation sequence (NGS) platform has facilitated the analysis ofinnate immunity-related genesfrom different lepidopterans, such as the tobaccohornworm, *Manduca sexta* (Zhang et al. 2011); the greater wax moth, *Galleria mellonella* (Vogel et al. 2011); the DBM (Etebari et al. 2011); the old world bollworm, *Helicoverpaarmigera*(Li et al. 2012), thebeet armyworm, *Spodoptera exigua* (Pascual et al. 2012); and the corn earworm, *Heliothisvirescens*(Shelby & Popham, 2012). Along with NGS, small-scale expressed sequence tag (EST) projects

havebeen performed on many lepidopteran species, for example, the fall armyworm,*Spodoptera frugiperda* (Duvic et al. 2012); the cabbage looper, *Trichoplusiani* (Freitak et al. 2007); the tasarsilkworm, *Antheraea mylitta* (Gandhe et al. 2006); and the cecropia moth, *Hyalophora cecropia*(Gudmundsson et al. 1991).

Conclusion

The emergence of multi-resistant bacterial strains has demonstrated the need foralternatives to synthetic antibiotics, and this has led to the discovery of natural antimicrobialpeptides from the order Lepidoptera to fight against microorganisms.Although with limited success in clinical trials, knowledge acquired in the past two decades has led to the identification of close to 1,750 natural AMPs, among which a significant number are isolated from insects. The insect AMPs will provide further insights fordesigning anti-infective drugs against cancer, wound healing, anti-inflamatorydisorders, antibacterial drugs, and food preservatives; moreover, much higher efficacy can be achieved through a synergistic approach. Interestingly, to date, clinicaltrials have already shown limited resistance against these peptides among pathogenicbacterial strains, greater ease of synthesis, and discovery of novel mechanisms of action. Such significant properties of AMPs make them a powerful arsenalof molecules that could be transformed into novel antimicrobial agents for the twenty-first century.

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Fig. 1.Workflow of the transcriptome assembly and analysisof insect's high-throughput sequencing data.

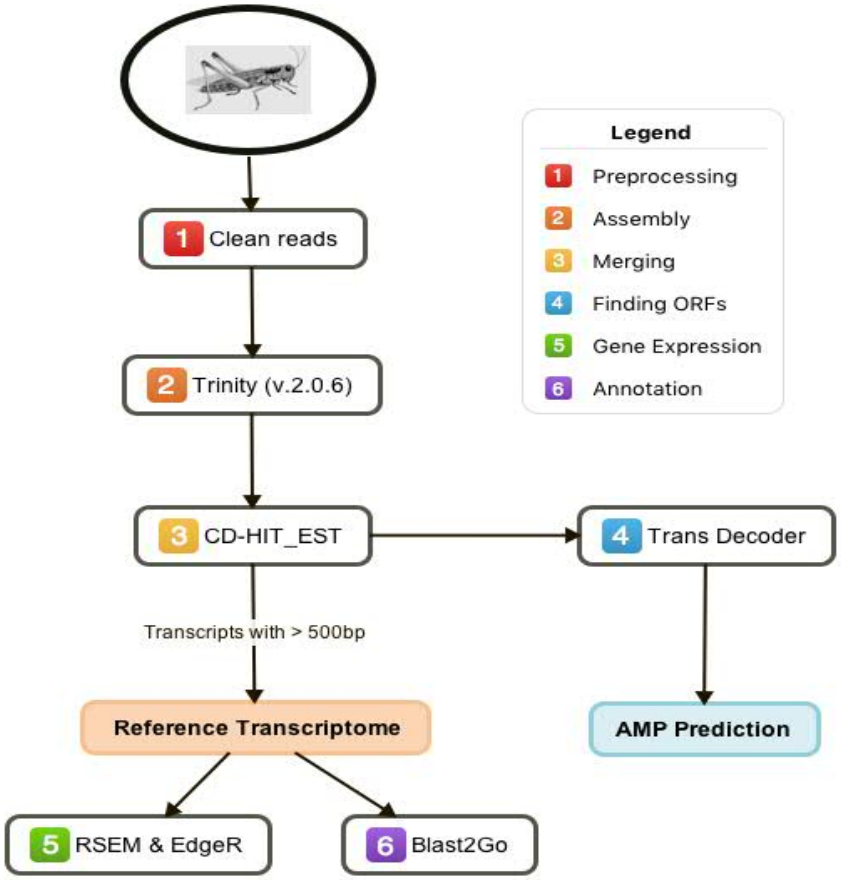
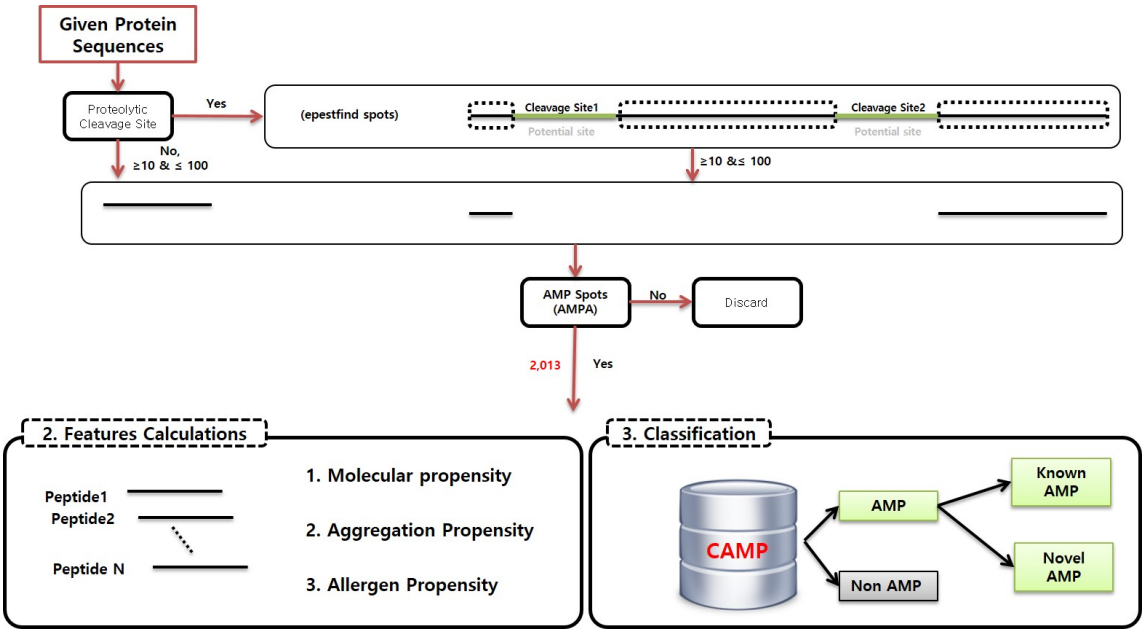


Fig. 2. Insect Anti-Microbial Peptide Prediction pipeline



SOLAR AND RF DRYING OF COPRA FOR THE CONTROL OF INSECT INFESTATION FOR EXTENDED SHELF LIFE & STORABILITY

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Abstract

India is the third largest country in coconut production. During storage copra is affected by microbes and stored pests. Sulphur fumigation is the predominant method used for copra storage.Use of plant extracts as an alternative method for traditional chemical methods and use of different storage structure, dryers (solar hybrid dryer and RF sterilizer) to reduce the moisture content of copra is the focus of the research.Copra is kernel (*Cocos mucifera*) used for coconut oil production. It is affected by coconut beetle and Aspergillus species. Study was conducted to evaluate the performance of Solar dryer and Radio Frequency sterilizer as a potential source for drying of copra at 58^0C(SD) and 65^0C(RF) to extend its storability and to assess the microbial activity. The treatment was carried out at various combinations of belt speed and electrode heights in order to get the reduced moisture content that can be achieved using the Radio Frequency setup and various temperatures in Solar dryer. It was observed that the rate of moisture content decreased from 58.95– 25.26% and initial relative humidity decreased from 84% to 26% in a temperature range of 65^0C and the variation in electrode height from 200 to 270 mm in RF sterilizer and in Solar hybrid dryer initial relative humidity decreased from 84% to 54% in a temperature range of 58^0C,initial moisture content 58.95% was reduced to 30.53%.Therefore, RF and Solar hybrid dryer holds potential to scale up to industrial applications in drying of copra which helps in reduced microbial spoilage and extends shelf life.

Keywords Copra, Radio frequency, Solar hybrid dryer,

Aim of the Study

To dry copra using the solar hybrid dryer and RF sterilizer. To use various temperatures to reduce moisture content in copra and to reduce microbial spoilage and extend shelf life of copra.

Background

Copra is a perishable food commodity and the deterioration is high. The length of time taken and the degree of deterioration are determined by the specific processing, handling, and storage conditions. (Lakshmanan et al., 2020). The most essential single component that initiates and progresses degradation is confined or reabsorbed moisture. The presence of moisture is always implied by bacterial and fungal infestation on copra. Bacterial activity is highest at moisture levels greater than 10%, while fungal/mould activity is highest at moisture levels less than 6%. (Aravindh & Sreekumar, 2015). From past decades, the control of pest in stored products particularly cereal grains and pulses, nuts and oilseeds by use of the chemicalsis a common strategy for post-harvest management. However, it is well known that this practice leads to the other problems including environmental pollution, human toxicity, development of insect resistance, quality concern and other associated damages. The increasing use of synthetic/chemical pesticides has resulted in a number of negative consequences, including toxic residue pollution of food, soil, groundwater, and air, as well as dangerous impacts on non-targeted insects and other creatures. Sulphites, such as sulphur dioxide, which works as a decolorant, bleach foods and prevent bacteria from growing (Garbati Pegna et al., 2017). To reduce toxic residual effects of sulphur which causes allergic reactions such as relatively mild, hives, or more severe such as difficulty breathing bronchoconstriction and Anaphylactic shock which is potentially fatal side effect of residues in copra. The need for alternative eco-friendly methods of controlling pest and microorganism infestation (Kraikaew et al., 2020).

Methodology

Coconut (***Cocos nucifera***) drying by using solar energy is the traditional method practiced in India (Aquino et al., 2005). But spoilage due to weather conditions causes liability during drying. An attempt was made to dry copra using RF sterilizer at 50 ^{0}c&65 ^{0}c and Solardryer 53 ^{0}c, 58 ^{0}c & 63 ^{0}c. Different temperatures used reduced the moisture content and microbial activity and the quality of the copra were assessed.

Fig 1. Solar Hybrid dryerand RFSterilizer treated copra sample

Results and Discussion

Table 1. Physical properties of copra treated with RF sterilizer; Solar dryer treated copra with Control sample

Control and dryers	Temperature(^{0}c)	Moisture content (%)	Relative humidity (%)
Fresh copra (control)	30	58.95	84
Solar dryer	58	30.53	54
RF Sterilizer	65	25.26	26

The solar dryer was used fordrying of copra at temperature 58^{0}c showed reduced relative humidity from 84% to 54% and moisture content 58.95% reduced to 30.53%. RF sterilizer electric post range from 200-270mm and oven temp of 65^{0}c showed marked decrease in moisture content from 58.95% to 25.26% and relative humidity from 84% to 26% which helps in reduced spoilage and extended shelf life of copra (Padmanaban et al., 2017) also reported the same result with RF treated dates which showed reduced moisture content and low microbial count.

Table 2. Proximate composition of copra treated with RF sterilizer, Solar dryer with Control sample

Control and dryers	Oil (%)	Carbohydrate (%)	Protein (%)	Ash (%)	Crude fibre (%)
Fresh copra (control)	38.2	9.7	4.6	1.2	2.3
Solar dryer	63.7	16.1	7.6	2.0	3.8
RF Sterilizer	8.1	45.1	21.0	5.5	10.5

The mineral constituents, as well as all three major food categories protein, fat, and carbohydrate, are all found in the copra kernel, with the oil/fat being the most important component. Coconut protein is biologically

beneficial, unlike plant proteins. The oil/fat present in thecopra consists of readily digestible edible fats and regarded as highest contribution to nutrition (Swain, 2013). Carbohydrates is said to be bulk and present in the form of cellulose and 7% cane sugar. The oil content of the solar dryer and RF sterilizer is 63.7%, 8.1% when compared with control sample 38.2%. The carbohydrate composition of the solar dryer and RF sterilizer is16.1%, 45.1% when compared with control sample 9.1%. The protein content of the solar dryer and RF sterilizer is (7.6 %, 21.0 %) and ash content is 2.0%, 5.5% when compared with control sample 1.2%. Crude fibre content of the solar dryer and RF sterilizer is (3.8% &10.5%) when compared with control sample is 2.3%.

Conclusion

The solar hybrid dryer and RF sterilizer were proved to be effective in reducing the moisture content of copra. RF treatment appears to be a viable technique for copra disinfestation because it ensures rapid heating throughout the fruit, bringing insects to their lethal temperature in minutes and limiting potential physical and chemical alteration of the copra, which would result in the loss of quality properties. Continuous RF processing is commonly utilized in the food sector to treat a variety of products. (Nukulwar & Tungikar, 2021). As a result, it may also be used for copra disinfestation, which can be done in 15-20 minutes at a temperature of 650°C and a diameter of 200-270 mm without affecting the nut's major physical features. Due to the lack of residuals in copra, it is possible to disinfest huge amounts of copra at a minimal cost and without causing harm to humans or the environment (Mohanraj, 2014). As a result, RF treatment may be advised as a disinfestation strategy for controlling eggs and young larvae in copra at harvest time, preventing N. rufipes establishment and debris. RF energy could be a feasible alternative to fumigation and hot air because of the treatment's efficacy and the ultimate quality of the treated copra (Dubey et al., 2020).

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ASSOCIATION OF GENETIC MARKERS WITH PHENOTYPIC TRAITS BY USING SSR MARKERS IN FINGER MILLET [*Eleusine coracana* (L.) Gaertn]

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Abstract

Finger millet (*Eleusine coracana* (L.) Gaertn] is an important crop used for food, fodder and industrial purposes. With the objective of increasing the utilization of finger millet germplasm in crop improvement, a composite collection consisting of 1000 accessions was developed, evaluated in three environments for 15 agronomic traits and profiled using 20 SSR markers. In this study has reported the marker-trait associations by using Simple Sequence Repeats markers. Allelic data on 959 accessions and 20 markers based on quality index was used for further statistical analysis. A total of 231 (121 common and 110 rare) alleles were detected in the composite collection. Significant variation of all the agronomic traits was observed. Marker UGEP8 in LG3 and UGEP56 in LG9 showed strong association with days to 50 % flowering in composite collection in over all the tree environments. Several other markers were associated with the traits but were not consistent across environments.

Key words: Finger millet, SSR markers, phenotypic traits, germplasm

Introduction

Finger millet (*Eleusine coracana L. Gaertn*) is an important crop in several countries of Asia and Africa used for food, fodder, and industrial purposes. Finger millet is a highly self-fertilized allotetraploid (2n = 4x = 36) derived from the wild tetraploid progenitor *E. coracana* subsp. *africana*. The A genome donor is believed to be *E. indica*. Both *E. floccifolia* or E. *tristachya* have been considered as potential B genome donors to *E. corancan* based on rDNA restriction pattern (Hilu et al., 1992) and genomic *in situ* hybridization (Bisht and Mukai, 2001).

In finger millet the diversity has been studied using morphological characters like growth habit, leaf architecture or floral morphology (Rachie and Peter., 1997). At molecular level, DNA markers such as RFLP (Muza *et al*., 1997), RAPD (Das *et al.,* 2007), SSRs (Dida *et al*.2007) have been used to determine genetic diversity. Comparative analysis of finger millet genetic map with rice genetic map was a novel attempt that reported high level of conserved co-linearity between the two genomes (Srinivasachary *et al.,* 2007). Low molecular variation was reported in the cultivated finger millet in the past as the results were based on limited number of germplasm and markers. With the discovery of large numbers of genomic SSR markers (Dida *et al*., 2007), it is now possible to conduct extensive molecular diversity and QTL analysis in finger millet. Population structure using 79 finger millet accessions and 45 SSR markers have been reported (Dida *et al.,* 2008). The present study aimed to assess the genotypic diversity, to dissect the population structure of global composite collectionand to find marker- trait associations in global finger millet composite collection.

Materials and Methods

All the 1000 accessions of the finger millet composite collection including four internal checks (VR708, VL149, PR202 and RAU8) were grown in the field. The DNA was extracted from single seedling of each accession by high throughput 96- well plate mini preparation method. From the preliminary screening of 31 SSR markers (Dida *et al.,* 2007) on an eight diverse finger millet genotypes (IE4709, IE6082, IE2921, IE5177, IE4057, IE4443, IE2564 and IE3025), 20 polymorphic SSR markers were selected to genotype the composite collection. Of these, 19 SSRs belong to dinucleotide repeats and one to trinucleotide repeats. The 20 SSR markers used for genotyping were mapped on nine chromosomes.

Polymerase chain reaction (PCR)

The PCR reactions were conducted in 96-well and 384-well micro-titer plates in a GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA, USA) Thermal Cycler. The PCR reactions were performed in 5 µl volume in 384-well PCR plates. The reaction mixtures contained 10 pmol of primer, 25 mM $MgCl_2$, 2mM dNTP, 0.3 unit of *Taq* polymerase and 1x PCR buffer (Applied Biosystems, Foster City, CA, USA). The touch down PCR protocol was used for the following reaction of following: three-minute denaturation cycle, followed by first five cycles of 94^{o}C for 20 seconds, 60 ^{o}C for 20 seconds and 72 ^{o}C for 30 seconds, then by 30 cycles of 94 ^{o}C for 20 seconds. After completion of 30 cycles, a final extension of 20 min at 72 ^{o}C to ensure amplification to equal lengths of both DNA strands. The amplified PCR products were tested on 1.2 per cent agarose gel to check for the amplification of the products.

Genotyping

The PCR products were size-separated by capillary electrophoresis using an ABI 3700 DNA analyzer (Applied Biosystems, Foster City, CA, USA). The PCR products of 4 primer pairs labeled with different dyes (FAM,

VIC, NED and PET) could be pooled (post-PCR), because of the different signal spectra of the fluorophores used. The products of the same fluorophore-labeled primers were also pooled, when they had non-overlapping amplicons in terms of size. The pooled PCR products were mixed with 0.25 µl of the GeneScan 500™ LIZ® internal size standard and 7 µl of Hi-Di™ Formamide (Applied Biosystems, Foster City, CA, USA). The final volume was made up to 12 µl with sterile double-distilled water. This mixture was denatured for 5 minutes at 95°C and cooled immediately on ice.

Fragment size fractionation

After denaturation, the plate with samples was placed into the sequencer machine (ABI Prism 3700 DNA analyzer). The capillary run was performed using the "GeneScan2_POP6 Default" run module and "G5" filter-set. The analysis module used was "GS500 analysis". The fragments were separated in a 50 cm capillary array using POP6 (Performance Optimized Polymer) as the separation matrix.

Data processing

After the capillary runs were over, the raw data were processed with Genescan 3.1 software (Applied Biosystems) to size the peak patterns in relation to the internal size standard GeneScan 500™ LIZ®. The principle behind this is that standards are run in the same lane or capillary injection as the samples, which contain fragments of unknown sizes labeled with different fluorophores. Genescan® analysis software automatically calculates the size of the unknown DNA sample fragments by generating a calibration sizing curve based upon the migration times of the known fragments in the standard. The unknown fragments are mapped onto the curve and the sample data is converted from migration times to fragment size. Genotyper 3.7 (Applied Biosystems) was used for allele calling. The peaks were displayed with base pair values and height (amplitude) in a chromatogram and the allelic data were exported in to Excel spread sheet for further analysis.

Association mapping

Phenotyping

Phenotyping of composite collection along with four check cultivars (VR708, VL149, PR 202 and RAU 8) was carried out in three environments, *viz.,* 2005-'06 post rainy at Tamil Nadu Agricultural University, Coimbatore (E1), 2006 rainy (E2) and 2007 rainy (E3) at ICRISAT, Patancheru. This experiment was conducted in augmented design with one of the four control cultivars repeated after every nine entries in all the environments. Data on 15 quantitative traits [days to 50% flowering (DF), plant height (PH), number of basal tillers (BTN), culm branching (CB), flag leaf blade length (FLBL) and width (FLBW), flag leaf sheath length (FLSL), peduncle length (PL), panicle exertion (PE), ear head length (EHL) and width (EHW), length and width of longest finger (LLF and WLF), number of fingers per ear head (NF) and plot yield (PY)] were recorded following finger millet descriptors. Mean, range and broad sense heritability were calculated for all traits to study the variability present in the germplasm material.

Association of markers with traits

All association tests were run with the mixed linear model (MLM) method in TASSEL 1.9.4 (http://www.maizegenetics.net/), a recently developed unified mixed-model method simultaneously taking into account multiple levels of both gross level population structure (Q) and finer scale relative kinship (K). The population structure matrix (Q) was identified by running STRUCTURE at K = 4. Only markers with an allele frequency of 5% or greater were included in the association analysis.

Results and Discussion

Substantial variation was observed for all traits and high heritability showed greater importance of the traits in revealing marker trait associations. The marker trait association of composite collection data was validated with reference set data. It was observed that the marker-trait association varied with the environments and population used.In the present study, association analysis resulted inconsistent association between the traits and markers for most of the traits mainly due to limited number of random and non trait specific markers. However, in the present study, QTL for days to 50 per cent flowering had consistent association with UGEP8 in LG3 (E2, E3 and pooled for both composite collection and reference set) and UGEP56 in LG9 (E2 and E3 in composite collection and E1 in reference set). It indicated relatively tight linkage between the trait and marker. Also the association varied in different sample size consisting of composite collection with 959 accessions and reference set with 300 accessions. It has been suggested that large numbers of molecular markers are needed to better cover the entire nuclear genome for such association studies (Jensen 1989). However, in our study only 20 markers were used. The marker UGEP3 on LG3 was associated with seven traits (PH, DF, BTN, CB, EHL, FLBL, FLBW) in composite collection and (BTN, FLBL, PL, EHW, PY, FL, PH) in reference set. Also majority of the markers were found to be associated with more than one trait, such an association may arise due to pleotropic effect of the linked QTL on different traits (Culp et al., 1979).

Conclusion

The global finger millet composite collection showed rich allelic diversity (231 alleles, 11.6 alleles per locus, 121 common alleles and 110 rare alleles at 1%). The markers UGEP8 and UGEP56 were consistently associated with days to 50 per cent flowering indicating relative strong association between marker and traits. Extensive study of these markers in mapping population would be helpful for confirmation of QTL.

Table 1.Association of 20 SSR loci with agronomic traits in finger millet composite collection in three environments and pooled.

Traits	Environment	Marker	Linkage Group	Position (cM)	*P*
Days to 50% flowering	E1				
		UGEP11	5A	63.5	0.034
	E2	UGEP56	9A	7.4	0.028
		UGEP8	3B	65.2	0.001
	E3	UGEP56	9A	7.4	0.025
		UGEP8	3B	65.2	0.016
	Pooled	UGEP8	3B	65.2	0.048
Plant height	E1	UGEP3	3A & 3B	75.8 & 64	0.013
		UGEP65	8A	31.6	0.044
	E2	UGEP68	9B	0	0.046
		UGEP104	3B	124.7	0.042
Basal tiller numer	E2	UGEP3	3A & 3B	75.8 & 64	0.039
		UGEP8	3B	65.2	0.003
Clum branching	E2	UGEP8	3B	65.2	0.047
		UGEP26	5B	121.1	0.017
Flag leaf blade length	E2	UGEP90	6B	23.3	0.035
	pooled	UGEP26	5B	121.1	0.038
Flag leaf blade width	E2	UGEP56	9A	7.4	0.019
		UGEP65	8A	31.6	0.001
	pooled	UGEP3	3A & 3B	75.8 & 64	0.028
Flag leaf sheath length	E2	UGEP26	5B	121.1	0.016
		UGEP18	1B	70.3	0.001
Peduncle length	E1	UGEP18	1B	70.3	0.024
	E2	UGEP65	8A	31.6	0.028
	Pooled	UGEP11	5A	63.5	0.02
Ear head length	E1	UGEP56	9A	7.4	0.001
	E2	UGEP8	3B	65.2	0.001
	E3	UGEP107	1A	9.5	0.001
	pooled	UGEP26	5B	121.1	0.032
Length of longest finger	E2	UGEP68	9B	0	0.054
	E3	UGEP3	3A & 3B	75.8 & 64	0.001
Plot yield	E3	UGEP104	3B	124.7	0.03

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CHARACTERIZATION OF PIGEON PEA (CAJANUS CAJAN (L.) MILLSPAUGH) GERMPLASM BASED ON MORPHOLOGICAL CHARACTERS

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Abstract

An experiment was conducted at NPRC,Vamban to evaluate the 169 germplasm for its quantitative character and plant ideotype. Out of 169 germplasm,31 germplasms falls in the category of above 400 cm ,111 germplasm in the category of 300-400 cm plant height whereas 14 germplasms recorded more than 30 no of branches per plant and 120 in the range of 20-30 no of branches and 10 germplasms in the category of 10no of branches.The character namely plant width observed maximum in the category of 40-60 cm. Only five plants recorded 20 cm. Pods per cluster maximum number falls in the category of two pods per cluster. Only 11 plants were recorded only thre4e no of pods per cluster. Number of clusters per plant was recorded in the range of 100 in 74 germplasms and 200 number of clusters in the 54 germplasms. Pods per plant was maximum in the category of 300 in 54 plants. A maximum of 101 germplasms falls in the category of pod length of 5 cm. Seed weight fall in the range between 2 to 4 gm to which maximum of 4 gm observed in 135 germplasm. Plant height was maximum in the germplam viz.,SKNP0926,AL 1770,GC-11-39,and ICP1627-recorded maximum plant height of above 400cm and the height of 100 cm was observed in PT 04-31.Branches per plant was maximum in P14,GRG 822-25,ICP 12942 and SKPN 0926 which is above 29 numbers. The plant width is maximum in ICP-87,ICP 7367,ICP 7898 and BRG 9-1.Pods per cluster was maximum in ICP 6997,P-72,ASG 133 and RPS 2007-109.VBN-2 ,ICP 12942,ICP 6997 and PPE-452.Pods per plant recorded maximum in VBN2, ICP12942,CRG 07-10,and ICP-382.The germplasm which clearly shows the maximum diversity in all the characters which forms the source for exploitation of desirable characters y utilizing it as parents in the hybridization programme.

Key words:Pigeon Pea -germplasm –Descriptors-Morphological Characters

Introduction

Pigeon pea (Cajanus cajan (L.) Millspaugh) is an important grain legume crop of the semi-arid regions and is also recognized as the second most important pulse crop in India. Realizing the significance of genetic resources, large number of germplasm lines have been collected, conserved, characterized and evaluated for various morpho-agronomical traits using the minimal descriptors by the National Bureau of Plant Genetic Resources New Delhi, India.(Mohar Singh et.al.2014) Genetic resources of Pigeon pea have also been screened for resistance to several biotic stresses like Fusarium wilt, Phytophthora blight and sterility mosaic disease and resistant lines have been identified.Crop improvment is based on genetic variation, in the frist place variation present in nature; both cultivated and wild and to a limited extent on variation created by induced mutation. The word germplasm covers both the collective genetic resources of a crop (or animal), as well as the genetic material in the cells. Plant breeders have always relied on germplasm collections, and often their work was most successful when the collection at their disposal included many diverse samples from a wide range of origins. Crop germplasm evolved through recombination and natural mutation, and has been subjected to selection pressures under domestication. (Van der Maesen ,1986)

The purpose of this work was to characterize morphologically seventeen previously selected pigeon-pea lines that went, after selection, through processes of self pollination, selection and multiplication, to obtain pure lines. It also had the objectives of checking the efficiency of the descriptors and describing its vegetative cycle. It was possible to perform the description and to conclude that the seventeen lines have distinct characteristics and can easily be identified by these traits. The used descriptors could properly perform this task and it was concluded that they can be simplified. The seeds of the commercial lines used in this work had mechanical mixture.

Materials and methods

One hundred and sixty nine germplasm accessions were raised in non replicated augmented design during kharif 2014 season and the details are furnished belowOne hundred and sixty nine germplasm were documented according to USDA/ARS with bioversityinternational by using the descriptors viz., growth habit, plant habit ,plant height, plant type,plant width, flower color ,flowering ,pod length, seed per podand stem color

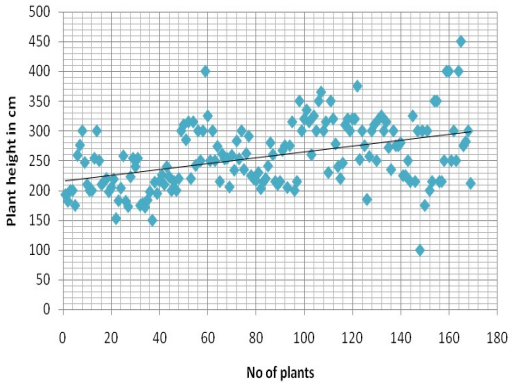
Results and discussions

Out of 169 germplasm,31 germplasms in the category of above 400 cm ,111 germplasm falls in the category of 300-400 cm plant height whereas 14 germplasms recorded more than 30 no/branches and 120 in the range of 20-30 and 10 in the category of 10.The character plant width observed maximum in the category of 40-60 cm. Only five plants recorded 20 cm. Pods per cluster maximum number falls in the category of two pods per cluster. Only 11 plants were recorded only thre4e no of pods per cluster. Number of clusters per plant was recorded in the

range of 100 in 74 germplasms and 200 number of clusters in the 54 germplasms.Pods per plant was maximum in the category of 300 in 54 plants. A maximum of 101 germplasms falls in the category of pod length of 5 cm. Seed weight fall in the range between 2 to 4 gm to which maximum of 4 gm observed in 135 germplasm.

Descriptor details	Redgram germplasm(n-169)
Plant Height(cm)	257.58(100-450)
Branches Per plant	14.67(4-29)
Plant width	39.00(10-72)
Pods/ cluster	2.04(1-3)
No of clusters	143.80(25-400)
Pod number	224.05(42-694)
Pod length(cm)	4.30(3-6.3)
Seeds wt in gm	3.89(3-5)
Flower colour	Red-3%
	Yellow -97%
Stem colour	Reddish green-0.60%
Plant Habit	Semi spreading-39.64%
	Spreading -51.48%
	Erect-8.88%
Growth Habit	Indeterminate-100%

Plant height was maximum in the germplam viz.,SKNP0926,AL 1770,GC-11-39,AND ICP1627-recorded maximum plant height of above 400cm and the height of 100 cm was observed in PT 04-31.Branches per plant was maximum in P14,GRG 822-25,ICP 12942 and SKPN 0926 which is above 29 numbers. The plant width is maximum in ICP-87,ICP 7367,ICP 7898 and BRG 9-1.Pods per cluster was maximum in ICP 6997,P-72,ASG 133 and RPS 2007-109.VBN-2 ,ICP 12942,ICP 6997 and PPE-452.Pods per plant recorded maximum in VBN2, ICP12942,CRG 07-10,and ICP-382.The germplasm which clearly shows the maximum diversity in all the characters as indicated the earlier study of Chang et.al.(2017) that food legumes have positive effects ondiversification of diets and agro-ecosystems. Phenotypic dataare highly valuable to facilitate utilization of gene banks.Large germplasm collections may be used in detectingpatterns and general trait relationships. The Exploration and Germplasm Collection Database revealed that collected germplasm possesses variation with respect to crop duration (annual/ semi-perennial/ perennial), maturity behaviour (synchronous/ asynchronous), growth habit (erect and compact/ semi-spreading/ spreading or plant type), pod and seed size, and seed coat colour (white/ cream/ orange/ light brown/ grey or dark grey (Majumdar and Singh, 2005; Remanandan, 1990). Variability in these and other parameters of the pigeonpea collections from India has been reported by others (Singh *et al*. 2014). Perennial forms are collected from hilly tracts of peninsular and north eastern India, while vegetable types (use of immature pod) from north-eastern states of Nagaland, Manipur and Tripura. Mehra and Arora (1982) also reported vegetable types from tribal areas of Karnataka (Bellary, Mysuru and Raichur) and Maharashtra, and have noticed very long pods, with 7-8 seeds per pod.
GIS mapping of 107 selected trait-specific germplasm (with regard to eight important morpho-agronomic traits) identified few areas – Akola (Maharashtra) for pod bearing length; Srikakulam (Andhra Pradesh) for bold seed; and Banaskantha (Gujarat) for high pod number. Unexplored and underexplored areas as well as crop wild relatives belonging to genepool one and two are identified for future collection(Semaval et.al.,2017)
Scatter plots for plant height.



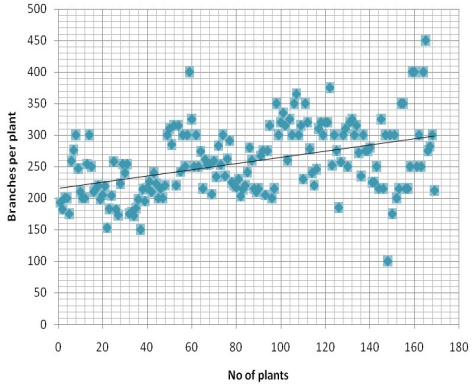


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Table 1. Descriptors for pigeon pea germplasm

		Quantitative Descriptors								Qualitative Descriptors			
S.No	Germplasm	Plant Height (cm)	Branches Per plant	Plant width	pods/ cluster	No of clusters	Pod number	Pod length	Seeds wt in gm	Flower colour	Stem colour	Plant Habit	Growth Habit
1	ICP7898	193	14	67	2	110	275	4.4	4	yellow	green	semi spreading	indeterminate
2	ICP12387	182	16	15	2	83	210	4	4	yellow	green	semi spreading	indeterminate
3	ICP7367	200	14	71	1	210	275	4.3	4	yellow	green	spreading	indeterminate
4	ICP10993	200	16	45	1	81	134	5	4	yellow	green	semi spreading	indeterminate
5	ICP7906	175	11	49	1	31	57	3.4	3	yellow	green	semi spreading	indeterminate
6	ICP10932	259	17	44	2	95	169	4.3	4	yellow	green	semi spreading	indeterminate
7	ICP11125	276	10	39	3	147	224	4.3	4	yellow	green	semi spreading	indeterminate
8	VBN 2	300	27	44	3	400	694	3.5	3	yellow	green	spreading	indeterminate
9	ICP3689	247	11	33	2	50	86	3.7	4	yellow	green	semi spreading	indeterminate
10	ICP7984	210	8	43	2	93	167	4.5	4	yellow	green	semi spreading	indeterminate
11	ICP12942	200	26	51	2	395	587	4.5	4	yellow	green	spreading	indeterminate
12	ICP12471	200	13	36	2	100	133	4.3	4	yellow	green	spreading	indeterminate
13	ICP1126	254	6	43	2	130	210	4	4	yellow	green	spreading	indeterminate
14	ICP12569	300	16	43	2	85	105	4.5	3	yellow	green	spreading	indeterminate
15	ICP11174	250	15	28	2	70	100	4.3	4	yellow	green	erect	indeterminate
16	ICP9274	210	9	37	2	88	132	5.2	4	yellow	green	spreading	indeterminate
17	ICP6698	215	11	28	2	115	200	4.3	3	yellow	green	spreading	indeterminate
18	ICP13575	220	7	26	2	73	112	3.2	4	yellow	green	erect	indeterminate
19	ICP491114	198	18	42	2	90	135	4.3	4	yellow	green	spreading	indeterminate
20	ICP11007	205	19	47	2	119	181	3.7	4	yellow	green	semi spreading	indeterminate
21	ICP11957	219	13	49	2	76	105	3.4	4	yellow	green	spreading	indeterminate
22	ICP13208	153	16	49	2	144	278	4.3	4	yellow	green	spreading	indeterminate

23	ICP11206	183	12	44	2	35	232	4.2	4	yellow	green	spreading	indeterminate
24	BAHUR	204	13	41	2	90	195	4.3	4	yellow	green	spreading	indeterminate
25	ICP7085	258	10	33	2	110	178	4.2	4	yellow	green	erect	indeterminate
26	P3474	182	16	53	2	126	171	4.2	4	yellow	green	spreading semi	indeterminate
27	P1174	173	23	41	2	195	240	4.3	4	yellow	green	spreading semi	indeterminate
28	VRG17	223	18	39	2	84	260	4.2	4	yellow	green	spreading	indeterminate
29	SMR-1693	254	14	34	2	165	205	4.2	4	yellow	green	spreading	indeterminate
30	ICP13938	240	13	45	2	110	294	4.3	4	yellow	green	spreading semi	indeterminate
31	L.NO.78049	254	10	25	2	105	252	3.2	4	yellow	green	spreading	indeterminate
32	ICP14505	175	17	33	2	159	325	4.2	3	yellow	green	spreading	indeterminate
33	ICP8864	178	9	20	2	56	110	4.3	3	yellow	green	spreading	indeterminate
34	P-11227	172	15	44	2	154	212	3.5	4	yellow	green	spreading semi	indeterminate
35	ICP1106	184	9	38	2	121	158	4.3	4	yellow	green	spreading semi	indeterminate
36	RG50	198	13	35	2	110	210	3.5	4	yellow	green	spreading	indeterminate
37	ICP1617	150	14	26	2	99	235	4.3	3	yellow	green	spreading semi	indeterminate
38	PLS476-A	214	9	31	2	53	85	4.2	4	yellow	red	spreading	indeterminate
39	ICP13918-A	195	12	43	2	100	194	5	4	yellow	green	spreading semi	indeterminate
40	RC83	216	8	32	2	46	105	3.8	4	yellow	green	spreading	indeterminate
41	RC129	226	8	44	2	91	175	4.4	4	yellow	green	spreading semi	indeterminate
42	ICP11119	210	17	42	2	155	250	3.5	4	yellow	green	spreading semi	indeterminate
43	P557	240	11	44	2	185	260	4.5	4	yellow	green	spreading semi	indeterminate
44	ICP1129-13	223	15	32	2	289	543	4.3	4	yellow	green	spreading	indeterminate
45	ICP10175	200	12	35	2	115	210	4	4	yellow	green	spreading	indeterminate
46	ICP-763-C	215	18	30	2	311	420	4.3	4	yellow	green	spreading semi	indeterminate
47	ICP12116	200	16	51	2	225	304	4	4	yellow	green	spreading	indeterminate

48	ICP806-1	220	14	42	2	86	105	4.2	4	yellow	green	semi spreading	indeterminate
49	D7-10	300	13	39	2	115	260	4.3	4	yellow	green	spreading	indeterminate
50	RM-18	310	23	42	2	193	272	3	4	yellow	green	semi spreading	indeterminate
51	ICP763-A	285	18	52	2	244	335	5	3	yellow	green	semi spreading	indeterminate
52	ICP763-B	315	16	53	2	80	175	4.3	4	yellow	green	spreading	indeterminate
53	ICP8501-1	220	24	41	2	254	385	5.2	4	yellow	green	spreading	indeterminate
54	BAHUR	315	16	34	2	137	205	4.3	4	yellow	green	semi spreading	indeterminate
55	IIRG-101	242	24	50	2	320	555	5	4	yellow	green	semi spreading	indeterminate
56	PLS-9170	300	12	41	2	95	120	4.3	4	yellow	green	semi spreading	indeterminate
57	PLS9176	250	17	42	2	159	215	3.2	4	yellow	green	spreading	indeterminate
58	ICP12727	300	19	37	2	215	320	4.3	4	yellow	green	semi spreading	indeterminate
59	ICP1627-A	400	14	45	2	250	380	4.2	4	yellow	green	semi spreading	indeterminate
60	P14-14	325	29	55	2	320	425	5.2	4	yellow	green	semi spreading	indeterminate
61	Phule T-25-5-26	250	20	53	2	275	321	4.3	4	yellow	green	erect	indeterminate
62	VRG-61	300	16	41	2	95	125	4.2	4	yellow	green	spreading	indeterminate
63	BSMR 736	250	16	38	2	70	110	4	4	yellow	green	semi spreading	indeterminate
64	MAL 19	274	14	52	2	125	248	4.3	4	yellow	green	spreading	indeterminate
65	WRG 47	215	19	37	2	215	285	4.5	4	yellow	green	semi spreading	indeterminate
66	S-1	261	13	27	2	75	105	5.3	4	yellow	green	spreading	indeterminate
67	WRG 55	253	11	21	2	45	68	4.3	4	red	green	semi spreading	indeterminate
68	GAUT 9802	254	17	34	2	56	78	5.2	4	red	reddish green	spreading	indeterminate
69	VRG 17	206	13	12	2	150	230	4.3	4	yellow	green	spreading	indeterminate
70	RA 6	259	14	11	2	175	215	4.5	4	yellow	green	spreading	indeterminate

71	JSP 98-5	234	18	13	1	252	320	5.3	4	yellow	green	spreading	indeterminate
72	BSMR 736	283	18	32	2	320	475	4.3	4	red	green	spreading	indeterminate
73	MAL 18	253	13	30	2	315	420	5.2	4	yellow	green	spreading	indeterminate
74	ICP6997	300	18	55	3	400	620	4.3	4	yellow	green	spreading	indeterminate
75	ICP7624	235	15	34	2	215	320	5.2	4	yellow	green	semi spreading	indeterminate
76	ICP8863	262	18	32	2	75	86	4.3	4	yellow	green	spreading	indeterminate
77	DA322	291	11	35	2	152	188	5.3	4	yellow	green	spreading	indeterminate
78	VRG959-1	225	14	15	2	242	375	3.2	4	yellow	green	spreading	indeterminate
79	CORG99013-7	220	12	13	2	112	220	4.3	4	yellow	green	semi spreading	indeterminate
80	VRG-59-1	217	17	23	2	104	275	4	4	red	green	erect	indeterminate
81	CORG-990013-4	230	13	28	3	115	282	5.2	4	yellow	green	semi spreading	indeterminate
82	SKPIP-206	203	20	55	2	83	197	4.2	3	yellow	green	spreading	indeterminate
83	VRG-05-011	214	11	26	2	84	105	4.2	4	yellow	green	spreading	indeterminate
84	CORG-990014	220	7	10	2	65	102	3.2	3	yellow	green	erect	indeterminate
85	JKM209	241	10	24	2	102	215	4.3	4	yellow	green	spreading	indeterminate
86	MDA05-1	280	8	21	2	82	105	6.3	4	yellow	green	spreading	indeterminate
87	WRGE-39	260	16	15	2	117	204	5.3	4	yellow	green	erect	indeterminate
88	ICP8863	215	19	32	2	124	240	4.2	4	yellow	green	spreading	indeterminate
89	RG12	210	8	30	2	98	124	5.3	3	yellow	green	spreading	indeterminate
90	JKE-110	215	14	34	2	37	55	4.3	3	yellow	green	erect	indeterminate
91	PT-05	267	10	42	2	54	88	4.2	3	yellow	green	erect	indeterminate
92	PTN-2001-4	275	13	35	2	95	187	4.3	5	yellow	green	spreading	indeterminate
93	WRGE-123	205	16	20	2	80	195	5.2	4	yellow	green	semi spreading	indeterminate
94	AL-15711	275	17	33	2	300	525	4.3	3	yellow	green	spreading	indeterminate
95	ICP-11124	315	20	30	2	305	530	4.2	4	yellow	green	spreading	indeterminate
96	SKNP0205	200	16	43	2	250	353	4	3	yellow	green	semi spreading	indeterminate
97	HDM-O4-1	215	11	27	2	95	115	3.5	4	yellow	green	spreading	indeterminate
98	PA-325	350	13	35	2	115	205	3.5	4	yellow	green	spreading	indeterminate

99	ICP-87	300	18	72	2	175	207	4.3	4	yellow	green	spreading semi	indeterminate
100	BRGE-3	320	16	36	2	75	95	4.2	5	yellow	green	spreading	indeterminate
101	BRG27	335	15	30	2	210	350	3.4	4	yellow	green	spreading semi	indeterminate
102	VRG-05-010	315	13	37	2	300	475	4.2	4	yellow	green	spreading semi	indeterminate
103	VRG-05-011	260	14	29	2	185	215	4.3	3	yellow	green	spreading semi	indeterminate
104	VRG-05-012	325	12	26	2	77	105	3.5	4	yellow	green	spreading semi	indeterminate
105	VRG-05-013	300	20	41	2	176	205	3.5	3	yellow	green	spreading semi	indeterminate
106	VRG-05-014	350	13	37	2	54	75	5.3	3	yellow	green	spreading semi	indeterminate
107	VRG-05-015	365	15	53	2	200	290	4.2	4	yellow	green	spreading	indeterminate
108	VRG-05-016	300	18	31	2	310	420	3.4	4	yellow	green	spreading semi	indeterminate
109	VRG-05-019	315	21	37	2	195	248	4.2	5	yellow	green	spreading	indeterminate
110	GRF 95	230	13	47	2	65	97	4.2	4	yellow	green	spreading	indeterminate
111	JKT 240	350	21	42	2	90	210	5.2	4	yellow	green	spreading semi	indeterminate
112	WRP 1	320	14	47	2	150	285	4.3	4	yellow	green	spreading	indeterminate
113	SKNP 0226	278	16	37	2	75	165	5.2	4	yellow	green	spreading	indeterminate
114	SKNP 505	240	11	50	2	110	178	5.3	4	yellow	green	spreading	indeterminate
115	P-72	220	19	57	3	45	92	5.2	4	yellow	green	spreading	indeterminate
116	YOG-08	246	11	54	2	39	68	4.5	4	yellow	green	spreading semi	indeterminate
117	ICPHRR4985 -10	310	13	38	2	55	98	4.2	3	yellow	green	spreading	indeterminate
118	ICPHRR4989	320	19	37	2	37	58	4.2	4	yellow	green	spreading semi	indeterminate
119	ICPL-20036	300	13	53	2	145	220	4.5	4	yellow	green	spreading semi	indeterminate
120	ICPL-84060	320	14	48	2	90	185	5.3	4	yellow	green	spreading semi	indeterminate
121	ICPL-84063	320	13	34	2	210	265	4.3	4	yellow	green	spreading	indeterminate
122	ICPL-97253	375	18	31	2	47	89	4.3	4	yellow	green	semi	indeterminate

												spreading	
123	ICPL-98023	252	12	30	2	52	75	4.5	4	yellow	green	semi spreading	indeterminate
124	ICPL-77303	300	12	35	2	232	315	4.3	3	yellow	green	semi spreading	indeterminate
125	PPE-452	275	19	52	2	351	84	4.2	4	yellow	green	semi spreading	indeterminate
126	ICP-382	185	22	43	2	350	475	5.2	3	yellow	green	semi spreading	indeterminate
127	ICP-8719	257	17	49	2	195	285	4.5	4	yellow	green	spreading	indeterminate
128	ICP 7035	300	15	39	2	45	89	4	4	yellow	green	semi spreading	indeterminate
129	ICP-10531	310	10	41	2	180	282	4.2	4	yellow	green	spreading	indeterminate
130	ICP13198	250	12	49	2	295	325	4.5	4	yellow	green	semi spreading	indeterminate
131	ICP13212	320	15	45	2	275	358	3.5	4	yellow	green	spreading	indeterminate
132	ICPHRL-49785	325	13	43	2	210	275	4.2	3	yellow	green	semi spreading	indeterminate
133	ICPHRL-4985-1	300	10	45	2	270	315	4.5	5	yellow	green	semi spreading	indeterminate
134	ICPHRC-4979-2	315	8	43	2	35	80	4.3	4	yellow	green	spreading	indeterminate
135	BRG 9-1	272	13	62	2	145	215	3.2	4	yellow	green	spreading	indeterminate
136	AL 1780	235	9	45	2	175	280	5.2	4	yellow	green	spreading	indeterminate
137	SKNP 0703	300	20	59	2	270	325	4.3	4	yellow	green	spreading	indeterminate
138	HO4-24	275	21	54	2	45	92	4.3	4	yellow	green	erect	indeterminate
139	HO4-26	275	18	43	2	310	520	3.2	4	yellow	green	spreading	indeterminate
140	CRG 07-10	280	13	40	2	345	580	4.3	3	yellow	green	spreading	indeterminate
141	AL 1688	225	7	57	2	242	380	4.3	5	yellow	green	spreading	indeterminate
142	PUSA 2009-05/4	225	12	48	2	195	270	4.2	5	yellow	green	erect	indeterminate
143	SKNP 0716	250	9	46	3	65	123	4.5	4	yellow	green	spreading	indeterminate
144	SKNP 0703	215	8	18	3	95	140	3.5	4	yellow	green	erect	indeterminate
145	ASG 133	325	13	42	3	235	463	5.2	3	yellow	green	spreading	indeterminate
146	PUSA 2009-05/1	215	16	45	2	220	463	4.2	4	yellow	green	spreading	indeterminate

147	PT 0012	300	16	40	2	45	73	5.2	4	red	green	semi spreading	indeterminate
148	PT 04-31	100	16	55	2	55	68	4.3	4	yellow	green	spreading	indeterminate
149	GC 11-39	300	4	24	2	110	235	4.3	4	yellow	green	spreading	indeterminate
150	RPS 2007-109	175	16	46	3	150	233	5.5	4	yellow	green	semi spreading	indeterminate
151	SKNP 0528	300	10	59	2	145	257	4.2	4	yellow	green	erect	indeterminate
152	ICP 8863	200	13	27	2	95	276	4.2	4	yellow	green	semi spreading	indeterminate
153	WRG 166	215	17	40	2	175	289	4.3	4	yellow	green	erect	indeterminate
154	VRG-09-107	350	7	54	2	69	110	4.3	4	yellow	green	erect	indeterminate
155	AL 1779	350	20	27	2	45	82	4.2	3	yellow	green	semi spreading	indeterminate
156	UPAS 120	215	13	26	1	25	54	4.2	5	yellow	green	spreading	indeterminate
157	PUSA 2011-1	215	13	27	2	170	205	4.5	4	yellow	green	semi spreading	indeterminate
158	SKNP 808	250	9	26	2	125	185	4.3	4	yellow	green	spreading	indeterminate
159	AL 1770	400	11	54	2	95	157	4	4	yellow	green	spreading	indeterminate
160	GC 11-39	400	15	44	2	76	94	4	3	yellow	green	semi spreading	indeterminate
161	GJP 1002	250	20	42	2	55	103	3	4	yellow	green	semi spreading	indeterminate
162	AKT 10-01	300	15	49	2	38	54	4	4	yellow	green	spreading	indeterminate
163	RPS 2007-10	250	12	38	2	27	42	4	4	yellow	green	spreading	indeterminate
164	GRG 818	400	17	28	2	75	107	4	4	yellow	green	spreading	indeterminate
165	SKNP 0926	450	24	39	2	115	184	4	4	yellow	green	semi spreading	indeterminate
166	ASR 001	275	21	36	2	85	106	4	4	yellow	green	semi spreading	indeterminate
167	WRG 171	282	19	32	2	252	310	4	4	yellow	green	spreading	indeterminate
168	GJP 1003	300	14	51	3	54	75	5.3	4	yellow	green	spreading	indeterminate
169	GRG 822	212	25	43	3	78	97	4.3	4	yellow	green	spreading	indeterminate
	Maximum	450	29	72	3	400	694	6.3	5				
	Minimum	100	4	10	1	25	42	3	3				
	SD	56.39	4.36	11.77	0.31	89.49	131.6	0.56	0.47				

GENOME-WIDE ANALYSIS AND CHARACTERIZATION OF SNF1-RELATED PROTEIN KINASES GENE FAMILY IN COWPEA (*Vigna unguiculata* (L) Walp.)

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Abstract

Plants owing to their sessile nature have evolved mechanisms to adapt and overcome various abiotic stresses by activating different signaling pathways triggering accumulation of stress associate proteins. A key regulator in ABA signaling pathway, sucrose non-fermenting-1-related protein kinase 2 (SnRK2) is a plant-specific serine/threonine kinase family involved in plants' response to osmotic stress. Analysis of the members of this protein family has been reported in some plant species. Cowpea (*Vigna unguiculata*), a tropical food grain legume of Africas and Southeast Asia has been subjected to hampering of growth and productivity due to drought stress. The genes encoding SnRK2s in cowpea and their detailed characterization remain unexplored. The present study attempts to identify and characterize *SnRK2* gene families in cowpea using bioinformatics tools. Analysis of the draft genome of *Vigna unguiculata* in NCBI and Phytozome databases revealed sixteen *SnRK2* genes. *In silico* analysis was done to determine gene structure, transcript length, chromosomal mapping of the genes to *Vigna unguiculata* genome. Domain architectures of the SnRK2 proteins were predicted. Physico-chemical characterization revealed these proteins in sizes ranging 53 to 112 kDa with pI values of 4.99 to 9.59. All identified cowpea SnRK2 proteins are hydrophilic in nature. Analysis of the evolutionary relationship of *SnRK2* with other related families showed three clusters based on the relatedness to *Arabidopsis thaliana* and thirteen other crops. Findings of this study provide insights into cowpea *SnRK2* gene family and its possible implications in plant stress tolerance.

Keywords: Cowpea, *Vigna unguiculata*, SnRK2, Genome wide, abiotic stresses

Introduction

Cowpea (*Vigna unguiculata*), native from Africa, has been widely cultivated in tropical and subtropical regions (Carvalho *et al*., 2019) and is a diploid member of the Fabaceae family with a chromosome number 2n = 22 and estimated genome size of 613 Mb (Lonardi *et al*., 2019). Cowpea has gained more attention recently from consumers and researchers worldwide due to its exerted health beneficial properties including anti-diabetic, anti-cancer, anti-hyperlipidemic, anti-inflammatory and antihypertensive properties (Jayathilake *et al*.,2018). Wide adaptability of Cowpea to low fertile soils, pH, high temperatures and drought makes this crop choicest for facing the changed climate scenario. Plants responses to drought are complex and envisaged with different mechanisms to adapt and survive under drought stresses (Carvalho *et al*., 2017). Drought stressed plants reveal several morphological, physiological, biochemical, and molecular changes that adversely affect their development, growth and productivity (Carvalho *et al*., 2019).

Among these tolerance mechanisms, phosphorylation and dephosphorylation are key events modulated by protein families. In plants, protein kinases include calcium-dependent protein kinases, mitogen-activated protein kinases, and sucrose non-fermenting-related protein kinases (Fatima *et al*., 2020). Abiotic stresses trigger a complex osmotic-stress and abscisic acid (ABA) signal transduction network. SNF1-related protein kinase2s (SnRK2s) are core ABA signalling components that are activated by ABA-triggered inhibition of type- 2C protein-phosphatases (Yohei Takahashi *et al*., 2020). Plant-specific *SnRK2* genes play crucial roles in the coordination of plant growth and development and responses to stress and are dubbed as equivalent of mammalian AMP-activated protein kinases and SNF-1 proteins 1 from yeast. SnRK (or SNF1-related protein kinase) family are specific types of serine/threonine protein kinases that exist widely in plants and function significantly in a host of processes, including growth and development, defense against various stresses, and hormone-mediated signaling (Zhiming Wu *et al*., 2020). In higher plants, the SnRK family consists of three subfamilies *viz*., SnRKl, SnRK2 and SnRK3 based on their sequence similarity and C-terminal domain structure characteristics (Fatima *et al*., 2020). The SnRK2 kinases phosphorylate and thus regulate the activity of downstream components including transcription factors and ion channels (Yohei Takahashi *et al*., 2020). Interaction of SnRK2 and protein phosphatase 2C (PP2C) in ABA-dependent signal transduction causes activation of stress-responsive genes(Fatima *et al*., 2020).

Genetic approaches have been playing a fundamental role in exploring key genes of osmotic stress and ABA signaling pathways. SnRK2 family members have been found in many plant species like Arabidopsis, maize, cotton, rice, wheat, soybean, Rapeseed, crabapple and grapevine (Fatima *et al*., 2020). However, the SnRK2 family remains unexplored in cowpea. The present study was attempted to identify and characterize the SnRK2 gene family in cowpea with the following objectives.

Objectives

1. Genome-wide identification and analysis of SnRK2 protein gene family in *Vigna unguiculata.*
2. *In silico* characterization of SnRK2 protein coding genes using bioinformatics tools.

Materials and Methods

Collection of datasets

The whole proteome of cowpea (*Vigna unguiculata* (assembly ASM411807v1) was downloaded from NCBI (https://www.ncbi.nlm.nih.gov/genome /). HMMER and keyword search in Phytozome V12.1 database (https://phytozome.jgi.doe.gov/pz/portal.html) was performed. For reference search using heterologous sequences, *Arabidopsis thaliana* SnRK2 (AtSnRK2) protein sequences were obtained from The Arabidopsis Information Resource (TAIR) (https://www.arabidopsis.org/index.jsp)

Chromosomal Mapping of SnRK2 genes

Mapgene2chrom, a tool to draw gene physical map based on PERL and SVG languages.(http://mg2c.iask.in/mg2c_v2.0/). The obtained *SnRK2* sequences were mapped on the *Vigna unguiculata* chromosomes using MapGene2Chrom (Jiangtao *et al*., 2015) with chromosome co-ordinates (gene id, gene start position, end position, chromosome id and size of chromosome).

Identification of sequence homologs using Hidden Markov models

The HMMER program version 3.3 (http://hmmer.org/), using hidden Markov models, was run to identify SnRK2 sequences in the cowpea genome by using AtSnRK2 sequences as bait sequence.

Identification of domain architecture in SnRK2 proteins

The domain and domain architecture of AtSnRK2s were obtained by Simple Modular Architecture Research Tool (SMART) (http://smart.embl-heidelberg.de).

Multiple Sequence Alignment

ClustalW like the other Clustal tools is used for aligning multiple nucleotide or protein sequences in an efficient manner. Then multiple sequence alignments of *AtSnRK2* genes were built by Clustal Omega to validate the results. MSAs were built on MEGA X to confirm the presence of conserved SnRK2 domain in identified cowpea SnRK2 proteins

Phylogenetic analysis

The evolutionary relationship of cowpea SnRK2 proteins was predicted by building a phylogenetic tree. Fifteen different crop's SnRK2 protein sequences were aligned by ClustalW and tree was built by Mega X the neighbour joining method with 1000 bootstrap replications. Further analysis of this tree was performed by exporting this tree to MEGAX, and was run by choosing default parameters such as Gap Penalty and Gap Extensions with default values

Physico-Chemical analysis of SnRK2 proteins

The theoretical isoelectric point and molecular weight were determined by ExPASy pI/Mw tool. (https://web.expasy.org/compute_pi/)

Results

Whole genome characters of cowpea in NCBI

The results shows the chromosome number, Reference sequence I'd, Size of genome sequences, GC Content, Number of protein, RNA and gene encoded (Table1). Gene prediction showed 29,773 total loci containing 42,287 protein-coding transcripts and 12,514 total alternatively spliced transcripts in cowpea.

Table 1. Whole Genomic information of Cowpea (*Vigna unguiculata*)

Type	Name	RefSeq	INSDC	Size (Mb)	GC%	Protein	Rrna	tRNA	Other RNA	Gene	Pseudogene
Chr	1	NC_040279.1	CM014066.1	42.13	32.8	3,567	-	79	489	2,849	120
Chr	2	NC_040280.1	CM014067.1	33.91	33.3	2,987	10	73	390	2,299	40
Chr	3	NC_040281.1	CM014068.1	65.29	32.0	6,200	-	146	777	4,804	112
Chr	4	NC_040282.1	CM014069.1	42.73	32.9	2,869	3	77	589	2,424	121
Chr	5	NC_040283.1	CM014070.1	48.75	32.7	4,192	-	78	754	3,285	100
Chr	6	NC_040284.1	CM014071.1	34.46	32.8	3,282	-	81	565	2,694	107
Chr	7	NC_040285.1	CM014072.1	40.88	32.0	4,243	-	105	490	3,182	72
Chr	8	NC_040286.1	CM014073.1	38.36	32.9	3,187	-	79	357	2,563	83
Chr	9	NC_040287.1	CM014074.1	43.93	32.7	4,019	32	84	516	3,072	68
Chr	10	NC_040288.1	CM014075.1	41.33	33.2	2,888	282	92	542	2,696	113
Chr	11	NC_040289.1	CM014076.1	41.68	33.2	3,079	123	109	538	2,694	130
	Pltd	NC_018051.1	-	0.15	35.2	84	8	38	-	131	1
Un	-	.	-	45.46	36.6	576	828	94	236	1,690	147

Characteristics feature of SNF protein in the cowpea genome

The SNF related proteins are characterized and was identical across the Cowpea genome. There are 16 protein identities of SnRK2 observed (Table 2).

Table 2. SNF related protein families in Cowpea

Name	Accession	Start	Stop	Std	Gene ID	Locus	Protein product	Length
VuSnRK1	NC_040279.1	18482681	18484506	+	114192996	LOC114192996	XP_027938484.1	471
VuSnRK2	NC_040279.1	27455241	27459193	-	114181284	LOC114181284	XP_027923498.1	832
VuSnRK3	NC_040289.1	37980286	37985690	+	114168947	LOC114168947	XP_027909735.1	1036
VuSnRK4	NC_040281.1	50643408	50646050	+	114178934	LOC114178934	XP_027920886.1	511
VuSnRK5	NC_040281.1	61287418	61298513	+	114179887	LOC114179887	XP_027922179.1	703
VuSnRK6	NC_040282.1	11541389	11543515	-	114181894	LOC114181894	XP_027924343.1	547
VuSnRK7	NC_040286.1	13343970	13349236	-	114194176	LOC114194176	XP_027940068.1	777
VuSnRK8	NC_040287.1	32398570	32403138	+	114164083	LOC114164083	XP_027904391.1	537
VuSnRK9	NC_040287.1	33057516	33064380	-	114162893	LOC114162893	XP_027902685.1	785
VuSnRK10	NC_040287.1	42775720	42778372	-	114164364	LOC114164364	XP_027904808.1	499
VuSnRK11	NC_040287.1	17565838	17567763	+	114162141	LOC114162141	XP_027901727.1	528
VuSnRK12	NC_040287.1	42901634	42905741	+	114163745	LOC114163745	XP_027903839.1	510

Chromosomal Mapping

The identified SnRK2 genes were mapped on six chromosomes (chromosomes 1,3,4,8,9 and 11). Four genes, namely 114192996 and 114181284 were predicted to be located on chromosome 7, whereas 114178934 and 114179887 had predicted locations on chromosomes 3 and 114181894,114168947 and 114194176 are located chromosome 4, 8 and 11 respectively. 114162141,114162893, 114163745 and 114164364 are located in chromosome 9 (Fig.1).

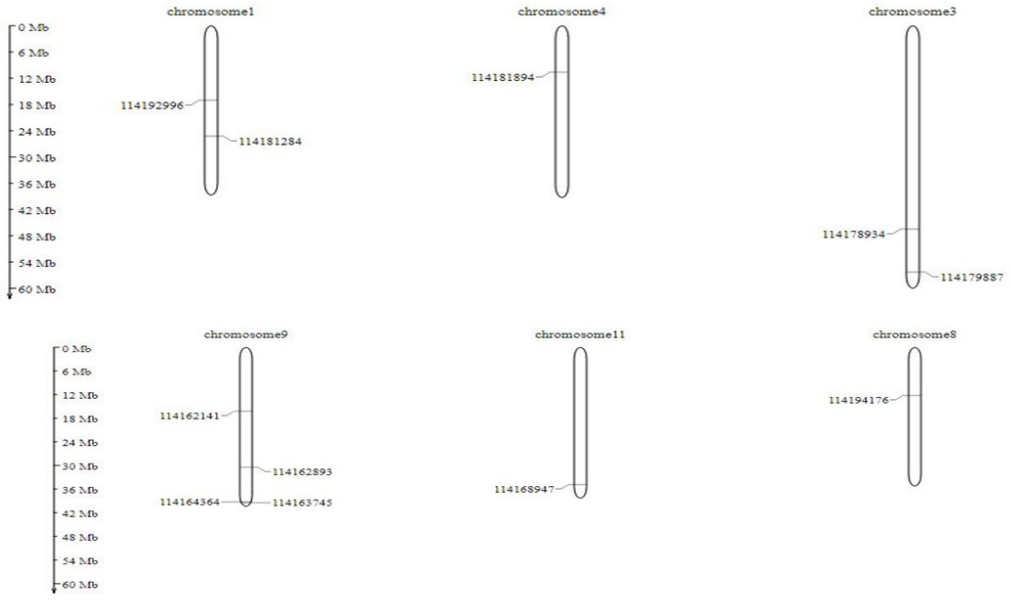


Fig. 1 Chromosomal mapping of Cowpea *SnRK2* genes

Sequence homologs of cowpea SnRK2 genes

By using the HMMER program, the domain architectures of Arabidopsis SnRK2s were studied. Search predicted the protein kinase domain belonging to PF00069 super family.

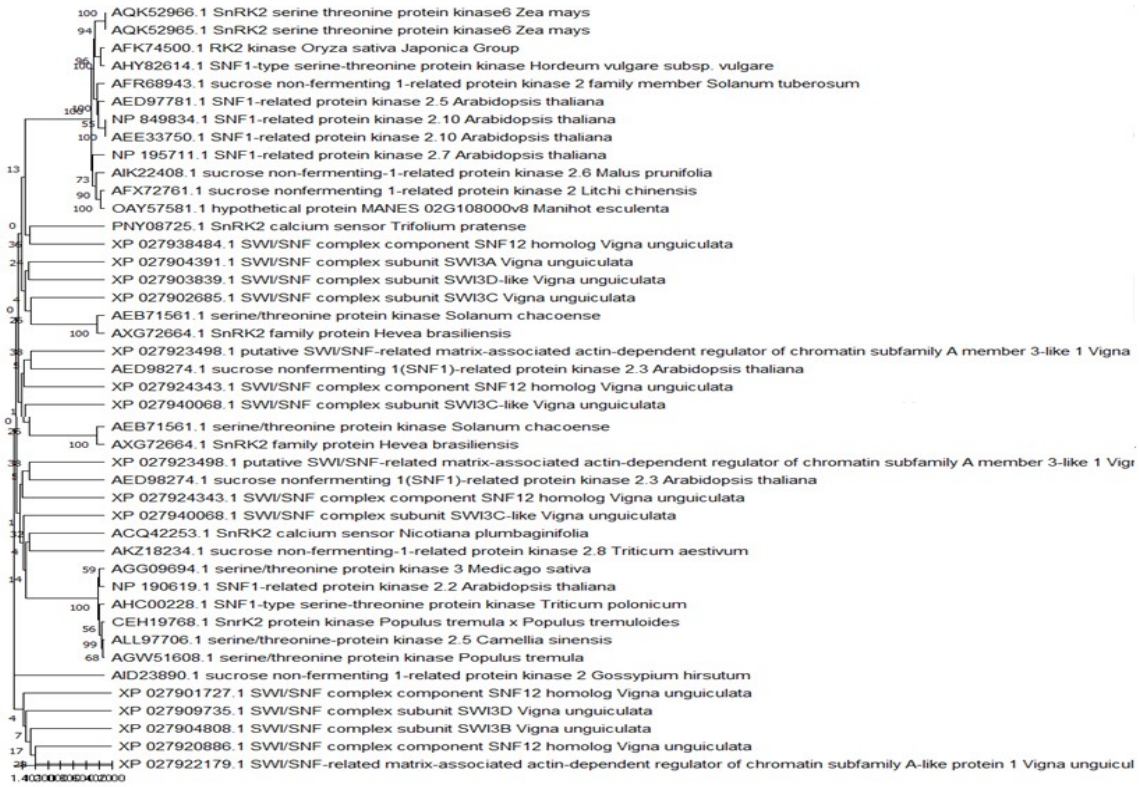
Multiple Sequence Alignments

The multiple sequence alignments (MSAs) of AtSnRK2 genes and Cowpea SnRK2 genes were built by Clustal omega to validate the results. MSAs were built on Clustal omega to confirm the presence of conserved SnRK2 protein sequence in identified cowpea SnRK2 related proteins.

Phylogenetic Analysis:

The evolutionary relationship of Cowpea SnRK2 proteins was predicted by building a phylogenetic tree. Cowpea SnRK2 and AtSnRK2 protein sequences were aligned by Mega X and tree was built by the neighbor joining method with 1000 bootstrap replications. The phylogenetic relationship was done among the 12*VuSnRK2* and 14 different crops SnRK2 protein were determined by constructing a phylogenetic tree by using complete protein sequences of AtSnRK2 and cowpea SnRK2 genes. Based on similarity with AtSnRK2, the identified cowpea SnRK2 were placed into four different groups (I, II, III and IV).

Fig. 2 Evolutionary relationship of cowpea SnRK2 proteins- Phylogenetic tree constructed using MEGA X tool



Physico-Chemical Analysis

The theoretical isoelectric point and molecular weight were determined by ExPASy I/Mw tool. The isoelectric point ranged from 4.99 to 9.59, which was slightly acidic, and the molecular mass ranged from 53259.04 to 112344.7

Conclusion

In the present study, sixteen SnRK2 genes in *Vigna unguiculata* were identified and *in silico* analysis has determined the gene structure, transcript length, chromosomal location and evolutionary relationships of this *SnRK2* gene family in *Vigna unguiculata* with other crop species with similar functions. These findings shed light on the possible role of *SnRK2* genes in *Vigna unguiculata* and lay a strong foundation for further detailed functional characterization of these genes.

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PHYTOCHEMICAL SCREENING OF ULTRASONICALLY TREATED KARUPUKAVUNI RICE (*Oryza sativa L. indica)* by using GC-MS

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Abstract

The phytochemical screening is the important assessment which reveals the bioactive compounds present in the sample, in this study the karupukavuni rice is ultrasonically treated at the temperature of 60ºC, and it examined for the phytochemicals by using the GC-MS more than ten compounds are analyzed after the treatment which are found at the different peak heights. The highest peak height was found at the 18.1, 18.2 and 15.7, which was found as cis-13-Octadecenoic acid, Oleic acid, 9,12-Octadecadienoicacid (Z,Z)-and n-Hexadecanoic acid respectively, in the untreated kavuni rice samples similar types of the compounds was identified, hence this study proves that the ultrasound treatment on the rice, retains the bio active compound than other methods, even when it is treated at the high temperature.

Key words: Karupukavani rice, Ultrasound treatment, GC-MS, Phytochemical screeing

Introduction

The black rice which is called as "KaruppuKavuni" in tamil is a type of rice variety which is naturally fortified with immense proportion of nutrients and antioxidants. In ancient days, it was believed that the regular consumption of black rice will prevent ageing and increase the longevity of the royal society. Due to this belief, the consumption of black rice was restricted only to the royals of ancient kingdoms and was forbidden to common civilians and thus got several names like Forbidden Rice, Imperial Rice, King Rice and Prized Rice. Moreover, due to its rich anthocyanin content on its outer layer of the kernel, it is also called as Black Rice or Purple Rice.(Hu et al., 2018) Several researches in the recent decade have found that the black rice have excellent antioxidants that effectively quenches the reactive oxygen species (ROS) in the body and thus, by acting as functional food, it reduces the incidence of diabetes, obesity, cardiac attack etc., and also by acting as a potential chemo preventive agent, it hinders the cancer growth.(Umamaheswari, 2019) Many studies have linked some of these beneficial health effects with the anthocyanin content of the black rice. Among several anthocyanin pigments, the Cyanidin 3-glucoside (which constitutes 93% of its anthocyanin content) and Peonidin 3-glucoside are found to be predominant pigments identified in black rice (Noorlaila, Nur Suhadah, Noriham, & Nor Hasanah, 2018). It is also observed that one tablespoon of black rice equals or exceeds the amount of anthocyanin present in blueberries. Some established health effects of black rice (KaruppuKavuni) are as follows: Rich in antioxidants particularly anthocyanin, Iron and Protein. Natural gluten free grain enriched with beneficial phytochemicals comparatively more than blueberries Apart from its health benefits, the black rice is adored by the common people for its stunning black coloured outer bran and, the high anthocyanin and fiber content along with a pack of aromatic natural phytochemicals present in the black rice gives as an excellent and appealing visual and textural feel and a specific appetizing aroma after cooking. Though the rice is the predominant staple food in several countries, its potential contribution in the prevention or management of several chronic diseases is not so widely recognized. In contrary, several researches prove that the consumption of fermented traditional pigmented rice varieties helps in bringing immense health benefits and makes the consumers to lead a nutritious life. Fortunately, the research on the effects of black rice consumption has gained popularity in recent decade or so and thus, enabling us with good research data to know its effects on different organs of the body. A huge number of scientific studies have shown that, the black rice powder is one of the nature's most well balanced super food and its abilities are truly remarkable. In some studies, the quality of the black rice can be improved by the appropriate technologies and treatments (Meng et al., 2018). when compared to other methods such as pre-gelatinization, enzymatic treatment, germination treatment, and soaking treatment, ultrasound treatment on rice will reduce the whole process time, enhance cooking and eating properties of white and brown rice varieties (Rice et al., 2015). The main advantage of ultrasound is, it requires only a minimal processing time, inflated productivity with lower energy utilization. So it is highly recommended to several processing methods such as extraction process, emulsification methods, homogenization, crystallization, filtration, separation, viscosity alteration, defoaming, and extrusion. For the effective solubilization, modification, and purification of starch, the ultrasound treatment is highly suitable. In this study the phytochemical screening of the ultrasonically treated kavuni rice was analyzed and compared with untreated rice samples.

Objective

1. Ultrasound treatment of the karupukavuni rice at the temperature of 60ºC
2. Identification of the metabolites present in the treated and untreated kavuni rice by using GC-MS

Materials and methods

Sample collection

The freshly milled Karupukavuni*(Oryza sativa L. indica)* rice is procured from the farmer in Thiruvarur district, Tamilnadu, India. After the procurement, the grains are stored at room temperature sealed in polyethylene bags for future analysis.**Ultrasonic treatment**

Karupukavuni rice of 250g is soaked in 300 ml of water. Then the soaked grains are sealed in the zip lock polyethylene pouches. After packing, the PE pouches are placed inside the ultrasonic equipment (SONICA 45L EP S3 *) treated at the temperature of 60º C, for 30 minsafter the treatment, rice samples are kept at room temperature.

GC-MS analysis

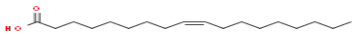
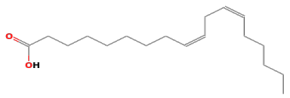
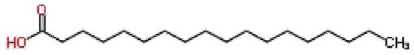
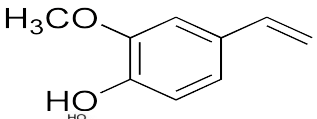
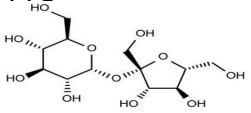
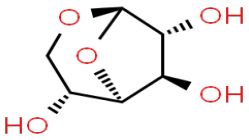
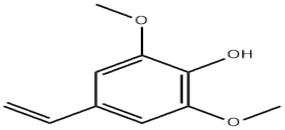
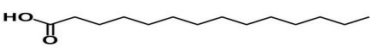
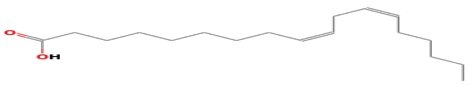
Sample extraction

The ultrasonically treated samples are grinded and it was extracted with methanol, and analyzed through Gas Chromatography – Mass Spectrometry for identification of different compounds.

GC Method

Table 1: Structure of isolated compound present in the untreated kavuni rice

The 8890GC/5977B GC/MSD equipped with Column Rtx-5MS (5% Diphenyl / 95% Dimethyl poly siloxane), 30m x 0.25mm ID x 0.25µm. The carrier gas was flowed at the rate of 1ml per min, with the Split ratio of 10:1. The Single Quadrupole Mass Spectrometer was used as the detector with the sample injection volume of 2µl, the Oven temperature Programmer was set at 110° C hold for 3.50 min Up to 200° C at the rate of 10 ° C/min, No hold Up to 280 ° C at the rate of 5° C / min followed by 12 min hold. For the data analysis OpenLab CDS Software was used. The temperature of the injector is 280° C.

S.no	Name of the compound	structure
1	Oleic Acid	
2	9,12-Octadecadienoic acid (Z,Z)-	
3	n-Hexadecanoic acid	
4	2-Methoxy-4-vinylphenol	
5	Sucrose	
6	β-D-Glucopyranose, 1,6-anhydro	
7	Phenol, 4-ethenyl-2,6-dimethoxy-	
8	Tetradecanoic acid	
9	Pentadecanoic acid, 14-methyl-, methyl ester	
10	Hexadecanoic acid, methyl ester	
11	9,12-Octadecadienoic acid (Z,Z)-	

Method for detection

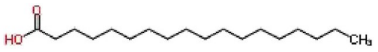
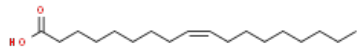
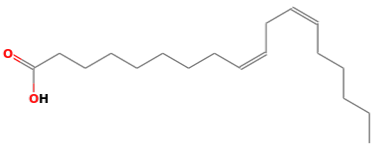
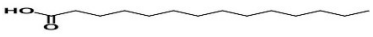
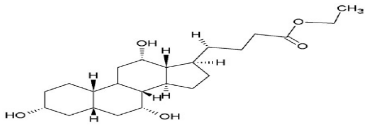
NIST Version-2020 was used as the library. For the detection, the Inlet line temperature is 290° C with source temperature of 250 ° C, Electron energy at 70 eV, the ionization was happens at 50-550 amu Mass scan (m/z) . 0 - 3.5 min is needed for the solvent delay, the Total GC- MS running time is about 40.50 min.

Result and discussion

The signal provided by a compound that elutes from the GC column into the detector is represented by each peak in the chromatogram. The peak size is related to the amount of comparable compounds in the examined samples. The results are tabulated in the below tables, table 1 represent the compounds present in the untreated sample and table 2 represents the compounds present in the ultrasound treated (60º C) kavuni rice. For the untreatedand treated kavuni rice, the highest peak wasat 18.2, 18.1 and 15.7, the identified compound ascis-13-Octadecenoic acid, Oleic acid, 9,12-Octadecadienoicacid (Z, Z)-and n-Hexadecanoic acidrespectively.

The majority of the compounds are similar to the treated and the untreated sample. For both treated and untreated kavuni rice there are totally 20 compounds are found. the molecular formula for the oleic acid is $C_{18}H_{34}O_2$ and it is used for the replace of saturated fats in the diet and also it involves in lowering cholesterol and inflammation.9,12-Octadecadienoicacid (Z,Z) which helps in the production of the mammalian nutrition. (Marhamati, Marhamatizadeh, & Mohebbi, 2021) n-Hexadecanoic acid is called as palmitic acid, which act as the food additive and surfactant. (Krishnaveni, 2015.) Tetradecanoic acid act as the antibacterial and it has the role as human metabolite. Methyl esters are act as the excellent solvent. Ethyl iso-allocholate is act as the potent inhibitor for Dihydropteroate synthase. Sterols are used in the pharmaceutical industry used in the formation of the progesterone and corticoids. γ-Sitosterol helps in the lowering the cholesterol and helps in the heart disease and rheumatoid arthritis. Spirost-8-en-11-one, 3-hydroxy-,(3β,5α,14β,20β,22β,25R)- have anticancer, antiproliferative, and anti-inflammatory properties.(Malathi, Anbarasu, & Ramaiah, 2016) The figure 1&2 represents the obtained chromatogram from GC-MS analysis.

Table 2. Structure of isolated compound present in the ultrasonically treated kavuni rice (*Oryza sativa L. indica)*

S.no	Name of the compound	structure
1	n-Hexadecanoic acid	
2	Oleic Acid	
3	9,12-Octadecadienoic acid (Z,Z)-	
4	Tetradecanoic acid	
5	Hexadecanoic acid, methyl ester	
6	Ethyl iso-allocholate	

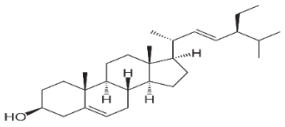
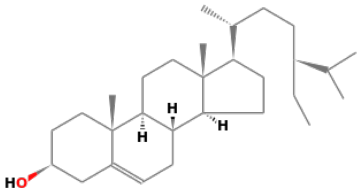
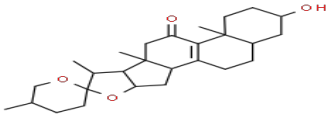
7	Stigmasterol	
8	γ-Sitosterol	
9	Spirost-8-en-11-one, 3-hydroxy-, (3β,5α,14β,20β,22β,25R)-	

Fig. 1. phytochemical screening of untreated sample

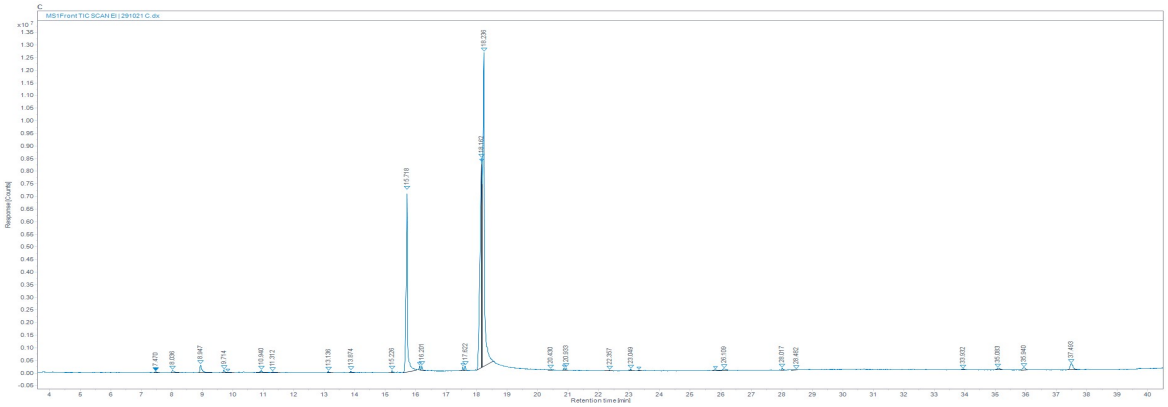
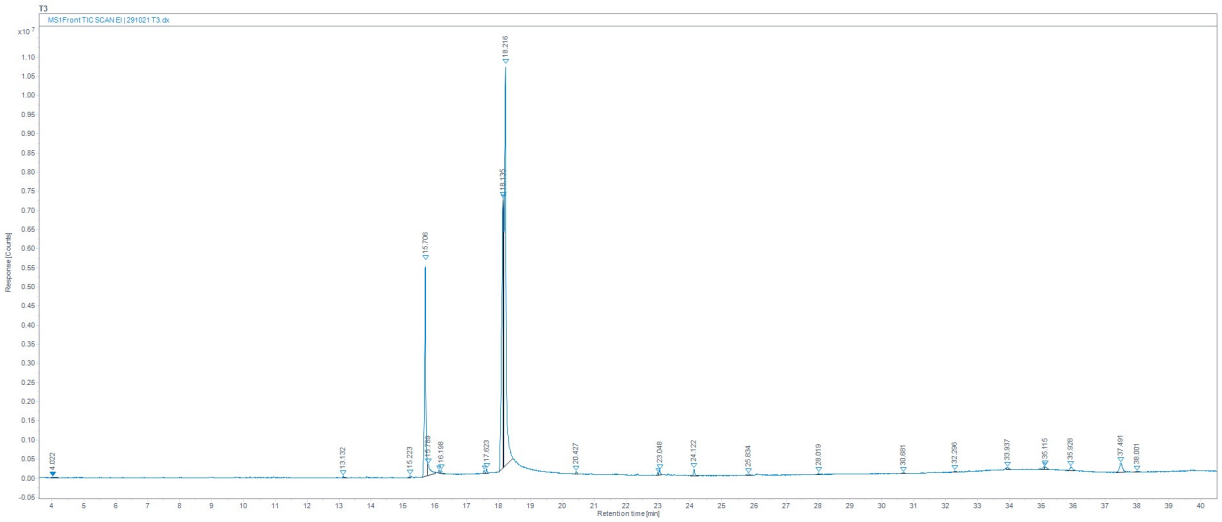


Fig. 2. phytochemical screening of ultrasonically (60^{o}c) treated sample



Conclusion

The black rice is immensely enriched with the lot of bioactive compounds, which act as the therapeutic food for various types of diseases. The ultrasound treatment is the non-thermal green technology which effectively retains the various bioactive compounds compare to other processing methods, hence the GC-MS analysis of the karupukavuni rice of treated and untreated kavuni rice samples, have found similar number of bio active compounds. Hence this method of green technology is highly recommended for the processing of rice varieties and food produce.

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FRAGMENT SHEDDING RESPONSE AND MODELLING TO CONTROL DEFECT ACTION LEVEL OF*TRIBOLIUM CASTANEUM* AND *LASIODERMA SERRICORNE*IN WHEAT FLOUR

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Abstract

Insect fragments are a major concern in flour and flour-based products. Storage pests shed their fragments during metamorphosis, *i.e.,* moulting, and the shedding of fragments varies based on the environmental conditions. The present research intends to determine the fragment shedding of two major storage insects (*Tribolium castaneum* and *Lasioderma serricorne)* in wheat flour at different storage parameters. The present study's three variables are insect population density, storage temperature, and the storage period, which were optimized using Box Behnken design in combination with Response surface methodology. Numerical optimization was performed to identify the optimal storage parameters to keep insect fragment counts below the Defect Action Level (DAL), i.e., 75 fragments/50 g of the flour as per Food and Drug Administration guidelines. The fragment counts were done by using the acid hydrolysis method. Single adult of T. *castaneum* attained DAL in 35 days of storage period compared to *L. serricorne* attained DAL at 23 days at 26 °C. Optimization results indicated that the predicted number of fragments shedded by the *T. castaneum* was 75, and *L. serricorne* was 140 at 26°C and 35 days of post infestation period under similar environmental conditions. Long term storage resulted in increase in fragments of both insects significantly. It was observed that fragment counts positively correlates with insect population density. The present study will help correlate IPD with DAL and determine the threshold level at which the commodities can be stored safely to prevent insect infestation and achieve zero tolerance of insects during storage.

Keywords*: T. castaneum, L. serricorne*, insect fragment counts, defect action level, optimization

Introduction

Stored product insects, most commonly infest food grains and processed foods. Coleopteran and Lepidopteran pests attack during various stages such as pre harvest, processing, transport and storage (Moura, 2017). Insect infestation is an indication of unhygienic and unsanitary practices during the above stages. It results in qualitative and quantitative losses due to insects feeding and contamination (Mattos et al., 2020). It multiplies, grows and add fragments into a commodity. The US –FDA has set the maximum allowable limit of unacceptable filth in food, known as defect action level (DAL). Though insect fragments are allowed in the food materials to allowable limits, it is considered an essential criterion during export and import. The estimated monetary loss caused by insect infestation is more than $470 million (USDA,2020).

Tribolium castaneum; Herbst (Red flour beetle) and *Lasioderma serricorne;* Fabricius (cigarette beetle) are the serious pests of processed food commodities (Vidya and Awaknavar, 2004, Ravi Kumar et al., 2017). *L. serricorne* is considered the serious secondary pest after the *Plodia interpunctella*; Hübner (Indian meal moth). *T. castaneum* is ranked as the fourth important pest and is the most abundant in retail grocery and departmental stores (Arbogast et al., 2000). The total life cycle of *L. serricorne* ranges from 7-10 weeks. The female cigarette beetle lays approximately 9 to 42 eggs during its life span. Among the developmental stages of *L. serricorne,* the larval stage is considered the most active and damaging one as it results in boreholes in the food samples, reduces the aesthetic value and quality of stored products (David W. Hagstrum, 2006). Adults of *T. castaneum* are long-lived, often up to 3 years and its damage results in quantitative losses to wheat flour. The females lay up to 300-500 eggs during their life span. The complete life cycle of the insect ranges from 7-12 weeks, depending on temperature (35°C) and relative humidity (60-80%) (Devi and Devi, 2015). Some insects undergo complete metamorphosis *i.e.,* after hatching from eggs, insects experience several different developmental stages during the life cycle: larva, pupa, and adult. When entering a new developmental stage, insects must shed the old cuticle periodically to develop and grow (Zhang, 2014, Wittmann et al., 2018). This process is called moulting, or fragment shedding or ecdysis. Moulting adds the fragments (elytra) to the food commodity, ultimately affecting the products' quality (Wu et al., 2019).

Detection and control of stored pests are a very high priority in stored food commodities (Johnson, 2020). It is necessary to quantify the fragments produced by stored pests at various life stages to maintain high-quality stored food commodities, especially in flour and spice powders. Milling industries face considerable problem with insect fragments. Nil tolerance for insect or insect fragment is established in food and food products in most European countries (Solà et al., 2017, Stejskal et al., 2015). The US Food and Drug Administration (US-FDA) has set a defect action level (DAL) for wheat flour (75 insect fragments per 50g) for macaroni and noodle products, and the maximum permissible limit is one insect fragment per gram of flour (FDA, 2005). In Canada, the defect action level not exceeding more than 25 fragments in three samples per 50g of flour is recommended (Bhuvaneswari et al., 2011, Trematerra et al., 2011).

Wheat is one of the major commodities that grow in the India with a production that reached 105 million metric tons in 2020 (USDA, 2020). Wheat flour is widely consumed in India in the form of various food products. Wheat flour is documented as a favourable medium for the growth of the number of secondary pests of the stored product in relation to any other flour. The flour constituents also play a significant role, influencing insect's survival, growth, and reproduction potential. Flour rich in protein such as gram flour and wheat flour, the insects reproduce and grow faster. Insects are poikilothermic animals that are largely affected by various environmental factors. Among all the climatic factors, the temperature has probably the greatest effect on insect development (Ju et al., 2011).

To develop economical and effective control measures, it is essential to know the environmental conditions that lead to the possible prediction of DAL and insect fragments with respect to population levels under similar environmental conditions and post infestation period. Abiotic factors such as temperature, humidity and moisture influence the shedding of insect fragments by the stored pests. Several studies have investigated the effect of temperature on the development and survival of T. *castaneum and L. serricorne*(Runner, 1919, Howe, 1956, Skourti et al., 2019, Edde, 2019) .

The present research focused to study the comparative fragment shedding behaviour of *T. castaneum and L. serricorne* with respect to insect population density and storage period. *In addition,* three storage parameters, namely insect population density (IPD), storage temperature (ST) and the storage period (SP) were optimized, in order to keep fragment counts below Defect Action Level by using Box Behnken design in conjunction with Response surface methodology.

Materials and methods

Procurement and sample preparation

Wheat grains were purchased from Thanjavur local market and were sieved to remove dirt, stones and dust and were screened for visible contamination or internal contamination and were sun-dried for four days to disinfect them from any residual infestation (Jonfia-Essien, 2006, Tefera et al., 2011). Before grinding, the wheat grains were sterilized at 60 ℃ for 10 h in a ventilated oven (Ever flow Oven Scientific Instrument, Chennai) to disinfest it from any residual infestation. The sterilized wheat grains were left to acclimatize to room temperature before being ground. The wheat grains were grounded in flour mill, and the flour was sieved through 0.25-micron mesh (Jayant brand, Mumbai, India) to remove any eggs or fragments present in it and was stored in an airtight container for future study.

Insect culture and rearing

Red flour beetle, *T. castaneum* and cigarette flour beetle *L. serricorne* were reared at the Indian Institute of Food Processing Technology (IIFPT), Primary Processing, Storage, and Handling laboratory (DPPSH), Thanjavur, India. The cultures of *T. castaneum* and *L. serricorne* were maintained on wheat flour at room temperature at 28 ± 2 °C. All the stages of the insects, *viz.,* egg, larva, pupa, and adults, were maintained separately for the experimental study (Rolania et al., 2013).

Experimental setup

Two different experimental set up were planned in this study to know the comparative fragment shedding behaviour of two storage insects and optimization of selected storage parameters. In the first experimental setup (Table 1), wheat flour infested with *T. cas*taneum and *L. serricorne* (1,3, and 5 number of insects) separately and kept at a fixed storage temp of 30 ℃ (Technico environmental chamber) for the storage period of 7,14,21, 28 and 35 days (Jonfia-Essien et al., 2010, Abdullahi et al., 2018). In second experimental setup (Table 2), wheat flour is infested as per the experimental design discussed in section 2.5. In each experimental setup, the fifty grams of wheat flour were kept in 500 ml glass jars (7.5x13.5 cm) at different storage parameters as per the experimental plan at a fixed relative humidity of 70±2 % (Howe, 1956).

Fragment count analysis

Fragments shedded by *T. castaneum* and *L. serricorne* were examined at DPPSH laboratory. Uniform aged adults of both the insectswere introduced into the glass jars containing wheat flour (Abdullahi,2018) as per the experimental design and each series was replicated three times. These jars are covered with the muslin cloth and tightly fixed with a rubber band to allow ample aeration and insect escape prevention from the jars. Stored samples were taken out at timely intervals and sieved using 0.25-micron mesh (Jayant make, India). The fragments shedded by selected storage insects were analyzed using the standard AACC acid hydrolysis method (AACC- 2000) and observation performed under the Leica stereo zoom microscope (Leica S8AP0, CMS GmbH stereo-zoom microscope LAS version 3.8.0). Fragment counts in three replicates of each infestation level were determined.

Experimental design and data analysis

Accurate counting of insect fragments by the microscopic analytical method is tedious and cumbersome. Various process variables counting fragments for two insects are challenging, increasing the chances of experimental and manual error. Hence, the easy experimental design needs to be adopted particularly in fragment shedding that can maintain the accuracy of results reduce time and cost by reducing the number of runs for experimental trials.RSM tool was used to establish an experimental design model based on the least square model to investigate the consequences of various independent parameters on the response to locate the optimum conditions for approximation and optimization of response model. Among various RSM designs, the Box Behnken design offers some advantages in requiring few experimental runs

for three process variables. Considering these points, the 3-factor, 3-level Box Behnken design was applied as a RSM tool (Kim et al., 2007, Kramar et al., 2003, Nazzal et al., 2002) . The insect population density (1,3 and 5), storage temperature (26, 30 and 34 ºC) and storage period (7,21 and 35 days) was used as the factors while insect fragment counts (IFC) were defined as the response. The level of independent variables was decided through literature and preliminary experiments.

The levels of a factor in the coded and actual form are given in Table 2. A second order polynomial equation was chosen to fit the experimental results. The general form of the model chosen is represented as follows:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2$$

(Eq. 1)

Where Y is the predicted response, *i.e.*, insect fragment counts from *T. castaneum* (IFC-T) and *L. serricorne* (IFC-L). β_0 is constant. Moreover, β_1, β_2, and β_3 are linear regression coefficients, β_{12}, β_{13}, and β_{23} represent interaction coefficients, and β_{11}, β_{22}, and β_{33} denote quadratic coefficients. X_1 represents insect population density (IPD), X_2 represents storage temperature (ST), and X_3 represents a storage period (SP).

Statistical analysis was performed using Design Expert 12.0 (Stat-Ease, Minneapolis, Minnesota, USA) software. The model significance was estimated based on the variance (ANOVA) with 95% confidence level. In statistics, the sum of squares (SS), degree of freedom (DF), mean squares (MS), F-values, and p values are used to describe the model (Popov et al., 2016). The polynomial equation's fitness to the responses was estimated using the coefficient of determination (R^2), and the effect of variables at a linear, quadratic, and interactive level on the responses were described using significance at 1, 5 and 10% confidence level. Three-dimensional (3D) contour plots were generated using polynomial equation to visualize the correlation between the dependent variables and each independent variable's levels.

Optimization and validation

An optimization study is performed to determine the storage parameters within the experimental range, by which fragment counts of storage insects restrict to cross the Defect action level (DAL). The numerical optimization option of the module of Design Expert software was utilized for this purpose. During optimization, insect population density and storage temperature were set within the range and storage period was set as maximum for prolonging the days of storage of wheat flour and insect fragment counts was set as 75 fragments per 50g of wheat flour as per the Food and Drug Administration (FDA) limit. In order to validate the optimization results, additional experiments were performed at optimize storage conditions generated by the optimization module and the experimental value of fragment counts were obtained.

Results and discussion

Comparative fragments shedding of two major insects *T. castaneum* and *L. serricorne* in wheat flour at a fixed storage temperature of 30°C for the storage period of 7,14,21, 28 and 35 days, were studied in the first experimental setup. The second experimental setup was planned to optimize the storage parameters in order to keep the fragment counts of storage insects in wheat flour below the Defect action level (DAL). Fragments shedded by *T. castaneum* and *L. serricorne* are presented in Figure 1 (a-h).

Comparison of Fragment shedding behaviour of *T. cas*taneum and *L. serricorne*

To know the fragment shedding behaviour of *T. cas*taneum and *L. serricorne,* wheat flour was infested with 1,3 and 5 numbers of insects at a fixed storage temperature of 30°C (Fig 2). It was observed that both the insects produced almost the same number of fragments (12 and 15, respectively) at first seven days of the storage period. It can be seen from Fig. 2 that the increase of storage period after a week time fragment shedding was also increased. It is clear from Fig 2, the cigarette beetle *L. serricorne* shedded more fragments (42, 64, 105, 198 fragments) than *T. castaneum* (24,48,68,119 fragments) in successive storage period of 14, 21, 28 and 35 days respectively. The percentage shedding difference (Table 1) of *L. serricorne* from *T. castaneum* was gradually increased from third to fifth weeks of storage period. Our results indicate that *L. serricorne* sheds more fragments and fragment /elytra numbers were increased with increase in insect population density. Pattikava et al, 2014 demonstrated effect of temperature and photoperiod on growth, moulting and survival of marron *Cherax tenuimanus.* The study results showed that specific growth rates and molt increment were significantly higher at a higher temperature than at lower temperatures (Pattikawa and Wenno, 2014). The study conducted by Skourti et al., 2019 demonstrated *T. castaneum* completes egg to adult development at 20 to 32.5°C temperatures except 35 °C, displaying temperature-dependent development of species. The model's estimations for lower and upper developmental threshold for the total immature period were 20.2 and 35.3 °C, respectively. The study predicts that the median time period for fastest development of egg and larvae for *T. castaneum* decreased at temperature of 30°C. Egg to adult temperature-dependent survival of *T. castaneum* was well described by the extreme value function with R^2=0.84). White (1987) predicted that temperatures ranging between 30 and 35 °C would increase the populations of *T. castaneum* on wheat by 8 to 20 times monthly. The other insect, *L. serricorne* reared at 25−27 °C, in wheat feed, increased by 60 % every week (Howe, 1956). The population of the above increased seven times in 3 weeks' time on finely sieved wheat feed or

broad English bran at 30 °C and 60 % RH (Lefkovitch and Currie, 1967, Edde, 2019). There have been several reports on population growth of *T. castaneum* and *L. serricorne* based on temperature and diet. Our study provides data that are relating insect growth and development time with the fragment shedding by both the insects and attained DAL. Our study also provides data that shows that time taken to reach DAL at 30 °C is significantly less for *L. serricorne* as compared to *T. castaneum*. Also highlighting temperature tolerance and fragment shedding behaviour of *L. serricorne* in wheat flour.

The model equation and statistical evaluation

As shown in Table 2, Box Behnken design was applied to optimize the storage parameters and according to the design matrix, 17 experiments were conducted. The fragment counts for *T. castaneum* (IFC-T) and *L. serricorne* (IFC-L) were in the range of 9 to 223 fragments and 13 to 325 fragments per 50 g of flour, respectively. The selected independent variables, namely insect population density (IPD), storage temperature (ST) and storage period (SP), have a marked influence on the fragments produced by both the insects.

The ANOVA, regression coefficients, adequacy of models, and goodness of fit results are summarized in Table 3. The model F value of 54.67 (IFC-T) and 23.89 (IFC-L) shown in Table 3 implies that the model is significant. Table 3 reveals that significant effect on the IFC-T as shown by liner terms (IPD- X_1 and SP- X_3); quadratic variable of ST-X^2 and the interaction variable of X_1 X_3 at $p< 0.01$, whereas linear variable (X_2); quadratic variables of X_1 and X_3; and interaction variables X_1X_2 and X_2X_3 were insignificant terms ($p>0.1$). The most significant factors affecting the IFC-T were IPD and SP with an F value of 79.87 and 352.95, respectively, and p values were <0.0001. In case of *L. serricorne*, all liner terms (IPD- X_1; ST-X_2 and SP- X_3); quadratic variable of ST-X_2^2 and SP-X_3^2 at $p< 0.05$ showed a significant effect on IFC-L. SP with an F value of 145.19, which is highly significant compared to IPD and ST.

The adequate precision for IFC- T and IFC-L (27.558 and 16.955 respectively) value was higher than 4.0 implies that the proposed model could be applied to navigate the design space.

The fragments shedded by *T. castaneum and L. serricorne* can be described using the following regression equation in terms of coded factor:

$$\text{IFC-T} = 107.70 + 32.87X_1 - 3.16X_2 + 68.75X_3 - 4.52X_1^2 - 27.76X_2^2 - 9.05X_3^2 - 10.10\,X_1X_2 + 23.25\,X_1X_3 - 1.25\,X_2X_3 \quad \text{(Eq.2)}$$

$$\text{IFC-L} = 196.19 + 34.70X_1 - 30.62X_2 + 105.75X_3 - 44.27X_1^2 - 69.73X_2^2 - 12.44X_3^2 - 14.77\,X_1X_2 + 28.25\,X_1X_3 - 7.75\,X_2X_3 \quad \text{(Eq.3)}$$

The predicted values agree well with the experimental data with a coefficient of determination $R^2 = 0.986$ and $R^2 = 0.968$, indicating that both the models accounted for more than 95% of the total variability response.

The adjusted R^2 (Adj- R^2) is a corrected goodness-of-fit, and it was also close to the coefficient of determination R^2, which indicates that the regression predictions approximate well with the real data points, as shown in Table 3.

The terms that have a positive sign in Equation 2 and 3 positively affect the increase of IFC of both the insects in the wheat flour. The linear term storage temperature (X_2) has a negative sign in both models, and thus it can be a controlling parameter to restrict the increase of IFC. Among the linear term, SP (X_3) had the highest impact on the increase of IFC and comparatively more predominant in IFC-L. After the linear term, the interaction between IPD with SP (i.e.X_1 X_3) had the highest impact on IFC's increase. Among the square terms, the square of ST (X_2^2) had the highest impact on the insects' fragment shedding. These results imply that more extended storage infestation period with more population density will significantly affect the shedding of fragments. In contrast, reduction or increment in the storage temperature will decrease the fragment counts. Azrag et al., 2020 reported that the temperature significantly affects the development time of all immature stages coffee berry borer, *Hypothenemushampei* and the maximum threshold temperature 32 °C was required for egg to adult development. Our results are also in line with the findings of the previous work shows similar trend in terms of fragment counts of red flour beetle, at temperatures 34°C and 26 °C, fragment counts was decreased indicating that temperature affects the insect development time thus the moulting. While in case of *L.serricorne* fragment counts were more at higher temperature; as reported late fourth instar larval and pupal stages are the more tolerant stage to elevated temperatures (Powell, 1931, Yu et al., 2011, Edde, 2019).At high temperatures, *L. serricorne* attained DAL soon as compared to *T. castaneum*. The model developed using BBD design in the present study is in line with the life cycle data and models that previous researchers obtained for stored product pests. Our results contribute to the DAL of *T. castaneum* and *L. serricorne* during storage and predict the optimum storage temperature and period.

Response surface graph

Figure 3 (a-c) and Figure 4 (a-c) shows 3-D surface plots constructed based on the Eq. (2) and Eq. (3), which determine the optimum storage parameters to control the IFC of *T. castaneum and L. serricorne*. The effect of IPD and SP on the IFC's of the both insects are illustrated in Fig. 3a and Fig 4a. The effect of IPD and SP were more prominent at the centre value of storage temperature. It was expected because as the number of insects increased, the fragments shedded by

the insect in the sample also increased along with the increase of SP. In Fig 3b and 4b, the response surface plot depicts IPD and ST's interaction on the IFC at the middle value of storage period. The effect of IPD can be considered as highly significant at storage temperature lies between the centre zone. The maximum shedding of fragments by both insects was noticed with more numbers of insects and minimum fragments were noticed when ST was lower and higher from its centre value. IFC-T and IFC-L were maximum at IPD of 5 and a storage temperature of 30°C. The SP and ST interaction effect for the IPD at middle point is depicted in Fig 3c and 4c. A more extended storage period showed more significance on the IFC of *T. castaneum*. The interactive effect on fragment shedding of *T. castaneum and L. serricorne* indicated that for prolonging the storage period with minimum increase of IFC could be possible by keeping the storage temperature either lower or higher from the centre value of 30°C. From our studies, it is evident that the fragment shedding was more at temperature 30°C compared to 26 and 34 °C. This can be correlated with the study carried by Howe (1956) , Pattikawa and Wenno (2014), and Skourti et al. (2019) show that insect growth was high and the development time was less at this temperature. With the increase in the insect population density, more fragments are produced, which causes detrimental effects on the quality of stored wheat flour.

Optimization of storage parameters based on DAL

The optimization value of the selected storage parameters was obtained using the numerical optimization module of Design expert software in which fragment counts were set as per DAL limit, i.e. ,75 fragments per 50 g of wheat flour. It is clear from Table 4 that a single insect of *T. castaneum* attained DAL within 35 days of storage period compared to *L. serricorne* attained DAL within 23 days of storage period at 26 °C. The predicted fragment counts shedded by one insect of *T. castaneum* and *L. serricorne* are 75 and 140 fragments per 50 g of wheat flour, respectively, at 26 °C for 35 days of storage period. From Table 4, at optimum conditions at 26 ° C, even a single insect of *L. serricorne* is present; it is possible to keep the wheat flour safely well below the DAL only up to 23 days. For the above conditions, the predicted fragment counts of *T. castaneum* is 48 fragments per 50 g of wheat flour.

The accuracy of the numerical optimization was confirmed by conducting a verified experiment under the optimal conditions. The percentage deviation from the predicted value was calculated, and it was 2.59 % for *T. castaneum* and 1.31 % for *L. serricorne* (Table 4). The low deviation from predicted value is one more confirmation that the prediction ability of proposed model is adequate. It is clear from optimization study that in bulk storage of wheat flour if storage parameters will set to restrict the fragment counts of *L. Serricorne* is not to cross the DAL value, then it can be implemented to *T. castaneum* also, as in same storage parameters, *T. Castaneum* is shedding less number of fragments than *L. serricorne*.

Conclusion

A comparative study on the fragment shedding among two major storage insects of wheat flour *L. serricorne* produced more fragments than *T. castaneum* at similar storage conditions. The number of fragments imparted by *T. castaneum* than *L. serricorne* was directly proportional to the infestation level. Our study proves that even the presence of a single insect in the flour during storage could cross the DAL of the flour and it is necessary to keep the flour at zero tolerance, especially during longer storage. Based on the study results, it is suggested the frequent sampling of the flour especially when the temperature reaches approximately 30°C. When fragments are noticed, control measures should be followed quickly before they developed into more tolerant stages (*i.e.,* larvae and pupae). Therefore, at 30to34°C insecticidal treatments will maximize their impact against the bothinsect populationdensities. Accurate and timely identification and discrimination among different storage pests are essential for effective integrated pest management (IPM). Thus, the present study provides data on the DAL, effects of temperature on fragments shedding and safe storage parameters to be considered during wheat flour storage. The effect of storage parameters like insect population density, storage temperature, and storage period on the flour's quality attributes, which reached DAL, can be the future thrust area of the current research work.

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Fig. 1. Images (a to d) shows different fragments of *L. serricorne* and (e to h) *T. castaneum* isolated and quantified from the wheat flour during the storage period viewed under the Leica microsystem. Leica S8AP0, CMS GmbH stereo-zoom microscope LAS version 3.8.0 images; Dimension -2048x1536x24bpp, Width-204 pixel, Height-1536 pixel, Horizontal and vertical resolution 300 dpi, Camera focal length 35mm and under 80 x magnification

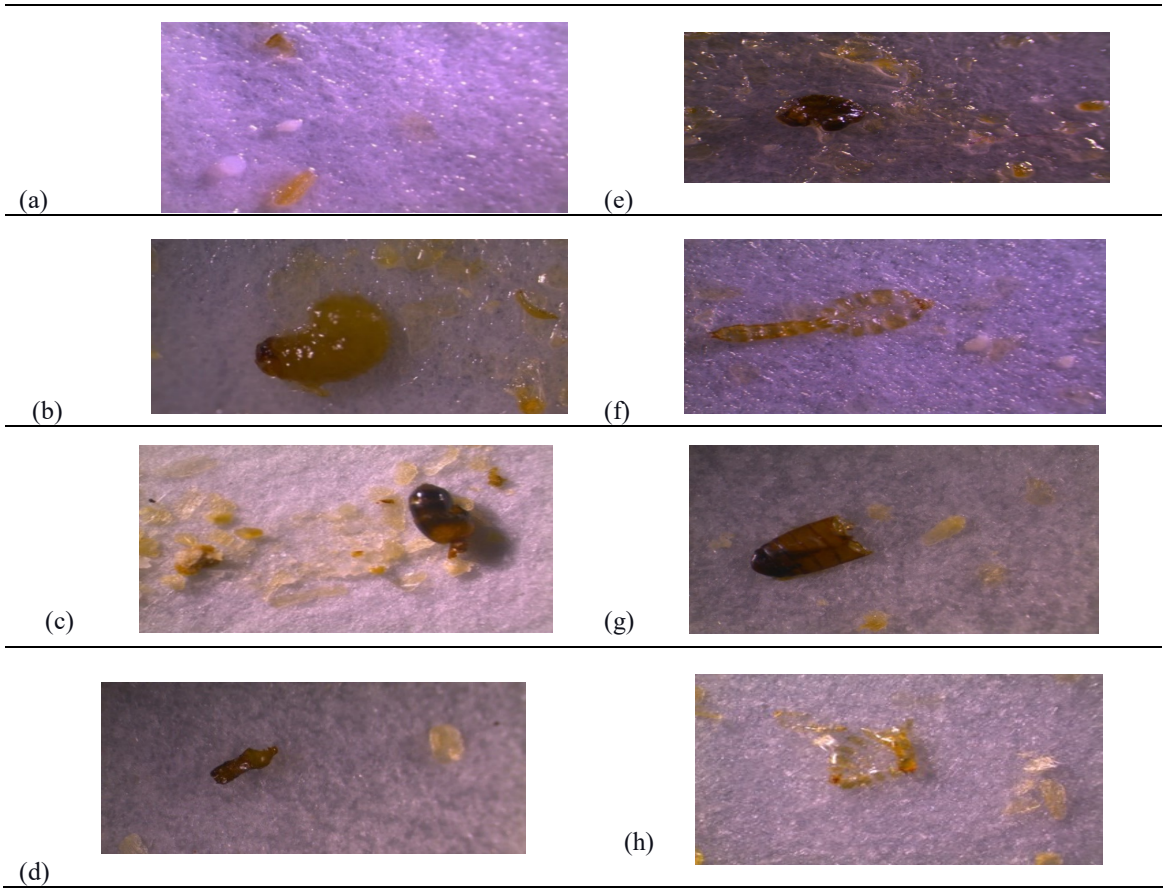


Fig. 2. Effect of storage days on fragment counts of *T. cas*taneum and *L. serricorne* in wheat flour with (Mean ± SD, n=3). Lines with markers denotes the flour infested with different insect population density of *T. castaneum* and *L. serricorne.*

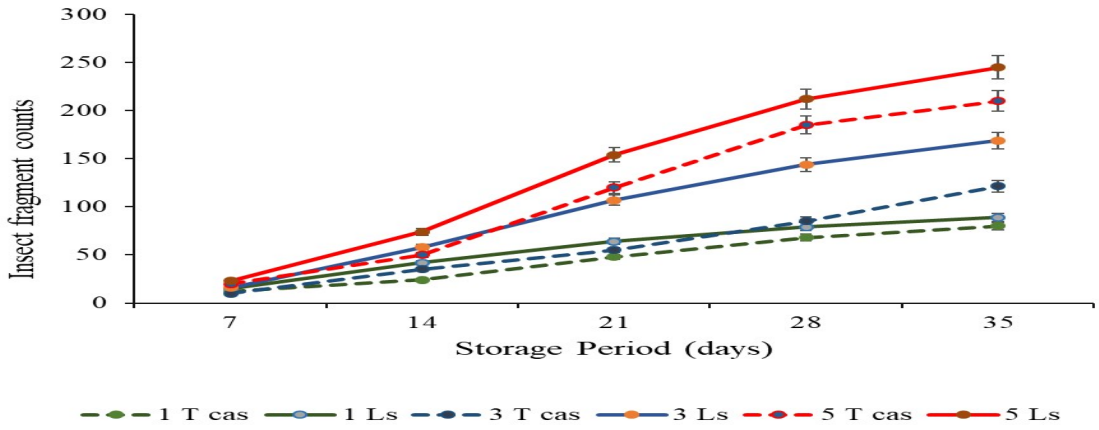
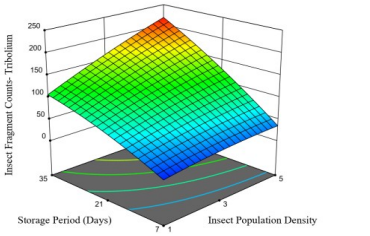
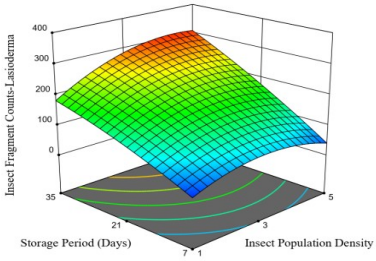


Fig. 3 and 4. Response surface 3D graph (a) Interaction effect of IPD and SP (b) Interaction effect of IPD and ST (c) Interaction effect of SP and ST on fragment counts of *T. castaneum and* Response surface 3D graph (a) Interaction effect of IPD and SP (b) Interaction effect of IPD and ST (c) Interaction effect of SP and ST on fragment counts of *L. Serricorne*



(a)



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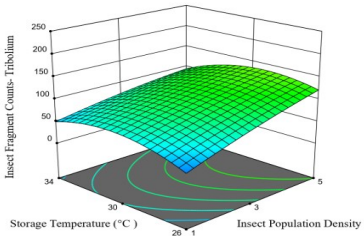

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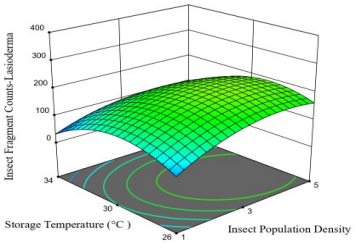

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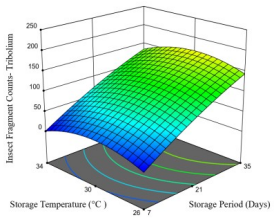

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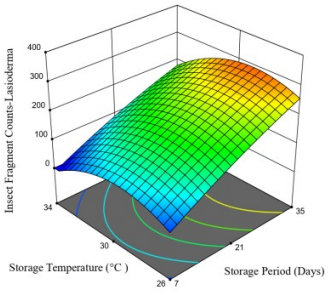

(c)

Table 1. Comparative fragments shedding behaviour of *T. castaneum* and *L. sericorne* at fixed storage temperature

Insect Population Density	Storage period	Insect fragment counts (IFC) of *T. castaneum*	Insect fragment counts (IFC) of *L. sericorne*	% Difference*
1	7	12±1.52	15±1.00	0.14
	14	24±0.57	42±1.00	0.41
	21	48±2.51	64±3.05	0.63
	28	68±1.15	105±2.08	1.04
	35	119±1.00	198±3.00	1.97
3	7	24±0.57	35±2.08	0.34
	14	52±2.51	68±3.60	0.67
	21	113±2.50	190±1.52	1.89
	28	125±2.00	205±4.00	2.04
	35	162±1.73	230±5.13	2.29
5	7	23±1.57	29±1.52	0.28
	14	50±2.51	74±5.00	0.73
	21	120±4.00	154±3.78	1.53
	28	145±3.51	212±1.52	2.11
	35	223±1.15	325±3.21	3.24

$$*\%\ \text{difference} = \frac{\text{IFC of } L.Serricornne - \text{IFC of } T.casteneum}{\text{IFC of } L.Serricornne} \times 100$$

Table 2. Box Behnken design matrix and insect fragment counts of *T. castaneum* and *L. serricorne*

Experimental run	Factor 1 X_1: IPD	Factor 2 X_2:Storage temperature (°C)	Factor 3 X_3:Storage period (Days)	Response IFC-T	IFC-L
1	1 (-1)*	30 (0)	7 (-1)	12	15
2	5 (+1)	30 (0)	7 (-1)	23	29
3	1 (-1)	30 (0)	35 (+1)	119	198
4	5 (+1)	30 (0)	35 (+1)	223	325
5	1 (-1)	26 (-1)	21 (0)	33	80
6	5 (+1)	26 (-1)	21 (0)	126	178
7	1 (-1)	34 (+1)	21 (0)	54	46
8	5 (+1)	34 (+1)	21 (0)	114	92
9	3 (0)	26 (-1)	7 (-1)	11	27
10	3 (0)	26 (-1)	35 (+1)	135	226
11	3 (0)	34 (+1)	7 (-1)	9	13
12	3 (0)	34 (+1)	35 (+1)	128	181
13	3 (0)	30 (0)	21 (0)	113	193
14	3 (0)	30 (0)	21 (0)	105	210
15	3 (0)	30 (0)	21 (0)	113	190
16	3 (0)	30 (0)	21 (0)	105	193
17	3 (0)	30 (0)	21 (0)	103	204

***Actual Value (Coded Value)**

X_1: Insect population density (IPD); X_2: Storage temperature (ST); X_3: Storage periods(Days);
IFC-T: Insect fragment count for *T. castaneum*. IFC-L: Insect fragment count for *L.serricorne*

Table 3. ANOVA model summary statistics for *T. castaneum* and *L. serricorne* fragments present in the wheat flour

Source	Insect Fragment Count- *T. castaneum* IFC-T			Insect Fragment Count- *L. serricorne* IFC-L		
	DF	**F-value**	**p- value**	**DF**	**F-value**	**p-value**
Model	9	54.67*	< 0.0001	9	23.89*	0.0002
IPD (X_1)	1	79.87*	< 0.0001	1	15.48*	0.0056
ST (X_2)	1	0.63	0.45	1	145.19*	0.0001
SP (X_3)	1	352.95*	< 0.0001	1	10.34	0.0147
X_1 X_2	1	2.95	0.129	1	5.18*	0.0570
X_1 X_3	1	20.18*	0.0028	1	1.09	0.3302
X_2 X_3	1	0.058	0.816	1	0.3899	0.552
X_1^2	1	0.7987	0.401	1	13.31	0.0082
X_2^2	1	24.39*	0.0017	1	1.02	0.345
X_3^2	1	3.12	0.120	1	26.75	0.0013
Residual	7			7		
Lack of Fit	3	9.44*	0.0275	3	18.23*	0.0085
Pure Error	4			4		
Cor Total	16			16		

*S= Significant
IFC-T: R^2 **= 0.986;** **Adj.** R^2 = 0.967 Adeq precision: 27.558
IFC-L: R^2 **= 0.968;** **Adj.** R^2 = 0.928 Adeq precision: 16.955
IPD: Insect population density(X_1); ST: Storage temperature (X_2); SP: Storage period (X_3)

Table 4. Optimized storage parameters for obtaining insect fragment counts of *T. castaneum* and *L. serricorne* within defect action level

Response Variable	Optimized storage conditions			Response Value		Deviation* (%)
	Insect Population Density (IPD)	**Storage Temperature ST (°C)**	**Storage Period SP (Days)**	**Predicted**	**Experimental [a]**	
Insect Fragment count of *T. castaneum*	1	26	35	75	77	2.59%
Insect Fragment count of *L. serricorne*	1	26	23	75	76	1.31%

* $\%\ \text{deviation} = \frac{\text{Predicted value} - \text{Experimental value}}{\text{Experimental value}} \times 100$ [a]Mean (n=3)

A NOVEL BIOPOLYMER PRODUCTION OF *GLUCONACETOBACTER OBOEDIENS* SJU-1: GENOMIC AND BIOCHEMICAL APPROACH

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Abstract

The elite cellulose producing strains were isolated, identified and characterized based on colony morphology, pellicle type and amount of cellulose production. A total of 22 fermented fruit samples and 16 fermented sugarcane variety juices tested, 141 isolates from fruits and 58 from sugarcane fermented samples produced zone of hydrolysis in GYC medium and further screened by streak plate method in HS medium, finally 59 cellulose producing isolates from fruit juices and 29 isolates from sugarcane juices were obtained. These isolates were further screened according to the Gallardo scale of thickness and a bacterial strain isolated from fermented sugarcane juice of sugarcane variety Co1148 yielded a cellulose pellicle with maximum thickness of 16.02 mm and the isolate was further characterized by biochemical and molecular level and designated as *Gluconacetobacter oboediens* sju-1 and 16S rRNA gene sequences submitted to NCBI-GenBank with the accession number of KF 164613.

Key words: *Gluconacetobacter oboediens* (sju-1); Bacterial cellulose; Cellulose fibrils; *Gluconacetobacter xylinum* NCIM2526

Introduction

Acetobacter had been renamed as *Gluconacetobacter* and are known to produce cellulose exhibiting superior features over plant cellulose. It is a Gram negative, non-pathogenic, rod shaped obligate aerobic bacterium. *G. xylinum* has been used as a model microorganism for basic and applied studies on bacterial cellulose, because of its capability ability to generate relatively high levels of polymers from a wide range of carbon and nitrogen sources. Under strict aerobic condition in static culture of this bacteria forms an extracellular secretion which can form aggregated fibrils that crystallize into ribbons and assemble into a thick cellulosic mat known as a pellicle formed at the air-liquid interface of the culture medium.

Materials and Methods

The fruit samples and juice samples was added 1 g or 1 ml each separately to 9 ml of sterilized peptone water, mixed thoroughly and serial dilutions for each sample were done separately. Then 1.0 ml aliquots from 10^6 dilutions were taken and spread on Glucose Yeast Calcium carbonate (GYC) medium and incubated at 30°C for 48 h. Then the cultures were maintained in Hestrin Schramn medium. Biochemical characteristics such as cellulose production test, oxidase, catalase, indole production, urea utilization, citrate utilization, gelatin liquefaction, oxidation of ethanol, over-oxidation of acetic acid, dihydroxyacetone production test and special tests like, cellulose solubility test and sodium hydroxide treatment test of the cultures were performed. The higher cellulose producing bacterium was further characterised through 16S rDNA sequencing. Full-length 16S rDNA (1500 bp) were amplified from the isolates by PCR using the universal forward primer fp1 (5' AGAGTTTGATCGTGGCTCAG 3') and the universal reverse primer rp2 (5' ACGGCTACCTTGTTACCACTT 3'). The purified 16S rRNA genes from positions 9 to 1509 (approximately 1,500 bases) were sequenced by using four primers, 27F (5'-AGA GTT TGA TCM TGG CTC AG-3'; positions 27-46), 800R (5'-TAC CAG GGT ATC TAA TCC-3'; position 800-783), 518F (5'- CCA GCA GCC GCG GTA ATA CG-3'; position 518- 537) and 1492R (5'- TAC GGY TAC CTT GTT ACG ACT T-3'.

Results and Discussion

A total of 29 cellulose producers were obtained from the sugarcane juice of different varieties. Out of these, sugarcane juice from the variety Co1148 and Co 2000-12 produced three isolates each *viz*., sju-1, sju-2, sju-3 and sju-6, sju-7, sju-8 respectively. Invariably most of the sugarcane juice varieties i.e. Co 2000-12, Co 86032, Co 99004, Co 99008, Co 0218, Co 8371, Co 671, Co 99008, Co 0218 and Co 8371 produced two isolates of cellulose producer, while Co 2001-15, Co 94008 and Co 94012 produced only one cellulose producing isolate each. Among the sugarcane juices from different varieties, sju-1 produced maximum pellicle thickness of 16.02 mm followed by sju-6, sju-7, sju-12, sju-16, sju-17, sju-18, sju-19 and sju-25 which formed 10 mm thickness pellicle each.

Biochemical characteristics of cellulose producing isolate

The most important and the key characteristics of formation of water insoluble pellicle was tested positive for all the isolates. All the cellulose producing isolates exhibited positive response to catalase test that was evident when production of bubbles was documented in the test media on the addition of hydrogen peroxide. The isolate tested were oxidase, gelatinase and urease negative. In general, the isolate showed no growth in citrate medium. The isolate tested negative for indole production. In the Congo red test, formation of red coloured colonies in Congo red incorporated LB medium was observed. Formation of dihydroxyacetone in glycerol medium was tested positive which was confirmed by the

production of blue colour precipitate upon addition of Benedict's solution. Oxidation of ethanol was confirmed by the presence of clearing zone in the ethanol containing GYC medium and over oxidation of acetic acid to CO_2 and H_2O were found to be positive by the change of colour of the indicator bromocresol green to yellow.

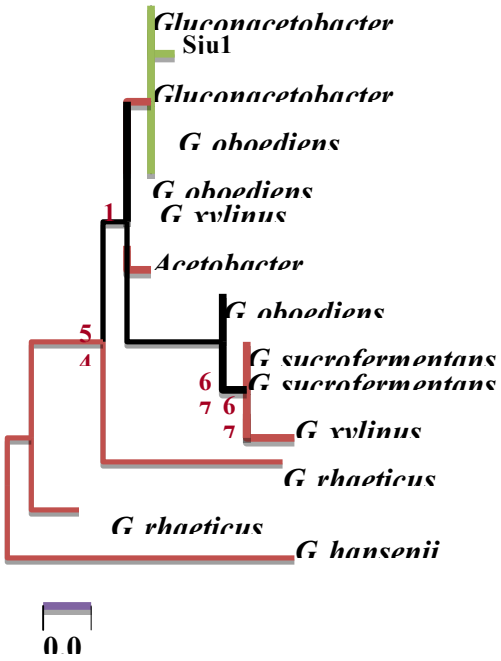
Molecular characterization of cellulose producing isolate

The sequence similarity of sju-1 with other *Gluconacetobacter* strains analysed using BLAST is presented in Table 6. The Neighbour-Joining tree of partial (341 bp) 16S rDNA sequence of *Gluconacetobacter* sp. (sju-1) obtained in this study together with selected sequences downloaded from GenBank database is shown in fig.1. The sequence analysis for the 16S rDNA gene of the isolate G*luconacetobactersp.* sju-1 was analysed in BLAST + of NCBI, GenBank to find similar nucleic acid sequences. The 16S rRNA gene sequence of *Gluconacetobacter oboediens* sju-1with 1456 bases, possessed 99.02 per cent similarity to *G. obediens*, and 99 percent similarity to *G. intermedius* and *G. xylinum respectively* and the unique sequences of *G. oboediens* sju-1 was deposited in the NCBI GenBank database under the accession number KF164613. Hence the exact species of the isolate sju-1 was identified as *Gluconacetobacter oboediens* sju-1.

Conclusion

The present study demonstrates the new isolate *Gluconacetobacter oboediens* sju-1 was identified from fermented sugarcane juice of sugarcane variety Co1148 based on biochemical and molecular level. The 16S rRNA gene sequences submitted to NCBI-GenBank with the accession number of KF 164613. *Gluconacetobacter oboediens* sju-1 yielded a cellulose pellicle with maximum thickness of 16.02 mm, very valuable, high strength cellulose fibrils as biopolymers and can be exploited by mass production to produce medically important biomaterials.

Fig.1. Phylogenetic tree for the 16SrDNA sequencing of *Gluconacetobacter obediens*



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EFFECT OF CARPET INDUSTRY EFFLUENT ON YIELD OF TOMATO (*Solanum lycopersicum*L.)

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Abstract

Effluent of carpet industry discharged on land and into water bodies induced environmental pollution. However, these are used for crop production because those effluents contain several plant nutrients. The aim of the present study is to observe the effect of carpet industry effluent on yield of very important vegetable crop tomato (*Solanum lycopersicum*L.). Effluent of carpet industry was procured from district Bhadohi and used in this study. A pot experiment was conducted adopting Completely Randomized Design with five treatments and three replications in the natural open weather conditions for 60 days during the plant season. Five concentrations v*iz*; 0%, 25%, 50%, 75% and 100% were used for present experiment. Zero per cent concentration was treated as control. Observations related to yield (number of fruits) were recorded at harvest. Results reveal that plant yield parameters gradually decreased with increase in effluent concentrations and the maximum number was recorded at 0% concentration level whereas minimum was with 100% concentration.

Keywords: Carpet industry effluent*,* Tomato, Number of fruits.

Introduction

Industrialization plays a very important role in the growth and development of any country. However, environmental pollution has also been its byproduct. It is well established that effluents are non-degradable. When these pollutants become accumulated in animals through food chain, they become biomagnified in their cells. Carpet industry effluent includes as large variety of dyes and chemicals addition that make the environmental changes for carpet industry not only as liquid waste but also its chemical composition. Carpet effluents are high volume of water that eventually results, suspended solids. They can contaminate water with oil, grease and waxes while some many contain heavy metal such as chromium, copper, zinc and mercury. Industrial waste water is discharged untreated either on land or into water bodies. Resultantly this waste water pollutes the water resources and ultimately the agricultural land (Arjun et al; 2013).

The use of industrial effluents for irrigation has emerged in the recent past as important way of utilizing effluent, taking the advantage of the presence of the considerable quantities of N, P, K and Ca along with other essential nutrients (Niroula; 2003). Effluents discharged from the industries have either beneficial or harmful effect on germination, growth and development of agricultural crops (Ramanaet al; 2002, Saravanamoorthy and Ranjithakumari; 2007).

Previous studies suggested that effluents form industries inhibit seed germination and seedling growth. Wins et al. (2010) studied the effect of textile effluent on germination and growth of*Vignamungo* L. (Black gram). At lower concentrations, the germination ratio and growth were relatively higher than the control, but with the increase in the effluent concentration these parameters were decreased. The best germination and seedling growth was observed at 25% concentration along with the growth promoting effect, significantly better than control.

Sasikala et al. (2013) studied the impact of dye effluent at various concentrations (4%, 8%, 10%, 12% and 16%) on seed germination of black gram for a period of fifteen days. They reported gradual decrease in the shoot and root length of the seedlings with the increase in the dye effluent concentrations.

Therefore, it is necessary to study the impact of these effluents on crop system before they are recommended for irrigation (Thamizhiniyaetal; 2009). After assessment of the beneficial and harmful effect of the different concentration of effluents on crops, suitable dilution can be used as liquid fertilizer. In this present study, attempt has been made to identify the effect of carpet industry effluent on yield of tomato.

Materials and methods:

Effluent collection: For present study, carpet industries effluent containing municipal sewage were collected fromGhosiatown of district SantRavidas Nagar, Bhadohi.Bhadohi district is situated in Latitudes 25˚23ꞌnorth and Longitudes82˚34ꞌ East at the distance about thirty miles from west of Varanasi, twelve miles north-east of Gopiganj and about three miles south of the river Varuna.The district is known by the name “Carpet city”as it is home to the largest hand-knotted carpet weaving industry hubs in South Asia.The location map is given in figure 1.

Fig. 1. Location map of Bhadohi region

To find out the effect of carpet industry effluent on the yield of tomato, a pot experiment was conducted adopting Completely Randomized Design with five treatments and three replications in the natural open weather conditions for 60 days during the plant season. Pots were filled with normal soil without any effluent treatment. The seedling of tomato (*Solanum lycoperisicum* L.) was obtained from Indian Institute of Vegetable Research (IIVR), Varanasi. Seedlings were transplanted in the second week of September in the pots. Five concentrations of effluent *viz;* 0%, 25%, 50%, 75% and 100% were used for present experiment. Zero per cent concentration was treated as control. All the pots were uniformly watered with distilled water whenever required. In treated pots, effluent of various concentrations were given at the interval of 15 days. The data pertaining to plant yield was recorded 60 days after harvest of treatment. Number of fruits, per plant were counted. The experiment was terminated at 60 days after planting. At harvest the plants were carefully uprooted.

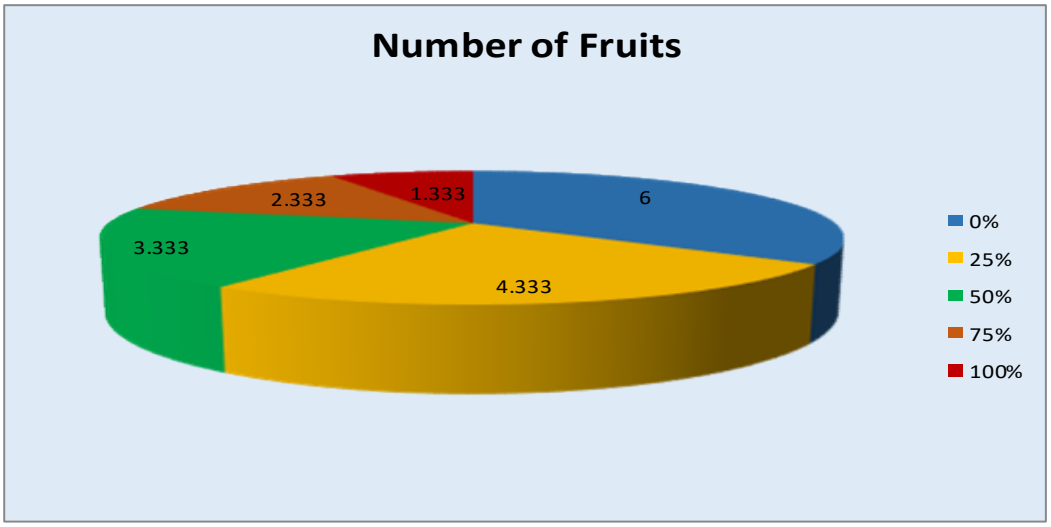
Result and Discussion

Effect of different concentrations of carpet industry effluent on the yield parameters under study have been presented in table 1 figure 2. Results presented in table 1 indicate that number of fruits decreased with increasing concentrations of carpet industry effluent. Maximum number was recorded in zero percent concentration of effluent (control). As the concentration of effluent increased there is continuous decrease in number of fruits. Minimum number was recorded with 100% effluent concentration as compared to control. Results are supported by study of Subramaniet al; (1998), they reported a progressive decrease in seedling growth with the increasing concentration of fertilizer factory effluent. Similar findings were also reported by Mishra et al; (1996). According them, the lower concentration of tannery effluent had a marked growth promoting effect while higher concentration of effluent showed reduction in seed germination, seedling growth and chlorophyll content in some crops.

Table 1. Effect of carpet industry effluent on number of fruits of tomato

Effluent Conc. (%)	Number of fruits at harvest
0	6.00
25	4.333
50	3.333
75	2.333
100	1.333
SEm±	0.360
CD (5%)	1.1094

Fig. 2. Effect of carpet industry effluent on number of fruits of tomato at harvest



Conclusion

This study concluded that effluent of carpet industry affects the tomato plant yield such as number of fruits. The higher concentration of effluent causes reduction in yield of tomato plant. Maximum yield is found in lower concentration of effluent and minimum yield in higher concentration of carpet industry effluent.

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CAN BIOSYNTHESIZED ZINC NANOPARTICLES INFLUENCE SEEDLING VIGOUR OF URDBEAN?

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Abstract

Urd bean or blackgram is an important pulse crop, and its establishment in the field is critical because of its poor seed quality. One of the opportunities to improve seed germination and seedling vigour is the use of nanoparticles. Therefore, the study was conducted to evaluate the effects of zinc oxide nanoparticles (ZnO NPs) on seed germination, and seedling vigour of blackgram (*Vigna mungo* L.). Zinc oxide nanoparticles were synthesized through the green synthesis method by using zinc acetate as precursor and *Tridax procumbens* leaf extract as reductant. The synthesized product has been characterized by Particle Size Analyzer (PSA), UV-visible spectroscopy, scanning electron microscope (SEM), and transmission electron microscope (TEM). Then, the blackgram seeds were treated with ZnO NPs, and seed germination and seedling vigour were quantified. The result indicated that the size of the ZnO NPs was 94 nm, assessed through a particle size analyzer. The UV–visible spectrum of synthesized colloidal ZnO NPs had the maximum absorbance peak at 298 nm. The SEM and TEM analysis indicates that ZnO NPs are spherical in shape. EDX confirms the purest form of Zn in ZnO NPs. The ZnO improved the seed germination and root growth of blackgram compared to untreated control. Thus, it is evident that seed treatment with ZnO NPs can be a potential approach to improve the seedling vigour of blackgram.

Keywords: Blackgram, Seedling vigour, Tridax daisy weed, ZnO nanoparticles

Introduction

Urd bean or blackgram (*Vigna mungo* L.) is the third most important grain legume cultivated in India accounting 12 percent of total pulse production. Blackgram crop is generally grown as a sequence crop after rice in rice fallow regions of Tamil Nadu because of its short duration nature. However, a major problem associated with blackgram is seed germination and seedling establishment, because of the unfavourable conditions prevails during seedling establishment.

Tridax procumbens L. (family Asteraceae), a weed of medicinal importance, native to the tropical America, which is a perennial creeper herb but it is growing worldwide. Green synthesis techniques make use of moderately pollutant free chemicals to synthesis nanometerials and embrace the use of benign solvents such as water, natural extracts. Green chemistry seeks to reduce pollution at source. It is enhanced to prevent waste than to treat or clean up waste after it is formed. Though physical and chemical methods are trendier for nanoparticles synthesis, the biogenic fabrication is a better choice due to eco-friendliness. Green synthesis of ZnO nanoparticles were agreed out using *Tridax procumbens* leaf extract for the eco-friendly development of novel technologies.

The phytochemical characterization of *Tridax procumbens L.* indicated the presence of alkaloids, flavonoids, carotenoids, fumaric acid, β-sitisterol, luteolin, glucoluteolin, n-hexane, tannin, quercitin, oxoester, lauric acid, myristic, palmitic, arachidic, linoleic acid and minerals such as sodium, potassium and calcium (Manokari and Shekhawat, 2017), which is suitable for green synthesis of nanoparticles. Therefore, the study was conducted to evaluate the effects of zinc oxide nanoparticles (ZnO NPs) synthesized from *Tridax procumbens* L. on seed germination, and seedling vigour of blackgram.

Methods

a. Green synthesis of ZnO nanoparticles:

Biosynthesis of zinc nanoparticles was done as per the procedure gave by Gnanasangeetha and Sarala Thambavani, (2013)**.** Zinc acetate dehydrate (99%) and sodium hydroxide (pellet 99%) were used as the precursor material and were supplied by Sigma chemicals, India. Weighed exactly100 grams of *Tridax procumbens* fresh leaves and grind the raw leaves in pestle and mortal without any liquid. 0.02 M Zinc acetate dihydrate solution was prepared and heated at 50-60°C for 30 minutes in a magnetic stirrer cum heater. Leaf extract of *Tridax procumbens* was slowly added drop by drop to zinc acetate solution under stirring for 2 hours & followed by the addition of 2.0 M NaOH at Ph 12. This resulted in formation of white suspension, which was covered in aluminium foil and kept undisturbed for 2 hours and got white precipitate which was washed three times with distilled water and finally washed with absolute alcohol (Gnanasangeetha and Sarala Thambavani, 2013).

b. Characterization of nanoparticles

Synthesized particles were characterized using the following equipments *viz.,* Particle Size Analyzer (PSA), UV- Visible spectroscopy, Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM) available at Department of Nano Science & Technology, TNAU, Coimbatore

. **Fig. 1. Particle size distribution of synthesized zinc nanoparticles**

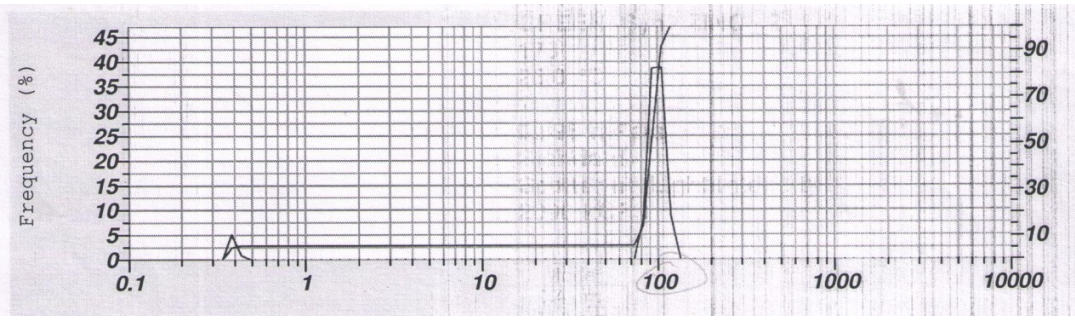


Fig. 2. The SEM image of ZnO NPs synthesized through biological method. The size of the ZnO NPs was 95 nm.

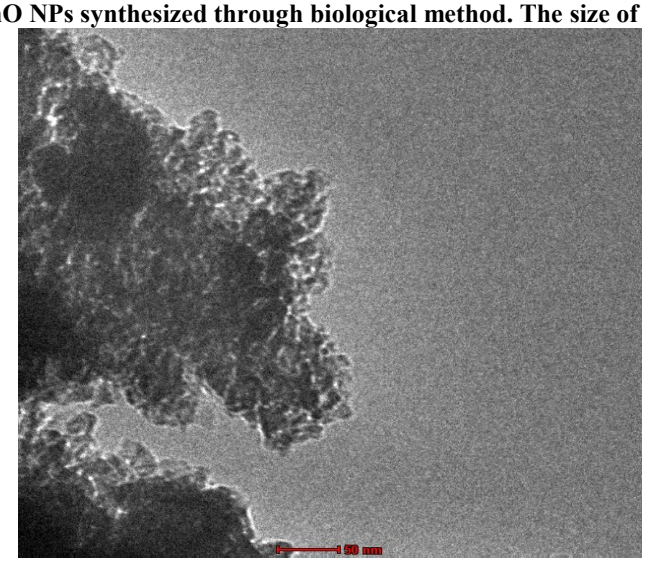


Fig. 3. Characterization of ZnO NPs using TEM. The size of the ZnO NPs was 60-75 nm.

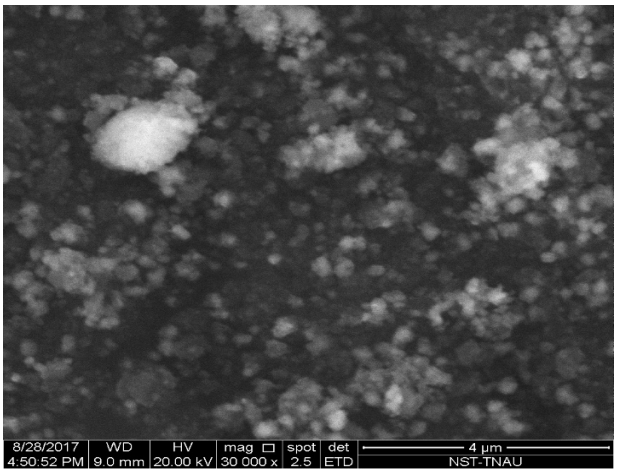
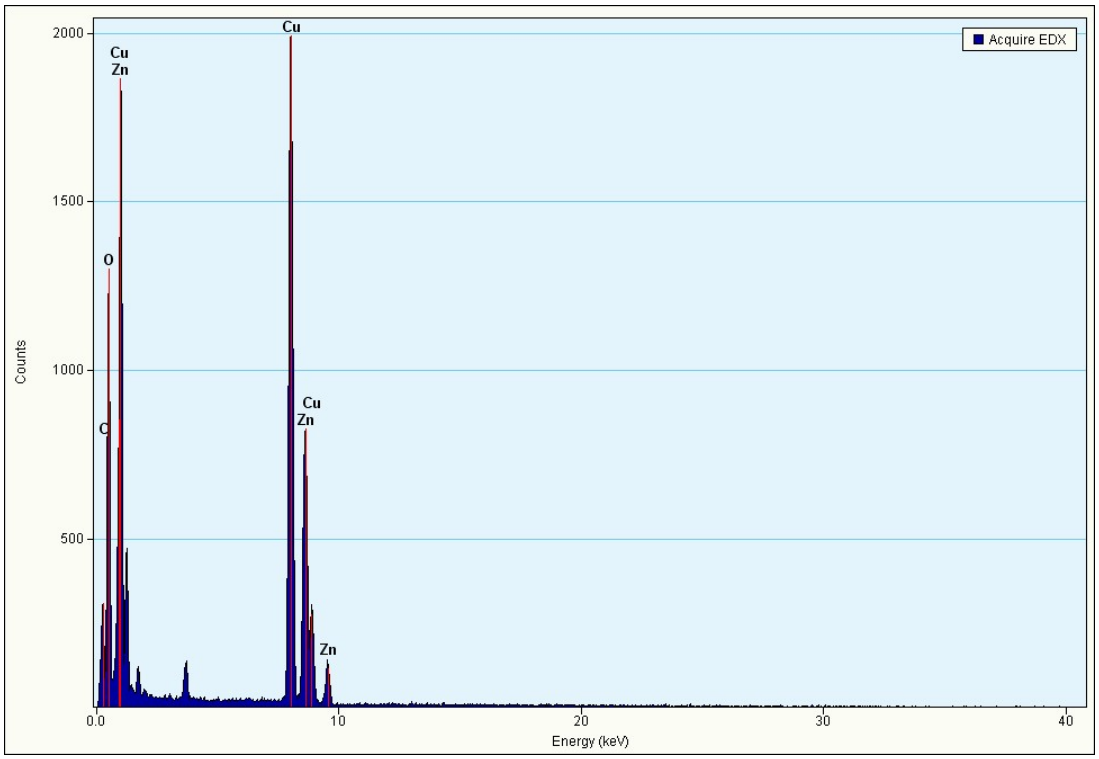


Fig. 4. EDX of ZnO NPs, showing the purest form.



c. Seed germination and viability study

A lab study conducted during Jan- March 2018 to study the effect of biosynthesized zinc nanoparticle from medicinal weed *Tridax procumbens* on the germination potential of blackgram *(Vigna mungo).* The synthesis and characterization of nanoparticles is explained in section b and c. The seeds of blackgram were soaked in Zinc nanoparticles (ZnO) suspension at various concentrations (250, 0.750, 1.00, 1.25, 1.75, 2.0 and Untreated control). Milli Q water was used in the soaking process. A filter paper (Whatman No.42, Maidstone, England) was kept in each petridish (90 mm x 15 mm). 5 ml of nanoparticle suspensions at various concentrations was added for all the treatments exeept the untreated control in the petriplate which contained 10 numbers of blackgram seeds. 5 ml of milli Q water was added to the untreated control.

Petriplates were covered by parafilm and placed in an incubator. Experiments were carried out in quadruplate and repeated twice and mean values were recorded.The seedling length was recorded and vigour index was computed (Abdul Baki and Anderson, 1973).

The data on various characters collected from the experiemnt were statistically analysed under CRD plot design as suggested by Gomez and Gomez (1984). Wherever the treatment differences were found significant ('F' test), the critical differences were worked out at five per cent (0.05) probability level and the treatment differences that were not significant was denoted as NS in the respective tables.

Results

Seed germination, seedling length and seedling vigour of treated blackgram seeds was significantly influenced by biogenic zinc nanoparticles (Table 1). Higher germination (100%) and vigour index (738.0) was observed with zinc nanoparticles 1.0 g L^{-1} concentration. Germination is normally known as a physiological process beginning with water imbibition by seeds and culminating in the emergence of rootlet. ZnO NP's is a metallic co-factor of an enzyme tryptophan that influences IAA synthesis and results in a positive response in seed germination. Earlier studies showed that ZnO NP's at 1000 ppm concentration can promote seed germination, seedling vigor in peanuts (Pandey *et al*., 2010).

Table 1. Effect of biogenic zinc nanoparticles (ppm) on seed germination and vigour index of blackgram

ZnO nanoparticle concentration (g L^{-1})	Germination (%)	Seedling length (cm)	Vigour index
T_1 : 0.250	96.7	7.1	683.7
T_2 : 0.750	96.7	7.3	702.7
T_3 : 1.000	100.0	7.4	738.0
T_4 : 1.250	100.0	6.9	689.0
T_5 : 1.750	100.0	6.6	658.8
T_6 : 2.000	96.7	6.2	601.7
T_7 : Untreated control	86.7	5.9	512.6
LSD (P=0.05)	**7.88**	**0.80**	**90.34**

Fig.1 Root length (cm) of blackgram as influenced by various zinc nanoparticle concentration.

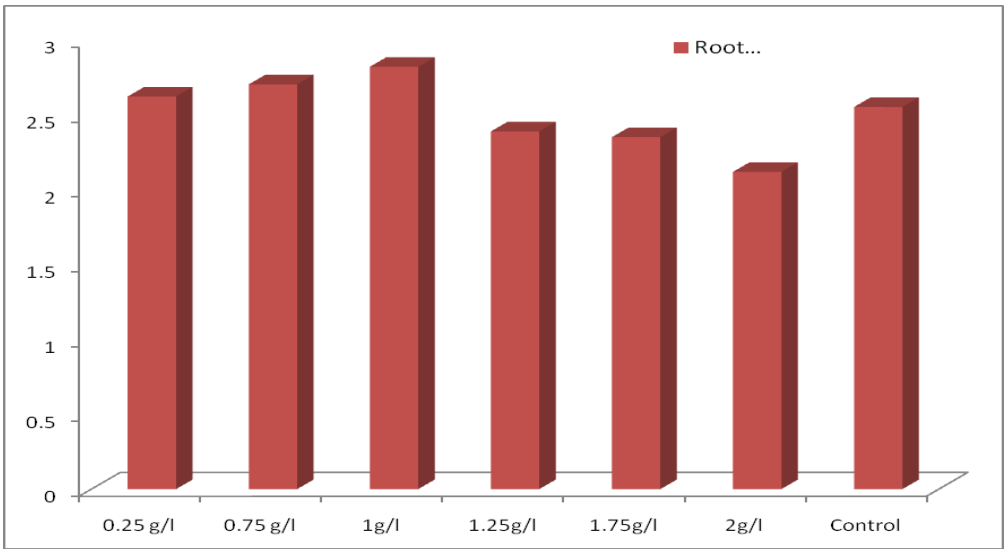
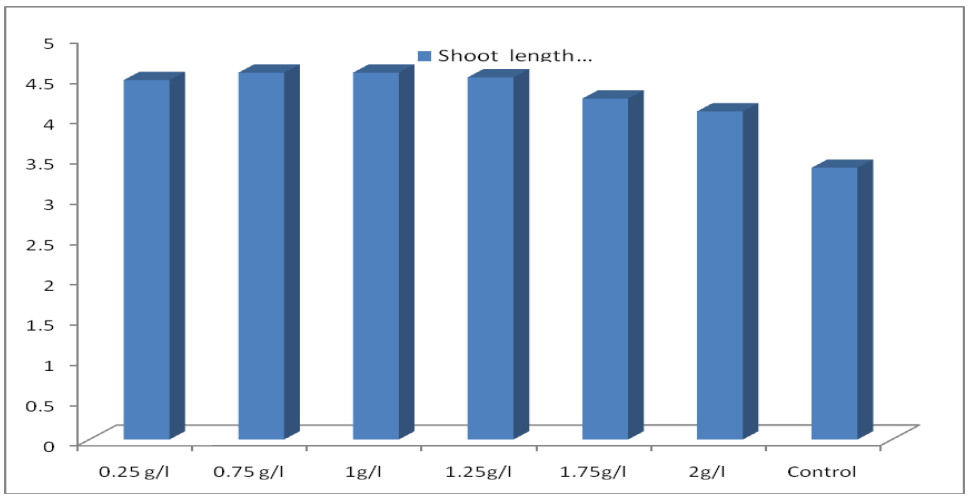


Fig.2 Shoot length (cm) of blackgram as influenced by various zinc nanoparticle concentration.



Significant differences in root length and shoot length was observed when treated with ZnO NP compared to control. Zn is a metallic cofactor of an enzyme Tryptophan monooxygenase, which is a rate limiting enzyme in auxin biosynthesis which is an important plant hormone in growth of seedling. However, improved root germination was observed with lower concentration of zinc rather than the higher concentration (Fig. 1 & 2). More pronouncing effect in reduction was noticed with 2.0g L^{-1} concentration. It is because of the toxicity effect of ZnO at higher concentrations. Roots are in direct contact with nanoparticles and accumulation in the root tissue or on the root surface is cause for shorter root length (Zafar *et al*., 2016).

Conclusion

It is concluded that ZnO NPs can be synthesised through biological method is more economical, efficient and environmentally safe. Zinc nanoparticles synthesised using *Tridax procumbens* extract-treated at the rate of 1.0 g L^{-1} had improved the germination and vigour index of urdbean or blackgram.

Acknowledgement

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FOOD ENTERPRENUERS FOCUS ON NUTRIMIX TO COMBAT ANEMIA

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Abstract

The average Hb was 9.8 g/dl among the girls showed that the average category of the girls comes under moderately anemic category among a selected adolescent group. The nutrimix formulated for improving haemoglobin had good significant impact on girls in the nutrimix which also had significant impact on the group. Thus it can be concluded that, there is a high significant increase in the Haemoglobin content with 90 days feeding trial among 56 girls in selective sampling method. Thus the formulated instant iron supplement rice powder will serve as a source of strength to the future reproductive group

Introduction

Anaemia, defined as a reduction in haemoglobin concentration, red-cell count, or packed-cell volume below established cut-off levels, is a widely discussed public health challenge that India is facing. The World Health Organization (WHO) defines adolescents as young people aged 10-19 years. Health of today's youth is hope for tomorrow's world, adolescent nutrition is therefore important for supporting the physical growth of the body and for preventing future health problems. During this time, physical changes affect the body's nutritional needs, while changes in one's lifestyle may affect eating habits and food choices. India is one of the countries with very high prevalence of anaemia in the world. Almost 58 per cent of pregnant women in India are anaemic and it is estimated that anaemia is the underlying cause for 20–40 per cent of maternal deaths in India. India contributes to about 80 per cent of the maternal deaths due to anaemia in South Asia.

Nutritional anaemia is a major public health problem in India and is primarily due to iron deficiency. The National Family Health Survey-3 (NFHS-3) data suggests that anaemia is widely prevalent among all age groups, and is particularly high among the most vulnerable – nearly 58 per cent among pregnant women, 50 per cent among non-pregnant non-lactating women, 56 per cent among adolescent girls (15–19 years), 30 per cent among adolescent boys and around 80 per cent among children under 3 years of age. The prevalence of anaemia among girls (Hb <12 g%) and boys (Hb <13 g%) is alarmingly high as per the reports of NFHS-3 and the National Nutrition Monitoring Bureau Survey (NNMBS). Percentage prevalence of anaemia among adolescent girls in the age group 15–19 years and in the older age group 20–29 years remains almost stagnant at 55.8 per cent and 56.1 per cent respectively. On the other hand, among adolescent boys, prevalence of anaemia for the age group 15–19 years is higher (30.2%) than the post-adolescence stage (19.3 per cent for the age group 20–29 years). According to world health organization (WHO) the haemoglobin level should be 12 g/dl for adolescent girls. Nutrition is a nucleus of human development and major source for meeting the nutritional requirements is healthy food. Adolescence would be the best investment for future. About one-quarter of India's population is adolescent group and 17.2 % of adolescents are in Tamil Nadu and their numbers are increasing rapidly. The prevalence of anemia among adolescents is 27% in developing countries every year. There is no nutritional awareness on the gregarious ill effects of anaemia among the adolescence. It has become an emerging need of the hour to eradicate anemia from the state and country. Prevention and management of IDA demands adequate iron intake and provision of bioavailable iron.

Entrepreneurship serves as a catalyst of economic development of the country. It is one of the largest sections for capital accumulation. The food processing industry is all set to drive Indian economy to higher growth, only need is to pay due attention on technological development of field, and generation of skilled manpower. The development of infrastructure facilities like cold chain, road facilities, and power will strengthen the food processing industry. It will have a very positive sign on perishable food products industry, such as fruit and vegetable, dairy industry, meat and poultry segment. Thus, this paper can help entrepreneurs to tackle all the nutritional challenges and become successful entrepreneur in food processing sector especially focusing on women health.

Key words: Anaemia, Haemoglobin content, girls

Methodology

Analysing Haemoglobin content of the girls

Prevalence of anaemia was found out by evaluating the haemoglobin content of the selected target group. The range values framed for anemia status in India given by Adolescent Division Ministry of Health and Family Welfare Government of India, 2015 has been taken as reference values. National Institute of Health and Family Welfare (NIHFW, 2015)

Standardization of iron rich nutrimix

A nutrimix rich in iron, folic acid and Vitamin C was standardised as six different trials and formulated with curry leaves, chekurmanis, agathi leaves and amla powder in the base of instant rice powder. Prevalence of anaemia was found

out by evaluating the haemoglobin content of the selected target group with the concern of selected girls and their parents. A Sample size of 56 (No.) were taken from adolescent girls in two batches. Feeding trial was given for a period of 90 days to the adolescents.

Food processing trainings for entrepreneurs

Entrepreneurs are being trained in a way to produce micronutrients rich food products to combat anemia at ICAR - Krishi Vigyan Kendra,Tiruppur. Therefore community will be benefitted by getting iron rich foods and entrepreneurs will be benefitted to supply the market demand high iron rich foods. Training programs like on campus, off campus, sponsored and paid trainings were conducted for the famers and entrepreneurs. Many enterprenuers were trained by demonstration through Frontline demonstrations (FLDs), On farm trials (OFTs) and Enterprenuership Development Program (EDP). Thus this approach is need of the hour for food entrepreneurship.

Results and discussion

Prevalence of anemia

The above table depicts the overall anemic status of the girl girls. The values looks alarmingly high but these values shall be taken as representative values of the adolescent girls.

Haemoglobin content of the girls

Hb Level	Anemic status	2018 N=41	%	2017 N=44	%	2016 N=38	%	2015 N=24	%	2014 N=32	%	Total N=179	%
≤8 g/dl	Severely anemic	0	0	0	0	0	0	3	12	2	6	**5**	**3**
8-10.9 g/dl	Moderately anemic	5	12	16	36	25	66	15	63	20	63	**81**	**45**
11-11.9 g/dl	Mildly anemic	36	88	28	64	13	34	6	25	10	31	**93**	**52**
≥ 12 Hg/dl	Normal range with no anemia	0	0	0	0	0	0	0	0	0	0	**0**	**0**
Total		41	100	44	100	38	100	24	100	32	100	**179**	**100**

Fig. 1. Combating anemia – the major nutrition problem screened among girls

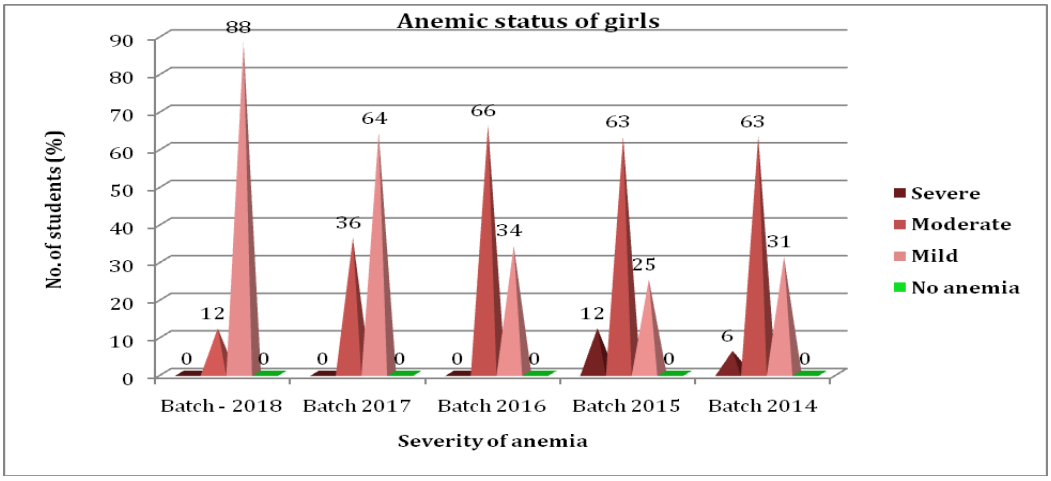
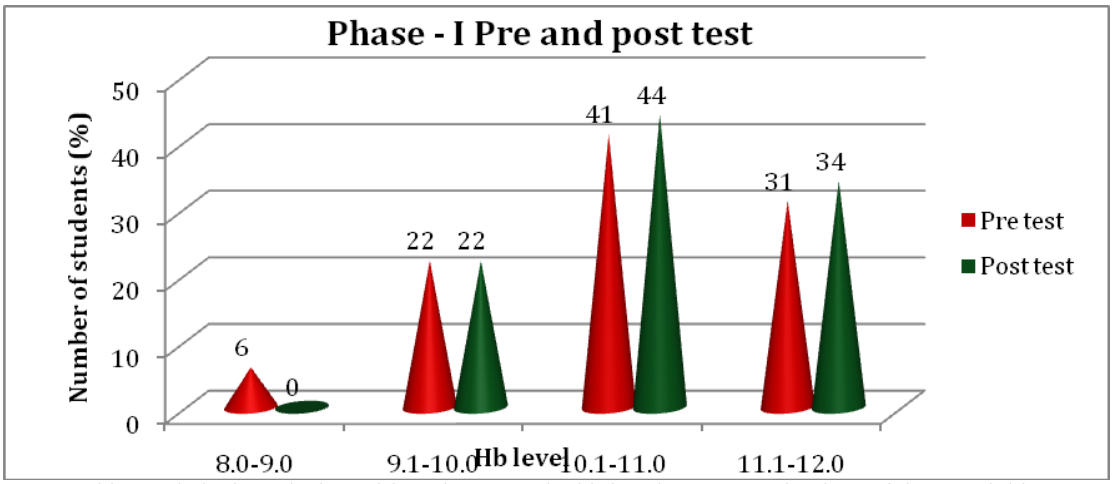


Fig.2. Comparison of pre and post evaluation

HB (gm/dl)	PRE EVALUATION N=32		POST EVALUATION N=32	
	No.	%	No.	%
8.0-9.0	2	6	---	---
9.1-10.0	7	22	7	22
10.1-11.0	13	41	14	44
11.1-12.0	10	31	11	34



From this statistical analysis, arithmetic mean is higher in post evaluation of haemoglobin test than pre-evaluation. So, it is statiscally proved that nutrimix powder formulated was very significant in the impact on the girls to improve haemoglobin level within a span of 90 days. Thus the study was continued to the next batch of adolescents.

Phase II Intervention

The study was continued with the second batch of girls to refine the nutrimix powder and also to let know the impact of the nutrient rich foods.

Standardisation of the mixture in Phase II

The mixture was standardised with the following ingredients in three trials by refining the first framed nutrimix and got finalised after consultation with the Head of the Institution and staff of Institution.

S.No	Materials	T1	T2	T3
1	Red Gram Dhal	20	40	40
2	Bengal Gram Dhal	30	35	20
3	Raw Rice	5	5	10
4	Moringa Leaves	5	10	10
5	Curry Leaves	20	5	10
6	Amla powder	20	5	10

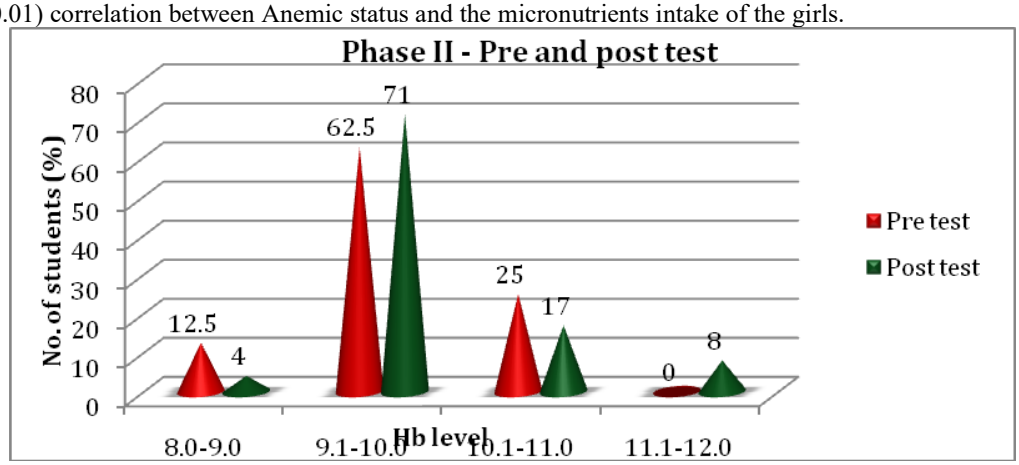
T3 was selected by the sensory evaluators and got finalised.

Feeding trial

Feeding trial was given for the period of 90 days to the sample size 24 adolescents (II batch). The nutrimix anaemic mix was given as 40g per day which supplements sufficient quantity of nutrients. After the study period of 90 days, the haemoglobin content of target group of 24 girls was analysed. The results are furnished below.

HB (gm/dl)	Pre evaluation n=24		Post evaluation n=24	
	No.	%	No.	%
8.0-9.0	3	12.5	1	4
9.1-10.0	15	62.5	17	71
10.1-11.0	6	25	4	17
11.1-12.0	0	0	2	8

Thus, the study had good significant impact on first batch of 32 girls (I batch) and thus the study was continued for the next batch of 24 girls (II batch) which also had significant impact on girls. From the table it can be observed that there is a high significant (0.01) correlation between Anemic status and the micronutrients intake of the girls.



Discussion

Prevalence of anemia

A total of 179 subjects were considered in this study and the results were drawn. It can be observed that three per cent of the girls were severely anemic and 45 per cent of them were moderately anemic. Majority of 52 per cent were mildly anemic. But none of the girls fall in the category of healthy range in anemic status. The prevalence of anaemia among girls (Hb <12 g%) and boys (Hb <13 g%) is alarmingly high as per the reports of NFHS-3 and the National Nutrition Monitoring Bureau Survey. It is reported that over 55 per cent of adolescent girls are anaemic. Percentage prevalence of anaemia among adolescent girls in the age group 15–19 years and in the post adolesent group 20–29 years remains almost stagnant at 55.8 per cent and 56.1 per cent respectively. India is one of the countries with very high prevalence of anaemia in the world. Almost 58 per cent of pregnant women in India are anaemic and it is estimated that anaemia is the underlying cause for 20–40 per cent of maternal deaths in India. India contributes to about 80 per cent of the maternal deaths due to anaemia in South Asia. (NNMBS,NIN,2006).

Entrepreneurs take up iron rich food products

Development of healthy nutrimix to combat anemia among the community were given through fifty two different training programs to the farmers and entrepreneurs by the Food Scientist at KVK,Tiruppur. Two successful entrepreneurs of KVK,Tiruppur have been developed to produce and market healthy nutrimix after obtaing FSSAI License. This iron rich nutrimix is designed and marketed especially for adolescent groups. Mrs.T.Loganayagi is an organic farmer holding 4 acres of land at Paranjervazhi, Kangeyam of Tiruppur district. She was struggling to market her organic produces due to the distance from her village to the city. She could not sell her agricultural and horticultural produces in the market in the name of 'Organic Products' though she is practicing organic agriculture and has its certification. She visited KVK, Tiruppur and got motivated on the Food Processing and Value addition technologies to enhance her income. Subsequently she had participated in various programmes related to Food Science oriented trainings of KVK and she is one of the FLD beneficiaries of 'Demonstration of value added products from Moringa leaves to SHG women'. Mrs. T. Loganayagi has started a food incubation unit named M/s Shree Murugai food products. Obtained FSSAI license and started up business as an Entrepreneur in food processing. She has started to produce 24 different food products from moringa and herbal dehydrated powders. She is selling dehydrated Moringa powder and Moringa incorporated food products.

Conclusion

The results revealed that the average Hb was 9.8 g/dl among the girls showed that the average category of the girls comes under moderately anemic category among a selected adolescent group. The nutrimix formulated for improving haemoglobin had good significant impact on girls in the nutrimix which also had significant impact on the group. Thus it can be concluded that, there is a high significant increase in the Haemoglobin content with 90 days feeding trial among 56 girls in selective sampling method. Thus the formulated instant iron supplement rice powder will serve as a source of

strength to the future reproductive group. This study helped to continue with large population particularly adolescents and further entrepreneurs are insisted to develop such healthy nutrimix to combat anemia among the community

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GREEN SYNTHESIS OF PALLADIUM NANOPARTICLES USING VARIOUS PLANT EXTRACTS

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Abstract

Green synthesized metallic nanoparticles are an evolving environment-friendly technique in recent years. Biological processing of palladium (Pd) nanoparticles by employing *Allium fistulosum* and*Tabernaemontana divaricate* leaf extracts were described in our current study. The prepared PdNPs were further undergoing characterization via Fourier transform infrared, Scanning and Transmission electron microscopy. Furthermore, validation of PdNPs creation was established through UV–visible spectrophotometer. Our SEM analysis results represented spherical morphology with a dimension of 2 µmfor both the extracts of *Allium fistulosum* and *Tabernaemontana divaricate* synthesized Pd NPs. TEM images of both the extracts designate synthesized PdNPs were moderately unvarying in diameter as well as its figure as a range of 2 to 5 nm. Eventually, anti-bacterial activity was also determined for both the extracts. Amongst, Pd NPs synthesised with *Allium fistulosum* demonstrated a good zone of inhibition against most of the bacterial strains. The findings are highly positive, demonstrating a significant increase in the activity of the undamaged fractions. The use of biological sources to synthesise NPs adds a new dimension to all application areas.

Keywords: *Allium fistulosum, Tabernaemontana divaricate*, Green synthesis, Antimicrobial activityand Palladium nanoparticles

Introduction

Recently, nanomaterial creation, mostly with the appropriate quality have increased attention in nanoscience and technology. Due to their intriguing physical, chemical and thermodynamic features, metal methods have attracted substantially increased interest, making them ideal options for uses in many domains like catalysis (Ha *et al.,* 2016; Bortolotto*et al.,* 2015), optical electronics (Cui and Lieber, 2001; Eryürek*et al.,* 2015) besides its biomedical applications (Anand *et al.,* 2016). Owing to their fruitful applications in the realm of bioscience, biomedicine and pharmacy, palladium nanoparticles, also known as PdNPs catalysts, has attracted considerable interest (Narayanan and El-Sayed, 2005). Progress in Pd NPs production has achieved tremendous importance due to its usage in both homogenous and heterogeneous catalysis, owing to its higher surface-to-volume ratio and enormous exterior strength. Normal formulated PdNPs delivery methods comprise electrical and chemical (Kumar*et al.,* 2013; Rajkumar *et al.,* 2014) and laser pulse ablation (Kim *et al.,* 2014) as well as sonochemical decline techniques (Nemamcha*et al.,* 2006). Because the synthetic chemical techniques of producing Pd NPs create a punitive action and decrease the catalyst performance of Pd, novel synthetic procedures need to evolve to meet a wide range of potential uses for the development of PdNPs with regulated size thickness. Perception of these literary works, biosynthetic strategy using plant resources has elevated in recent times a clear and acceptable alternative to the physicochemical method implemented so far. Throughout this analysis, PdNPs by employing floral extract of numerous plants, including Allium *fistulosm (*Bouqellah*et al.,* 2019) and *Tabernaemontana divaricate* (Purushothaman*et al.,* 2016), have been described in current time reports. Hitherto prospective of floral resources to be examined. *Tabernaemontana divaricate* and *Alliumfistulosm* are flowering plants belonging to Apocynaceae and Alliaceae's families, respectively. The extracts of these plants exhibited antibacterialcharacteristics (Kumar *et al.,* 2011).

This paper looks at the biogenic synthesis of PdNPs utilizing various leaf extracts, namely, *Allium fistulosm,* and *Tabernaemontana divaricate*. We have also characterized the processed PdNPs via UV-vis, FTIR, SEM and TEMspectroscopy. The antimicrobial activity of the Pd NPs was also examined.

Materials And Methods

Preparation of plant extract

Two plants namely, *Tabernaemontana divaricate* and *Alliumfistulosm* were collected from in and around the Perambalur district. The collected leaves were washed thoroughly to remove dust particles, fungal spores and shade dried to remove moisture. About 10g of eachleaf were transferred into a 250ml beaker containing 200ml distilled water and boiled for 45 minutes on the heating mantle. The extract obtained was cooled to room temperature and filtered.

*Synthesis of Pd*NPs

Palladium acetate solution was prepared and used for the synthesis ofPdNPs. Aqueous extract of*Allium fistulosum*and*Tabernaemontanadivaricata* were added separately into test tubes containing 2mM aqueousPalladium acetate solution in different proportions. The change in colour of the solution indicated the formation of Pd NPs. The change of colour from mild brown to dark brown when the *Tabernaemontanadivaricata l*eaf extract and *Allium fistulosum* were added

dropwise to palladium acetate indicated the formation of Pd NPs. The colour change in an aqueous solution is due to the excitation of surface Plasmon vibrations.

Characterisation of Pd NPs

UV- Vis spectral analysis

The reduction of Pd ion to metallic Pd NPs was spectroscopically identified by a double beam UV-Visible spectrophotometer at a different wavelength ranging between 200-1000nm. The optimum conditions of processing Pd NPs from the extracts were observed to be two mM palladium acetate at a temperature of 60°C after carrying out the experiments at different palladium acetate concentrations. The intensity of UV-Vis was gradually enhanced to constant as a function of the reaction time. The colour changes from light to dark brown and mild brown to green due to the excitation of surface plasmon resonance in Pd NPs. These optic-based characteristics of PdNPs were examined utilizing a UV-Visible absorption spectrophotometer by employing a Varian Cary-50.

Fourier transform infrared spectroscopy (FTIR)

A functional group of prepared PdNPs was determined using FTIR (Varian 800), and it was compared with an aqueous extract of *Allium fistulosum*and*Tabernaemontana divaricate*. The synthesized samples were added to potassium bromide (KBr) in the proportion of 1:99 to attain the pellet and it was scanned in between 400 to 4000 cm^{-1} within a resolution of 2 cm^{-1}.

Scanning electron microscopy (SEM) and Transmission electron microscopy (TEM)

The PdNPs were categorized using Scanning electron microscopy methods. The crystal-like nature and its dimension were further validated by employing SEM configuration for PdNPs exhibited indications for palladium metal material. Moreover, the shape, dimension and structure of PdNPs are evaluated by Transmission Electron Microscope. A one droplet suspension of PdNPs was incorporated into the carbon covered copper grid. An imageJ platform is utilized to identify the dimension and shape of PdNPs from the obtained TEM pictures.

Antimicrobial study

Well diffusion technique was employed to evaluate the antimicrobial action of *Allium fistulosum*and*Tabernaemontana divaricate* derived Pd NPs (Holtand Bard, 2005). The microbial strains such as *Bacillus cereus, Bacillus subtilis, E. coli* and *Enterobacter cloaca* were utilized as test organisms. The 24 hours grown strains were interchanged to the Petri plates on an exterior of a germ-free nutrient agar medium. A total of three wells in a diameter of 5 mm was completed in every plate and 50 µl of Pd NPs (1 mg/mL), distilled H_2O and antibiotic disc (Gentamicin sulphate) was incorporated as a control. Further, these plates are protected at room T for 24 hours, and the zone of inhibition was examined for the individual trial of bacteria (Sharmila*et al.,* 2016).

Results And Discussion

UV-visible spectral results provide 1° information regarding the creation of NPs. The extracts, *Allium fistulosum and Tabernaemontana divaricate* combined with the Pd NPs were perused below UV-visible spectroscopy. The reduction of Pd ion to metallic Pd NPs was spectrometrically identifiedby a double beam UV-Visible spectrophotometer at different wavelengths ranging between 200-1000nm. Two plant extracts, namely, *Tabernaemontanadivaricata*and *Allium fistulosum*combined with the Pd NPs, were subjected to UV-visible spectroscopy (Figure 1 and 2). Pd NPs shows a maximum absorption peak of 215 and 277 nm, respectively. The absorption peak intensity of *Allium fistulosum* was 225nm and 223nm respectively. In contrast, the peak intensity of their synthesized PdNPs was found as 275 nm respectively. The colour change occurred owing to its surface plasmon resonance, which was an inherent feature of metal-based NPs. There was a noteworthy improvement in peak in the PdNPs in comparison with crude plant extracts. Overall, the UV-spectral analysis of the PdNPs describes an increase in the absorption peak representing the more significant formation of Pd NPs.

FTIR analysis was performed to examine both the NPs and floral extracts exhibited absorption peaks: while PdNPs were allocated as symmetry and anti-symmetry frequency of broadening. The results for all the two extracts of Pd NPs were explained in Figure 3. Figure 3(a), a spectrum of *Tabernaemontana divaricate* synthesized particle showed a total of 3 peaks and a more influential peak at 1070 per cm, where a lesser height at 1554 and 1411 per cm, respectively. Figure 3(b), a spectrum of *Allium fistulosum* synthesized particle showed a total of 3 peaks and a more influential peak at 1121 per cm, where a lesser height at 1457 and 1599 per cm, respectively.

SEM pictures of palladium nanoparticles synthesized using *Allium fistulosum*, and *Tabernaemontana divaricate*. Our analysis results represented spherical morphology with a dimension of 500 nm and 2 µm, respectively, for the extracts of *Allium fistulosum*, and *Tabernaemontana divaricate* synthesized Pd NPs. The palladium nanoparticle, which is synthesized from the extract of *Allium fistulosum* was found to be better when compared to the other two extracts of processes nanoparticles. TEM images of both the extracts designate synthesized PdNPs were moderately unvarying in diameter as well as its figure. Pd NPs were exhibited as a range of 2 to 5 nm. TEM studies confirmed that the *Tabernaemontanadivaricata* extract was suitable and useful for forming highly stabilized Pd NPs. The particle size, morphology, and crystallinity were studied using TEM respectively (Figure 4).

The Pd NPs exhibited stronger bactericidal activity towards *Escherichia coli*, *Bacillus subtilis* and *Bacillus cereus* might be because of the point that PdNPs can simply enter within thinner outer layer speedily to suppress the metabolic action in comparison with the thicker sheath of the gram-positive microbes whereas it is not showing any activity against *Enterobacter cloaca*. Also, the various leaf extracts phytochemicals that exist as capping material in the PdNPs exterior might affect the antimicrobial activity towards gram-negative microbes (Figure 5).

Conclusion

Plant extracts such as *Allium fistulosum*and*Tabernaemontanadivaricate*have been successfully used to synthesise Pd NPs in a lesser price, eco-friendly approach. UV, FTIR, TEM and SEM were used to characterise the synthesised Pd NPs. Biosynthesised Pd NPs are nontoxic and have antibacterialproperties. Pd nanoparticles synthesised with Allium fistulosum (V-Ag) demonstrated a good zone of inhibition against both bacterial when compared to *Tabernaemontanadivaricate* sources of synthesis. The use of biological sources to synthesise nanoparticles adds a new dimension to all application areas.

Acknowledgement

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Fig.1. UV-visible spectral study of (a) Plant extract of *Tabernaemontanadivaricata*(b) PdNPs with *Tabernaemontanadivaricata.*

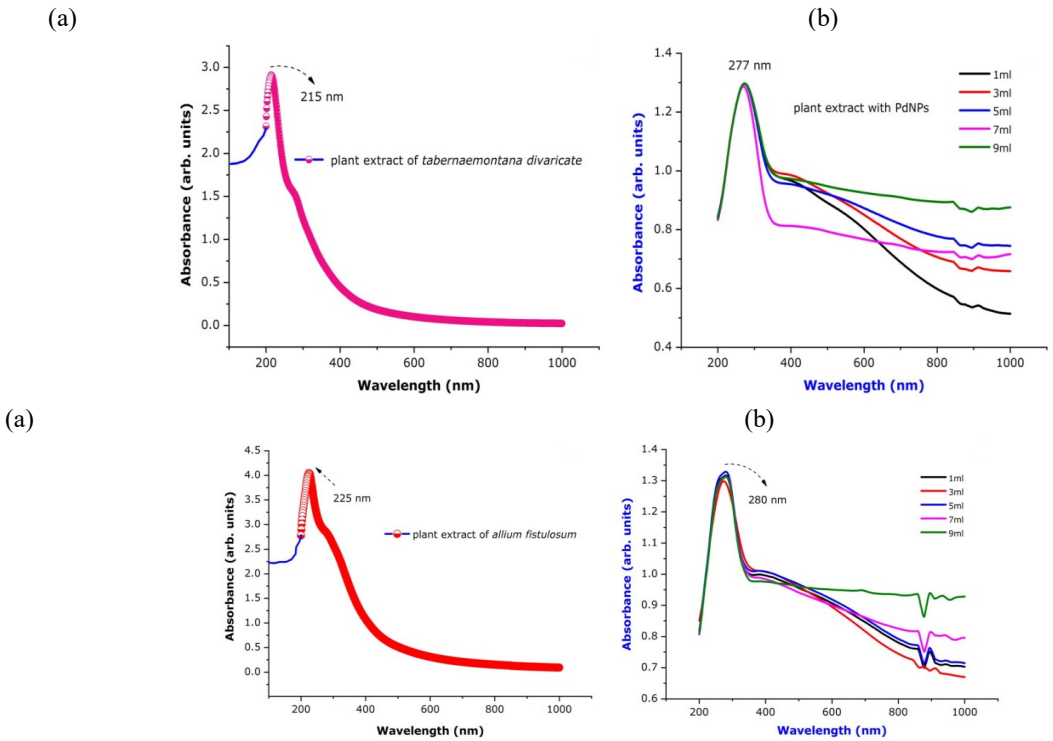
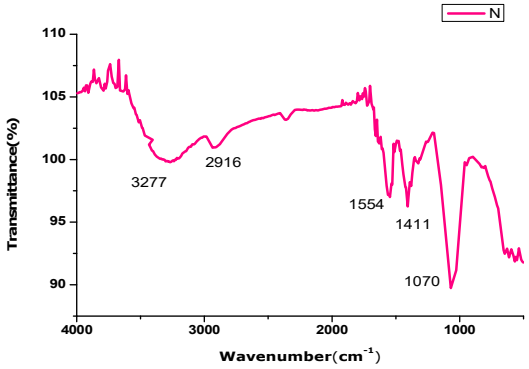


Fig. 2. UV-visible spectral study of (a) Plant extract of *Allium fistulosum*(b)PdNPs with *Allium fistulosum*

(a)



(b)

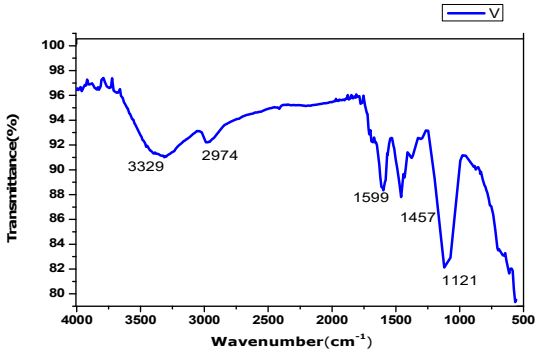


Fig. 3. FTIR study of (a) Pd NPs with *Tabernaemontana divaricate* (b) PdNPs with *Allium fistulosum*

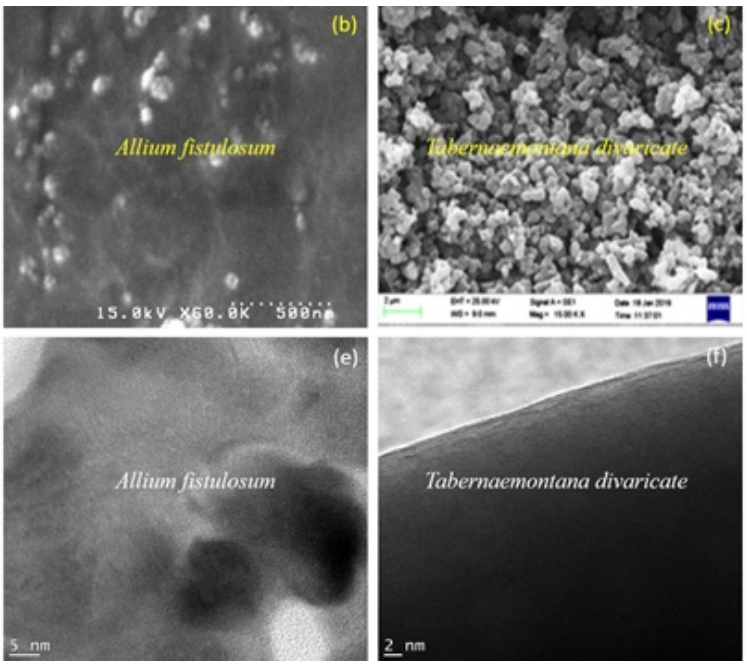


Figure 4. SEM and TEM image of synthesized Pd NPs

Fig. 5. Zone of inhibition of various bacteria (a) *Bacillus subtilis* (b) *Escherichia coli* and (c) *Bacillus cereus*

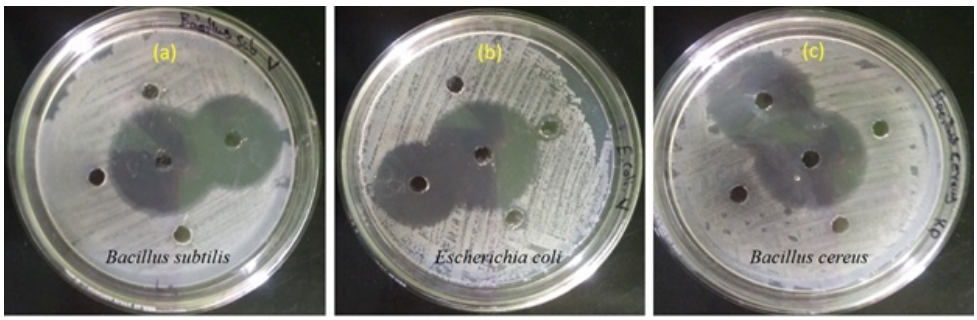


Table 1. Antimicrobial activity plates of various microbes

Microorganism	**Control – Gentamicin Sulphate**	***Allium fistulosum* (V)**	***Tabernaemontanadivaricate*(N)**
		Zone of inhibition in mm	
Escherichia coli	21 mm	16 mm	13 mm
Bacillus subtilis	25 mm	17 mm	18 mm
Bacillus cereus	26 mm	18 mm	15 mm
Enterobacter cloaca	-	-	-

ESTABLISHMENT OF A SIMPLE METHOD FOR THE SYNTHESIS OF A BIODEGRADABLE POLYMER FROM *Saccharum officinarum* L. and *Labeo rohita*H. scales

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Abstract

Globally, the plastic wastes have been a great concern of environmental pollution. Biodegradable biopolymers, the green alternatives to traditional synthetic polymers are gaining immense attention the recent years. Moreover, synthesizing valuable biopolymers at low cost is appreciable. The prime focus is to deliberately study the efficient methods of producing commercial biopolymers from biological resources, probably the wastes. Our study involves production of a biopolymer from sugarcane bagasse (SCB), *Prosopis juliflora*bark and *Labeo rohita* (rohu)scales. Structural differences between SCB cellulose, *P. juliflora* cellulose and rohu chitosan were studied using Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM). Characteristics of the polymer were found to be dependent on the source of origin and the degree of polymerization.

1. Introduction

Synthetic polymers are a great concern of environmental deterioration. Generally, they are made up of long chains of high-molecular weight monomers that provides flexibility, toughness and resistance to environmental degradation (Brigham 2018). Although there are several control measures employed like incineration, reusing, recycling etc., there are also concerns of pollution and economic loss which has to be addressed (Sohn et al. 2020). Synthetic polymers have innumerable applications in the area of electronics, medicine, packaging etc., (Feldman and Barbalata 1996). Biologically active polymers can be synthesized separately or in combination with natural polymers thus, finding their applications as biomaterials for tissue engineering (Sionkowska 2011).

Cellulose is recognized as a renewable and eco-friendly biodegradable homopolymer (Taylor 2008). It is made up of D-anhydro glucopyranose units connected by β-1,4-glycosidic linkages (Qiu and Hu 2013). Cellulose derived polymers have been long used for biopolymer production. There are three main strategies involved namely, cellulose deconstruction monomers-based, natural cellulose fibers-based and nanocellulose-based biopolymers (Shaghaleh et al. 2018). Apart from biodegradability, the cellulose biopolymers possess low density, high specific surface area and good mechanical properties. They also exhibit different structural forms such as nanoparticles, gels, copolymers and membranes to be largely applied as drug delivery systems, electronics, sensors, hydrogels, smart membranes and shape memory materials, etc. (Qiu and Hu 2013).

Chitosan, the deacetylated form of chitin isfound largely among lower animals like the crustaceans (Kurita et al. 1993). It is a pseudo cationic natural polymer with excellent physiochemical properties, biodegradability, biocompatibility, and antibacterial activity (Kurita 2001). Generally, the extraction protocol involves crushing up of the raw materials and obtaining chitin, which is followed by alkali deacetylation to yield chitosan (Abdou et al. 2008). Chitosan biopolymers has potential applications in the areas of medicine, hydrogels, food packaging, water treatment, adhesives, biosensors and membranes. They are also used as coating materials due to their proper binding and film-forming properties (Honarkar and Barikani 2009).

Both cellulose and chitosan can be easily obtained from various natural wastes. Sugarcane bagasse (SCB) and fish scales are some of them. SCB is the heterogenous fibrous by-product of the sugar industry and a rich source of natural cellulose (Jiménez et al. 2016). SCB contains 40-50% cellulose, 25-35% hemicellulose and remaining is lignin (Plermjai et al. 2018). SCB is generally used as source of biofuel, feedstock and as raw materials for construction and paper industry (Motaung and Linganiso 2018). It can be used as fillers as they have varying reinforcement properties (Jiménez et al. 2016). Thus, several million Tonnes of SCB produced as waste can be effectively used for the production of cellulose polymers.

Prosopsisjuliflora Sw. is considered as an environmental weed as it absorbs underground water to a greater extent. It suppresses vegetation by inhibiting the growth of other species of plants near them (Patnaik et al. 2017). It grows intrusively on the semi-arid land and becomes a serious threat as it tends to colonize on agricultural lands (Ilukor et al. 2016). Unlike other plants, *P.*

Fish scales are a rich source of chitin and chitosan. They are dumped as wastes near water bodies. They are characteristically high in proteins and suspended organic materials, thereby affecting the water quality.

Materials Required

Collection of samples

Cellulose extraction from SCB and *P.juliflora*bark

Extraction of chitosan from Rohu fish scales

Production of biopolymer

A total of 3 different combinations comprising of varying quantities of cellulose from two sources, chitosan and natural plasticizerswere taken to study the effective polymerization. It is depicted in table 1.

Table 1 Different combinations performed to study effective polymerization.

Combination	Cellulose	Chitosan	Plasticizer
I	1.4g	-	1mL (acetic acid)
II	1g	-	25mL (sorbitol)
III	1g	-	6mL (glycerol)

Combination I:

1mL of acetic acid was added to 0.8g of the chitosan powder obtained and the contents were physically mixed. To that, 0.6g of cellulose was added and ground well. Finally, it was incubated at room temperature for about 3 hours.

Combination II:

1g of cellulose was gently mixed in 25mL of sorbitol using a magnetic stirrer for 15 minutes, followed by incubation at 80ºC for 7 minutes. It was then incubated overnight at room temperature.

Combination III:

1g of cellulose was ground with 6mL of glycerol. It was subjected to magnetic stirring for 10min and incubated under room temperature.

and placed in a magnetic stirrer for 15 minutes. Solution was then incubated under sunlight for about 3 hours.

Results and Discussion

Extraction of cellulose and chitosan:

Figure 1 shows the powdered extracts of cellulose and chitosan. Figure 1a, shows the cellulose obtained from the alkali and acid treatment of sugarcane bagasse. Alkali extraction method provided with cellulose releasing out all other soluble polysaccharides like the hemicellulose. Further bleaching with acid helped in delignification as discussed by Mzimela et al. 2018. Similarly, figure 1b, shows the cellulose extracts obtained from *P.juliflora* bark. Figure 1c, indicates the chitosan extracted from the wastes of *L.rohita*scales.

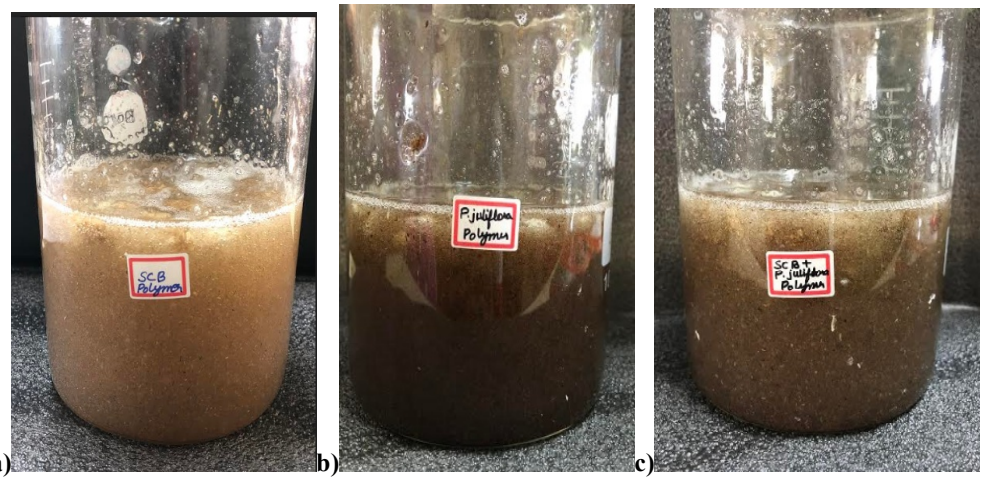
Figure 1: Powdered extracts of cellulose and chitosan polymers a) cellulose powder extracted from sugarcane bagasse b) Cellulose powder extracted from *P.juliflora* c) chitosan powder extracted from *L.rohita* scales

a) b)

Synthesis of biopolymer

The chitosan and cellulose extracts were subjected to physical mixing (Figure 2) with the plasticizers like sorbitol and acetic acid.

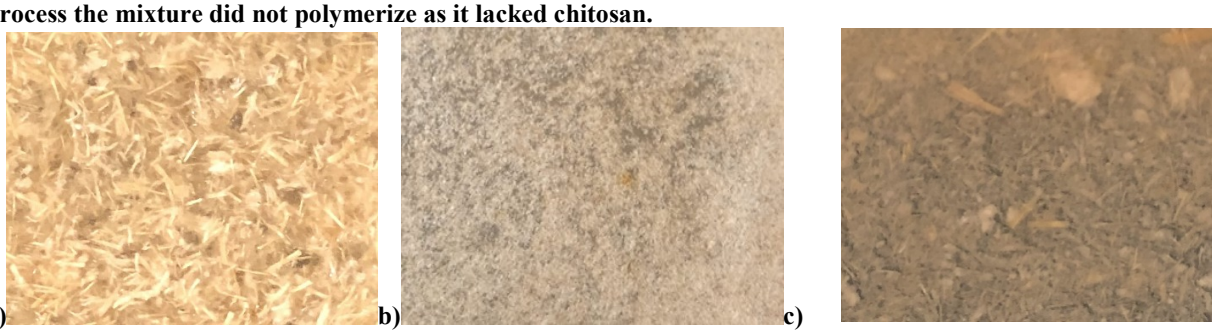
Figure 2: Physical mixing of cellulose and chitosan polymers with plasticizers a) sugarcane bagasse cellulose and rohu chitosan (b) *P.juliflora* bark cellulose and rohu chitosan c) combination of cellulose from sugarcane bagasse and *P.juliflora* and rohu chitosan



a) b) c)

Fig. 3, shows the six different combinations performed to standardize a simple method for the production of biopolymer.

Fig. 3a, indicates the unpolymerized product obtained from combination 1 which contains only cellulose and chitosan without the addition of any plasticizer. Thus, the final mixture was unable to form a biopolymer. Figure 3b, shows the product obtained from combination 2 that contains only the cellulose and sorbitol as the plasticizer. The final mixture failed may be due to the absence of chitosan in it. Figure 3c, shows the product obtained from combination 3 containing cellulose and glycerol as the plasticizer. Even after overnight incubation and drying process the mixture did not polymerize as it lacked chitosan.



a) b) c)

Fig. 3: Biopolymers obtained a) combination 1 b) combination 2 c) combination 3

Conclusion

Although, cellulose and chitosan are the most abundantly available natural polymers, obtaining a biopolymer from their combination involves intricated processes. In this method we were able to optimize a simple method for the production of biopolymer. Additional investigation is ongoing to determine the nature of its biodegradability, tensile strength and elasticity. General applications are highlighted in food packaging, natural bio-sealants for preventing concrete leaks and insulators for electrical devices. Thus, adaptation of the method is certain to bring improvements in the yield and quality of the product.

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SILVER AND SILVER-IRON NANOPARTICLES SYNTHESIZED BY PHOTOREDUCTION FOR APPLICATION IN CANCER THERAPY

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Abstract

Metal nanoparticles had been extensively studied for numerous functions inclusive of healing applications for most cancers. In this study, nanoparticles of silver and silver-iron with aminolevulinic acid (ALA) have been synthesized using (ALA:AgNPs and ALA:AgFeNPs) the photoreduction method with a three hundred W xenon lamp, characterised via way of means of UV/vis absorption, zeta potential, x-rays diffraction, FTIR and transmission electron microscopy. The sizes acquired have been ~ 23 nm for silver and ~ 12 nm for iron. Cytotoxicity assays have been completed on breast tumor cells (MCF-7) and prostate most cancers cells (LNCaP). The effects acquired confirmed that it become viable to synthesize silver and silver-iron nanoparticles via way of means of the photoreduction method, and to functionalize their surfaces with ALA, which become brought to the cells and transformed to protoporphyrin IX (PpIX).

Keywords: Silver Nanoparticle; Photoreduction; Cancer.

Introduction

Silver nanoparticles (AgNPs) entice widespread interest due to their applicability in numerous areas. The antimicrobial residences of AgNPs display efficiency towards greater than 650 pathogenic organisms, making these nanostructures relevant to merchandise withinside the medical-hospital area (tissues and implants), footwear and sneakers, meals storage containers, washing machines and air conditioners [2,3]. The outcomes confirmed the presence of AgNPs withinside the nucleus and withinside the mitochondria, which indicated a rupture of the mitochondrial respiration chain originating reactive oxygen species (ROS) and blockage of ATP synthesis inflicting DNA harm. Generation of ROS is likewise regarded to induce apoptosis/mobileular demise in numerous mobileular subculture models Silver nanoparticles have packages in most cancers treatment and are drug transporters which could supply healing agents. [7]. Aminolevulinic acid (ALA) is the primary metabolite in the heme biosynthesis pathway. Porphyrins are biosynthesized from aminolevulinic acid (ALA). Moan et. al [8] showed superior ALA-mediated protoporphyrin IX (PpIX) accumulation in tumor cells and powerful mobileular destruction after mild illumination.

Materials and Methods

A. Silver and silver-iron nanoparticles with 5

To put together ALA:AgNPs, forty five mg de AgNO3 had been mixed with 13.five mg de ALA and 30 mg of polyethylene glycol (PEG) in 30 mL of distilled water at 20oC. The manner turned into observed with the aid of using lively stirring for five mins, and 10 mL of the ensuing answer turned into uncovered to a three hundred W xenon lamp for 1 minute. After irradiation pH answer turned into adjusted to ~ 7.0.

To put together ALA:AgFeNPs, forty five mg of iron powderwere diluted in 30 mL of distilled water and the pH answer turned into adjusted to 12. After that, forty five mg of AgNO3, 13.five mg of ALA and 30 mg of PEG had been introduced in answer, homogenized for five mins after which uncovered to a three hundred W xenon lamp for 1 minute. After irradiation, the pH answer turned into adjusted to ~ 7.

B. Characterization

The UV-vis absorption spectra had been measured through a Shimatzu spectrophotometer, the usage of 1-cm quartz cells. The form and sizes of ALA:AgNPs and ALA:AgFeNPs had been acquired from transmission electron microscope (TEM) a Jeol (Zeiss, Germany). The powerful floor costs on the ALA:AgNPs and ALA:AgFeNPs had been measured the usage of zeta potential (Malvern Instruments Zetasizer, Worcestershire, UK). The structural identity of the ALA:AgFeNPs pattern changed into completed the usage of the X-ray diffraction analysis (XRD) size the usage of a Bruker D8 Advance 3kW diffractometer (Cu radiation tube, 250 mm goniometer, 40 kV, 30 mA) at Multiuser Center of the Nuclear Fuel (IPEN/CNEN-SP). The approach changed into completed for silveriron nanoparticles. The cloth changed into separated the usage of a magnet. The supernatant became then centrifuged for five minutes at 10.000 rpm, constantly setting apart the supernatant from the backside body, and centrifuging the supernatant again. The manner became repeated five times, and the contents acquired have been oven dried (60°C) for twenty-four hours. The spectra have been analyzed the usage of Qualx2 software program. Peaks have been as compared to the compounds databases of software program to make assumptions among acquired Crystal diffraction spectra as opposed to used the ones from known compounds. The complete width at 1/2 of most of peaks (FWHM) values became used to crystallite length calculations thru Scherrer's calculation (λ=0.154056, K=0.91).

C. Cell Culture

Two tumor traces had been evaluated: breast most cancers cells MCF-7 (ATCC® HTB22™) and human prostate most cancers cells LNCaP (ATCC®. CRL1740™). The cells had been maintained in RPMI 1640 medium supplemented with 10%fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37 °C with 5% CO2. The cells had been automatically cultivated each three days with 70-80% confluency and harvested the usage of 0.25% trypsin

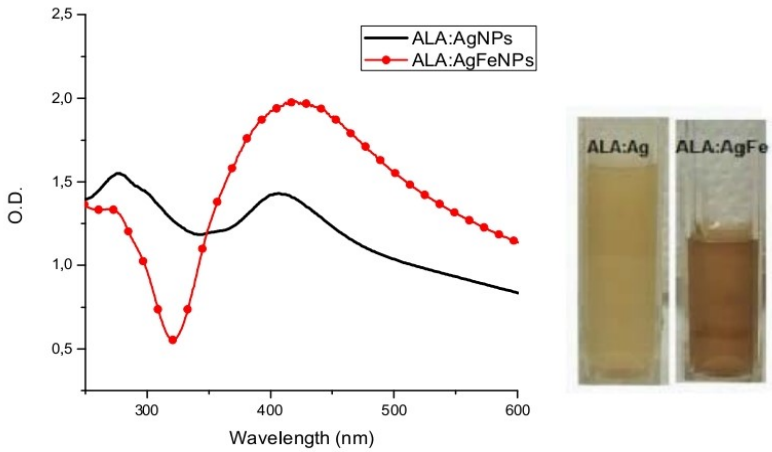
D. Cell Viability

Cells (8x103 cells/properly) have been plated on 96-properly flat backside plates. After 24 hours, the cells have been incubated with dilutions of the check compound (ALA:AgNPs and ALA:AgFeNPs) on the concentrations of 5, 10, 20, 30 and 40 mL diluted in RPMI medium to a very last quantity of 500 µL. The plates have been then incubated for twenty-four hours at 37 ° C and 5% CO2 atmosphere. Subsequently, the supernatant become removed, and the cells have been washed with PBS and the cells have been incubated for twenty-four hours with RPMI medium. Cell viability become evaluated through MTS (CellTiter 96® AQueous MTS Reagent) in formazan. The quantity of product is absorbable at 490 nm and is without delay proportional to the quantity of stay cells in culture. The effects have been statistically compared (ANOVA and Dunnett check) or negative (control cells, NaCl 0.9%) or positive (suspension of latex powder, 0.5 g/L)

Results and discussion

A. Silver and silver-iron nanoparticles characterization The absorbance spectra of the nanoparticles are proven in Fig. 1, wherein the feature band of silver nanoparticles (~ 420 nm) seems because of the SPR (floor plasmon resonance) effect, indicating the formation of silver nanoparticles. Not all metals have plasmon resonance, due to the fact it's miles essential for the presence of loose conduction electrons, and as a result the iron does now no longer gift distinguishable bands withinside the location of the located spectrum

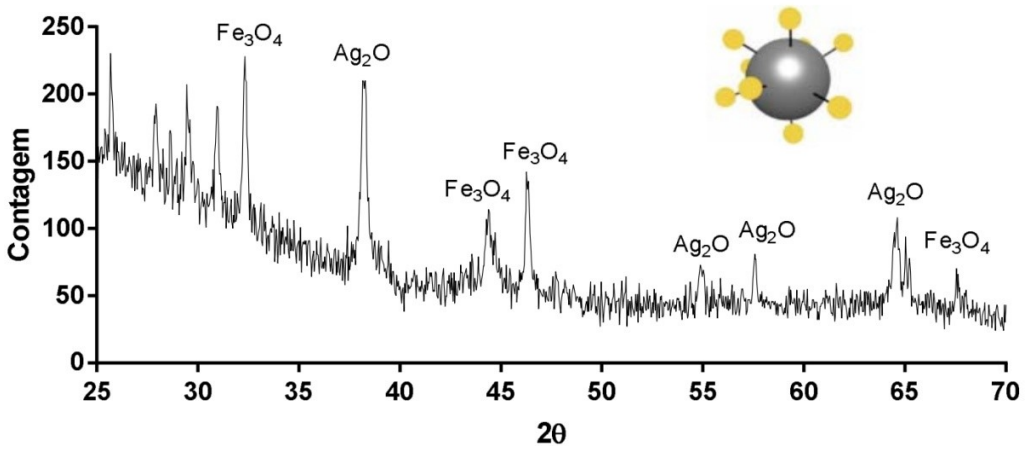
Fig.1. Absorption spectra of ALA:AgNPs and ALA:AgFeNPs



The silver and silver iron nanoparticles had been synthesized via way of means of the photoreduction technique with the usage of a Xenon lamp. White mild lights become critical for the formation of nanoparticles, heating and providing photons to the solution. The mild nevertheless interacts with ALA, which has a low pKa value (4.05), releases extra H+ ions. In this manner the mild irradiation acts as a catalyst for the steel reduction (oxidation / photoinduced reduction), supplying ions that culminate withinside the passage of the silver from the valence 1+ to zero, and consequently they had been exclusive for every material. The elevation of temperature promoted via way of means of the irradiation additionally performs an crucial function withinside the synthesis process [10].

The end result acquired with the X-ray (XRD) indicates the reflections regarding the interplanar distances characteristic of the crystalline levels of the magnetite and silver

Fig. 2. X-ray diffraction spectra of silver-iron particles (ALA:AgFeNPs - 2 mins of illumination - pH 7.0) and the powders acquired after centrifugation and drying of the sample (10.000 rpm for five mins and drying at 60 ° C for twenty-four hours)



Using the Scherrer equation and the values of the width at 1/2 of top of the maximum excessive magnetite and silver peak, thinking about λ=1.54056, it changed into feasible to calculate the crystallite size (Eq.1)

$$Zc = 0{,}9\ .\lambda\ /B.\ \cos\theta. \qquad (1)$$

The diameter received for the silver turned into 12 nm and for the magnetite, it turned into 23 nm, which confirms the results received through transmission electron microscopy (MTEM), with smaller debris for silver and large for iron. It is viable to word in Fig. 3a that the answers of ALA:AgNPs are quite heterogeneous with quantities of very big debris and quantities of very small debris. Figure 3b indicates greater homogeneity for the ALA:AgFeNPs samples

Fig. 3. TEM picture of the samples: (a) ALA:AgNPs and (b) ALA:AgFeNPs, with the decrease comparison nanoparticles are like silver with ~ 12 nm and the large ones with iron with ~ 23 nm.

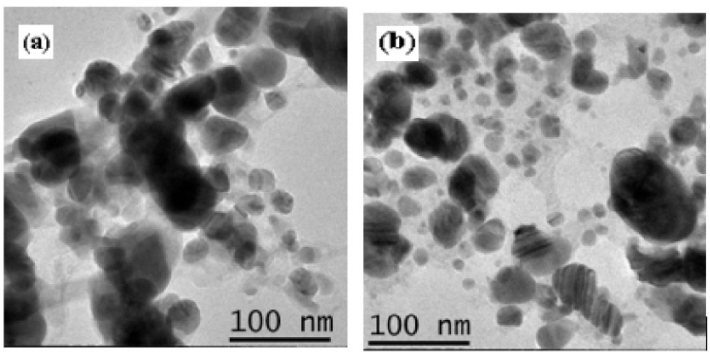


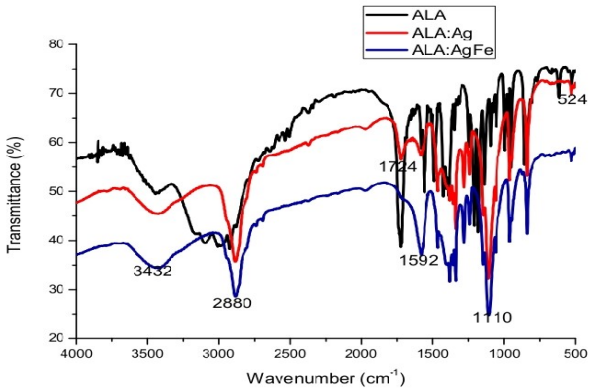
Table 1 presents the obtained values of zeta potential and polydispeity index, indicating moderate solution stability [10, 11].TABLE I. SIZE, ZETA POTENCTIAL AND POLYDISPERSITY INDEX OF SILVER AND SILVER-IRON NANOPARTICLES.

	Zeta Potential (mV)	*PDI*	*Hydrodinamic diameter (nm)*
ALA:AgNPs	-36.6 ± 8.45	0.319	77.02
ALA:AgFeNPs	-30.8 ± 4.40	0.336	3296

FTIR spectra confirmed specific bands for silver and silveriron. The nanoparticles display a few bands withinside the same regions (Fig. four): ~ 2880 cm -1 (C-H) and ~ 1110 cm -1 (C-H). The C = O band of the carboxyl (~ 1716 cm -1), which for ALA changed into strong, decreases and indicates the interplay of the nanoparticles with the purposeful group.

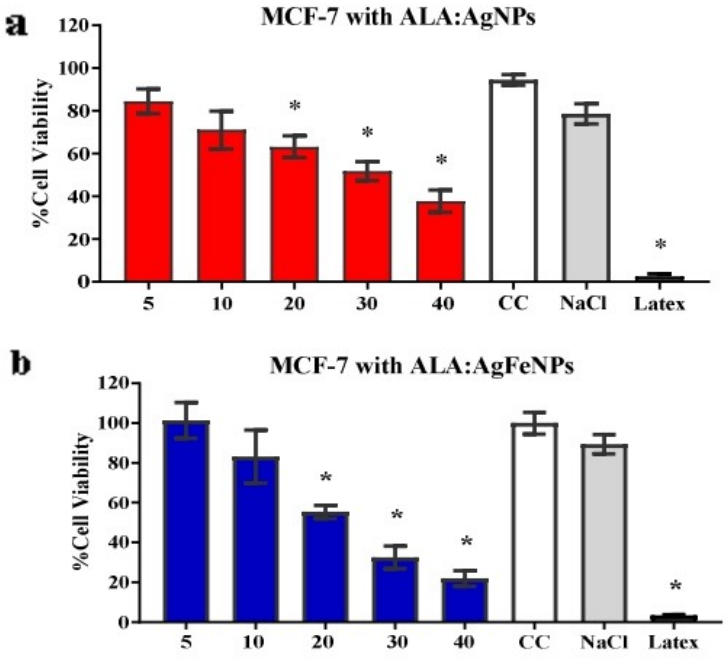
The response pH of the nanoparticles round 3.four favors the binding of the carboxyl group (pKa four.05) with the metallic ions Ag +.

Fig. 4. FTIR spectra of the nanoparticles: spectrum of ALA, ALA:AgNPs and ALA:AgFeNPs and their respective functional groups.



Cytotoxic assays of silver and silver-iron nanoparticles have been made with MCF-7 breast tumor cells cultured in a 96-properly plate. Figures 5a and 5b display the effects obtained. Silver and silver-iron nanoparticles did now no longer display excessive toxicity with volumes of 5, 10 and 20 µL of nanoparticles. The toxicity extended with the boom withinside the nanoparticles volumes and ALA:AgFeNPs turned into extra poisonous than ALA:AgNPs

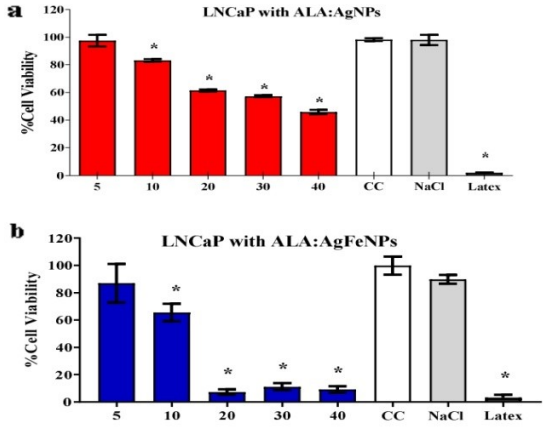
Fig. 5. Cell viability take a look at in MCF-7 cells, incubated for twenty-four hours with: (a) silver nanoparticles and (b) silver-iron nanoparticles. Data have been compared the use of the ANOVA take a look at accompanied with the aid of using the Dunnett take a look at, with p *<0.001.



For prostate tumor cells, the consequences indicated that silveriron nanoparticles have been a good deal greater poisonous than silver nanoparticles as confirmed withinside the Figures 6 a and b.

Iron is metabolized with the aid of using cells permitting the simpler supply of the silver nanoparticles into the cells, which as soon as interior the cells are poisonous and cause to cell death.

Fig. 6. Cell viability check in LNCaP cells, incubated for twenty-four hours with: (a) silver nanoparticles and (b) silver-iron nanoparticles. Data have been compared the usage of the ANOVA check observed with the aid of using the Dunnett check, with p* <0.001.



Conclusion

The synthesis of metal silver and silver iron nanoparticles become viable with the aid of using the photoreduction method, a simple, safe, price powerful and eco pleasant approach.The ALA become included into the floor of the nanoparticles, with the aid of using binding of the metallic with its carboxyl useful group, and its transport become evaluated. Iron silver nanoparticles had been more poisonous to the breast most cancers and prostate most cancers mobileular lines evaluated. ALA:AgFeNPs induces ROS technology inside cells, wearing the ALA, which become transformed to PpIX after 24 hours.

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SACCHARIFICATION OF *ERIANTHUS S*P. BIOMASS BY ENZYME MEDIATED THERMOPHILIC FUNGI

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Abstract

Biomass deconstruction is important for understanding the turnover of biological materials in nature and has important implications for biomass based biorefineries. In recent years there has been an increasing interest in elucidating the biological role of thermophilic fungi and in characterization of their industrially useful enzymes.Thermotolerant lignocellulolytic enzymes have become a subject of interest in industrial processes due to their ability to degrade lignocellulosic polysaccharides. In search of potential microbes producing these enzymes, four thermophilic fungal isolates were obtained from various agricultural wastes by selective enrichment and were screened based on hydrolysis capacity of carboxymethyl cellulose (CMC). In this research were evaluating the performance of cellulases of thermophilic fungi *Cheatomium thermophilum* and β-glucosidase of *Bacillus licheniformis* VCB4 in a cocktail of 40 U (1:1), significant reduction was observed in the treatment with cocktail than the control as well as commercial cellulase. Reduction in peak intensity was observed at wave numbers 1300, 1420, 1509, and1000 in case of saccharified residues.

Keywords: Thermophilic fungi, *Erianthus*sp. , saccharification and FTIR.

Introduction

Thermophilic fungi are important organisms for degradation of plant material in nature. In nature, thermophilic fungi are typically found in compost, wood chip piles, stored grains, paddy straw, forest soil, animal dung and other environments waste, that are self-heating due to degradation of plant materials.These same properties make the fungal enzymes suitable for industrial use. One example is fungal cellulases that are deployed in biorefineries for conversion of biomass to fermentable sugars and in the paper, textile and detergent industries (Karmakar *etal.,* 2011). Biomass decomposition by mesophilic fungi has been extensively studied (Dashtban *etal.,* 2009). Which might the typically effective at 50°C. Several thermophilic fungi produce more thermostable enzymes that can be used at temperatures up to 70°C (Voutilainen *etal.,* 2008).Any basic understanding of the association of culturable thermophilic microbes and usefulness of their metabolites in the field of biomass conversion would help us in designing and development of biobased processes or products.

Materials and Methods

Culture isolation and screening: Four thermophilic fungi were isolated from elephant dung, horse dung, paddy straw,forest soil and decomposed woodand were enriched by following bio-trap enrichment technique. The enriched samples were then transferred to minimal broth in 100ml conical flask containing 1% CMC and incubated at 50^{o}C for 8 to 15 days. After 15 days of incubation, the fungal mat grown on the enriched media were transferred to a Petridish containing minimal agar amended with 1% CMC.After 3-5 days, plates were flooded with 1% Congo-Red solution for 15-20 min then de-stained with 1M NaCl solution for 15-20 min. The diameter of zone of decolorization around each colony was measured.

Biomass conversion: saccharification was carried out by suspending it in sodium citrate buffer of 0.1 M (pH 5.0) in the capped polycarbonate flasks. Addition of buffer was done in such a way so as to maintain the substrate concentration at 10 % (w/v) after enzyme addition. The flasks were autoclaved for 15 min, cooled and supplemented with crude FPases (24 U) of thermophilic fungal strain *C. thermophilum*, β-glucosidase (40 U) and from a thermophilic bacterial strain *Bacillus subtilis* VCB4 and *Aspergillus niger* (40 U)commercial enzyme obtained from Biocatalysts lab, Department of Agricultural Microbiology, TNAU. Both the enzymes were loaded as a cocktail (1:1).

FTIR analysis

The FT-IR (Furrier Transform Infrared) spectra of the samples were obtained using a FTIR (FTIR-6800 JASCO, Japan). Absorbance spectra were recorded between 4000 to 400 cm^{-1} wave numbers with a spectral resolution of 4 cm^{-1} and 64 scans per sample.

Results and Discussion:

FT-IR analysis of Saccharified *Erianthus*

While evaluating the performance of cellulases of thermophilic fungi *C. thermophilum* and β-glucosidase of *B. licheniformis* VCB4 in a cocktail of 40 U (1:1), significant reduction was observed in the treatment with cocktail than the control as well as commercial cellulase. Reduction in peak intensity was observed at wave numbers 1300, 1420, 1509, and1000 in case of saccharified residues. In case of liquid fraction after saccharification, the peak intensity showed more reducing sugars at wavelength <1108.87 cm^{-1} and reduction in 1216.86, 1366.32, 1440 and 2576.43 to 1956.43 wave length which corresponds to cellulose and hemicelluloses respectively. The functional groups are presented. Finally an absorption peak at 880-805 cm^{-1} explains a C-O-C stretching present in the β-(1, 4)- glycosidic linkage in cellulose and hemicellulose (Das *et al*., 2013) and 1320 cm^{-1} which corresponds to CH rocking vibration of the glucose ring.

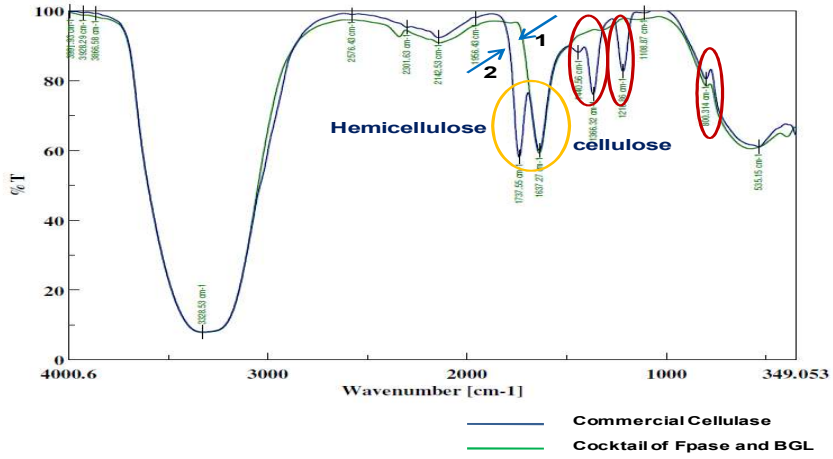
The changes in the compositions of *Erianthus* sp. after enzyme hydrolysis were evaluated using ATR-FT-IR from the wavelength range of 400 -4000 cm^{-1}. Results of the FT-IR functional groups are presented in (Table 1).

Table 1. Functional groups of various structural polymers of *Erainthus* sp. biomass

Wave number	Functional group assignment	Corresponding Polymer
3340	O-H stretch	Lignin
2833	C-H stretch	Lignin
1725	C=O stretch	Hemicellulose
1590	Aromatic ring vibration	Lignin
1502	Aromatic ring vibration	Lignin
1461	C-H deformation	Lignin
1425	C-H in plane deformation	Lignin
1323	C-O of syringyl ring	Lignin
1240	OH	Hemicelluloses
1028	C-O stretching	Cellulose

The FTIR spectrum of enzymatically saccharified *Erianthus* sp. residue, showed a considerable decrease in cellulose content in the treatment that received FPase and β- glucosidase cocktail number 1035 cm^{-1} corresponding to C-O stretching. It corresponds to cellulose and a remarkable decrease was observed when compared to the control. Peak at 1240 cm^{-1} with functional group of OH and 1725 cm^{-1} with ketone/aldehyde C=O stretching, corresponds to hemicelluloses had also decreased compared to control. Reduction in peak height at 3421 cm^{-1} corresponding to lignin was observed in treated compared to control (Fig 2). In case of the liquid fraction, spectral intensities corresponding to hemicelluloses and cellulose were markedly reduced at the wave number 1725, 1240 and 1028 cm^{-1} for cocktail compared to commercial cellulase of *Aspergillus niger* (SIGMA C1184-5KU). There is no variation noticed in the peak intensity at 3340 cm^{-1}, which corresponds to lignin.

Fig 1. FT-IR analysis of hydrolysate fraction of enzyme hydrolyzed *Erianthus* sp. biomass



Conclusion: The study suggested that the thermophilic cellulolytic fungi, *C. thermophilum* isolated from decomposed wood weres more efficient in deconstruction of *Erianthus* sp. biomass. The thermophilic cellulolytic fungi *C. thermophilum*, also possess multi substrate specificity which can be used as a thermophilic cellulase source for bioconversion.

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THEME 1V LIFE SCIENCES

IN VITRO REGENERATION OF PIGEONPEA (*Cajanus cajan* Walp) FROM COTYLEDONARY-NODE EXPLANTS

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Abstract

Multiple shoot buds induced from the cotyledonary-node region of imbibed seeds with seed coat intact gave the highest recovery. BAP (3 mg/l) in Modified MS medium (MMS) improved the frequency of bud induction. Shoot elongation (8 cm) was observed with BAP (2.5 mg/l) + NAA (1.5 mg/l) + GA_3 (0.5 mg/l) in combination. Sustained recovery of multiple shoots was observed until second subculture in the same MMS medium [BAP (2.5 mg/l), NAA (1.5 mg/l) and GA_3 (0.5 mg/l)]. Elongated shoots rooted in half-strength MMS (IBA 2.5 mg/l, NAA 0.5 mg/l) were successfully hardened with the survival percentage of 83%.

Keywords Pigeonpea · *Cajanus cajan* · organogenesis · histology

Introduction

Plant regeneration in pigeonpea, an important grain legume via organogenesis or indirect embryogenesis has been reported from immature and young seedling explants cultured on media containing different auxins and cytokinins. However, the associated low frequency of regeneration has been the major disadvantage for their use in transformation. Moreover, the excision of explants from immature zygotic embryos and young seedlings at an appropriate age is technically difficult. Genetic engineering with agronomically desirable foreign genes has so far not crossed the boundaries due to the recalcitrance in culture. However, here we demonstrate the effect of different cytokinins on the induction of multiple buds from cotyledonary-node cultures. BAP induced shoot organogenesis while with NAA and GA_3 caused a shift in regeneration from induction of adventitious shoots to elongation and sustained recovery of shoots with 2-3 subcultures (Chardra *et al*., 2003).

Materials and methods

Plant materials

Mature pigeonpea seeds Var. VBN1, rinsed with TWEEN 20, were immersed in 70% (v/v) EtOH for 5 min, surface-sterilized with 0.1% (w/v) $HgCl_2$ for 10 min and then completely washed 4-5 times in sterile distilled water. Following this, seeds were incubated in 50 ml of sterile distilled water for 16 h at 25±2 ºC and at 60% RH in dark. The effect of the intact seed coat in both imbibed and non-imbibed seeds on multiple shoot formation was examined by culturing the seed explants with or without seed coat. Seed coats were carefully removed without damaging the surface of the cotyledons. Thus the entire seed explants were cultured in half-strength MMS [MS salts (Murashige and Skoog 1962) with B5 (Gamborg et al. 1968) vitamins] and 0.4% Phytagel (SIGMA Cell culture^{TM}) at pH 5.7-5.8 supplemented with varying concentrations and combinations of plant growth regulators *viz*., BAP, Kn, TDZ, NAA and GA_3.

Induction of multiple shoot buds and micro shoots

Each seed (imbibed and non-imbibed) with or without seed coat was cultured separately in wide-mouthed plastic-capped glass tissue culture bottles (250 ml capacity) containing MMS medium + sucrose 3% + Phytagel 0.4% + BAP (1, 1.5, 2, 2.5, 3 and 3.5 mg l^{-1}), Kn (1, 1.5, 2, 2.5, 3 and 3.5 mg l^{-1}) and TDZ (0.05, 0.1, 0.5, 1.0, 1.5 and 2.0 mg l^{-1}) for induction of multiple shoot buds. Culture media was adjusted to pH 5.8 prior to autoclaving at 121 °C, 15 psi for 20 min. Bud formation frequency (BFC) was calculated as mentioned by Saborio et al (1997).

$$BFC = \frac{\text{Average number of buds per explant}}{\text{Per cent explants forming buds}} \times 100$$

After one-wk culturing, the main shoot was excised and the clumps of multiple buds were cultured for the induction of micro shoots. This contained MMS medium + BAP (2, 2.5, 3 and 3.5 mg l^{-1}) + NAA (1.0 and 1.5 mg l^{-1}) + GA_3 (0.5 and 1.0 mg l^{-1}). Observations on the mean number of micro shoots induced and shoots elongated with shoot length per explant were recorded after two-wks of culture in the same medium with regular subculturing.

Induction of new micro shoots in long term cultures

In a separate experiment, clusters of shoot buds, after the first excision of elongated shoots, were cultured on the respective fresh induction and elongation media, to examine the efficiency of long term cultures for continued induction of new micro shoots from the pre-existing adventitious buds. Elongated shoots were separated in at least four successive cycles

with regular subculturing at two-wk intervals. Mean number of micro shoots, elongated shoots and shoot length per explant were recorded after two-wks of culture.

Rooting *in vitro* and acclimatization

Proliferated and non-hyperhydrified shoots were dissected out carefully from the base of the multiple shoot stock and transferred to half MMS solidified (0.4% Phytagel) media supplemented with IBA (1, 2, 2.5 mg l^{-1}), NAA (0.5 and 1.0 mg l^{-1}) to develop roots *in vitro* for one-wk period. Shoots were also grown for a wk in liquid MMS medium containing IBA (5.0 mg l^{-1}) and NAA (0.5 mg l^{-1}) on filter paper support. Profusely-rooted shoots were cultured in the same culture environment in order to minimize the transplanting shock for a wk in potting mixture. It contained smooth and coarse sand mixed with autoclaved garden soil and 10% organic matter (3:2). Final hardening was done in the mist chamber. After hardening, plants were transplanted to plastic pots with soil bed. The survival percentage and the length of shoots were recorded.

Statistics

All the cultures were incubated at 25±2 ºC and at 60% RH under 16/8 h photoperiod regime (36 µmol $m^{-2}s^{-1}$). All experiments, with five replications were set-up in a completely randomized block design. For all treatments (media compositions), one wide-mouthed plastic-capped glass tissue culture bottle per treatment per explant was used each containing 8-10 explants. Explants were cultured in basal MMS medium, as control for all experiments. Data analysis was done by Analysis of Variance (ANOVA) following the General Linear Model procedure of the SPSS V10 statistical package. Mean values were separated by Duncan's multiple range test (DMRT) at 5% probability level (Duncan, 1955).

Results

Induction of multiple shoot buds

Mature, aseptic seeds swelled in culture within 3 d and were the primary response observed. Numerous green adventitious shoot initials arose from the swollen cotyledonary nodal region. Their number increased gradually at the end of the first wk. Influencing factors for maximum induction of adventitious shoot buds from cotyledonary node region were examined and the results are presented below.

Cause of imbibed seed and seed coat

The presence of seed coat in both imbibed and non-imbibed seeds, drastically affected the differentiation of shoot-buds irrespective of the cytokinin levels in MMS medium. Seedlings developed from imbibed or non-imbibed decoated seeds exhibited thickened seedlings but with stunted growth. In contrast, seedlings raised from non-imbibed seeds, with intact seed coat, failed to produce multiple shoot-initials. Instead, differentiated clusters of leafy structures appeared which suppressed further morphogenesis. However, imbibed seed cultures with intact seed coat, differentiated maximum frequency of adventitious shoot buds in the cytokinin-supplemented medium compared to control.

Dose-dependent response of BAP on morphogenesis

Seedlings from imbibed seeds with intact seed coat, swollen rapidly at the cotyledonary-node region followed by the induction of multiple buds in MMS medium supplemented with BAP (3.5 mg/l). MMS medium supplemented with BAP (3.5 mg/l) induced 92.3 per cent of cotyledonary-node explants to multiple buds with 27.5 per cent bud induction frequency. Low concentrations of BAP (1, 1.5 and 2 mg/l) in MMS medium, sustained significant stability in differentiating multiple buds but drastically reduced the bud formation to 16.59 per cent. In contrast, supplementing Kn and TDZ in cultures, inhibited the bud formation at the cotyledonary-node region irrespective of the nature of the explant. Thus, an exclusive dose-dependent response to BAP was observed in MMS medium for maximum induction of multiple adventitious buds of pigeonpea.

Induction, proliferation and elongation of multiple shoots

Multiple shoots obtained at four levels of BAP failed to proliferate on the same medium; instead it resulted in dense greenish nodular mass which occasionally differentiated rosetting of shoots. Hence, it is necessary to optimize a suitable medium for induction, proliferation and elongation of micro shoots. Clumps of multiple buds of adventitious origin from imbibed seed explants with seed coat were cultured at four levels of BAP (2, 2.5, 3.0 and 3.5 mg/l) and transferred for induction and elongation of micro shoots (Plate 15 F-G) in MMS medium containing NAA and GA_3. Results of this experiment with various growth regulators at different levels are presented in Table 1.

Both induction and elongation of micro shoots were observed at the same level of BAP (2.5 mg/l), only after the addition of NAA and GA_3, especially at low levels. Mean number of 30.15 micro shoots induced in MMS medium supplemented with BAP (2.5 mg/l), NAA (1.5 mg/l) and GA_3 (1.0 mg/l), elongated a mean of 12.08 shoots per explant, each with longer shoot of 8.15 cm, after two wks of culture. Similar response was also observed in BAP (2.5 mg/l), NAA (1.5 mg/l) and GA_3 (0.5 mg/l) supplemented cultures. Further increase in levels of BAP, though reduced the shoot length, improved the mean recovery of elongated shoots (17.80) in BAP (3.5 mg/l), NAA (1.5 mg/l) and GA_3 (0.5 mg/l) supplemented cultures (Table 1).

In majority of cultures, application of GA_3 showed an increased internodal length and expanded leaf. However, an increase in GA_3 (0.5 mg/l) level in BAP (3.5 mg/l) and NAA (1.5 mg/l) supplemented MMS medium, drastically reduced

both the mean recovery of elongated shoots and shoot length. Thus, there is a dose-specific synergistic effect of NAA and GA_3 in BAP supplemented MMS medium in enhancing the recovery of elongated shoots.

Sustained recovery of new shoots in cultures

Continued production of shoot-initials was observed from the cluster of shoot buds when cultured on the same medium. After the first harvest (first cycle) of the elongated shoots, the basal mass of shoot buds still contained many shoot primordia. Elongated shoots were separated at 15 d interval for four successive cycles. However, an intervening undesirable basal callus was induced innately after the excision of elongated shoots in all the cultures. The harvested shoots showed 3-4 internodes and normal leaves. Improved and continued shoot recovery was observed in BAP (2.5 and 3.5 mg/l) supplemented MMS medium containing NAA (1.5 and GA_3 0.5 mg/l), respectively (Table 2). However, BAP 2.5 mg/l supplemented cultures were competent enough in increasing the internodal length of micro shoots compared to those of BAP 3.5 mg/l. Stabilized recovery of healthy shoots longer than 7.5 cm was possible in almost all four cultures maintained in BAP 2.5 mg/l supplemented MMS medium when compared to other treatments. However, a decrease in mean recovery of elongated shoots was observed from the third round of subculture (22.52 to 7.09) to the fourth round. Similar response was also observed in BAP 3.5 mg/l supplemented cultures, but with shoots lesser than 7 cm in length. Cultures maintained in BAP (3 mg/l), NAA (1.5 mg/l) and GA_3 (1.0 mg/l) and BAP (3.5 mg/l), NAA (1.0 mg/l) and GA_3 (0.5 mg/l) supplemented MMS medium exhibited poor recovery of healthy shoots associated with leaf curling, yellowing and induction of translucent tissues even after first subculture (Table 2).

Rooting of elongated shoots and establishment of plantlets

Elongated and well-developed shoots (> 6 cm long) with at least two nodes were excised from the shoot clumps and transferred to half strength MMS rooting medium containing varying levels of IBA and NAA (Table 3). Innumerable adventitious roots were initiated from the basal end of the individual shoots after one wk of culture. However, shoots cultured at low concentration of IBA (1 mg/l) induced basal callusing as well, which ultimately reduced the number and length of adventitious roots. About 20 per cent of rooting of shoots was observed within 4-5 d followed by another 60 per cent at the end of first wk. Remaining 20 per cent of cultures developed roots at the end of second wk and are termed as late roots. Rooting percentage was greater (92.5) in half-strength MMS containing IBA (2.5 mg/l) and NAA (0.5 mg/l) compared to control. Greater recovery of rooted shoots (92.5%) with 8.67 roots per shoot was observed in IBA (2.5 mg/l), NAA (0.5 mg/l) and IBA (2.5 mg/l), NAA (1.0 mg/l) supplemented cultures (Table 3).

Some abnormalities were also noticed in culture as roots formed all over the basal region of shoot, if the shoot length was less than 6 cm, especially those from BAP 3, 3.5 mg/l supplemented cultures. Even when exposed to high concentration of IBA 5 mg/l in liquid medium, tissue at the basal part of the shoot showed browning, necrosis and root clumping. Generally, shoot growth slowed down until the newly formed roots started to elongate. Prolonged exposure of rooted shoots to IBA in culture killed the whole shoot. Hence, rooted shoots with 6 cm root length were hardened. Thus, a brief exposure of shoots in rooting medium enhanced the recovery of healthy rooted shoots. Survival percentage was maximum (83.33%) with primary hardened shoots in both garden soil and soilrite compared to those which were directly subjected to hardening (control). Emergence of fresh leaves followed by an increase in plant height was witnessed after 2 wks of hardening. Entire acclimatization process required 3 wks

Discussion

Plant tissues regenerate shoot buds or embryos *in vitro* either directly from the explant or indirectly through an intervening callus phase. Pigeonpea like many other grain legumes does not regenerate through callus phase. However, direct shoot bud differentiation has been reported from a variety of explants on media containing cytokinins like BAP, Kn and TDZ. The sequence of morphogenetic events has been summarized in Fig. 5. Shoots have been recovered from cotyledons (Metha and Mohan Ram, 1980; Geetha *et al*., 1998; Mohan and Krishnamurthy, 1998), cotyledonary-nodes (Ignacimuthu *et al*., 1997; Prakash *et al*., 1994; Singh *et al*., 2002; Singh *et al*., 2003), epicotyls (Kumar *et al*., 1984; George and Eapen, 1994), embryonic axes (Rathore and Chand, 1999; Franklin *et al*., 2000) and primary leaf explants (Eapen and George, 1993; Eapen *et al*., 1998; Singh *et al*., 2002; Dayal *et al*., 2003). Moreover, the excision of explants from immature zygotic embryos and young seedlings at an appropriate age is technically difficult, cumbersome and time consuming. Even the associated low frequency of regeneration in callus cultures (Eapen and George, 1993) and its ultimate repeatability has been the major disadvantage in pigeonpea transformation. Hence the present study was aimed for a high rate of multiple shoot induction from cotyledonary-node explants of pigeonpea (Var. VBN1), with a view to develop an efficient, reproducible shoot regeneration procedure.

Morphogenesis in cotyledon cultures of pigeonpea takes place by differentiation and redifferentiation from organized structures and not *via* the formation of an organized callus. Cytokinins are in general required to induce shoot buds from cultured tissues (Mok *et al*., 2000). Those that initiated multiple buds on cytokinin-supplemented MMS medium exhibited thickened seedlings, stunted growth with enlarged cotyledons, swollen cotyledonary-nodes and reduced root growth and proliferation. Whitish mass of basal calli was seen at positions where either the shoot or the root came in contact with the medium. A large number of adventitious buds got induced from intact seed coat in imbibed seed cultures. This

distinctive behavior of explants with intact seed coat in enhancing the bud induction frequency was analogous to those observed by Prakash *et al*. (1994) and Geetha *et al*. (1998) in other varieties of pigeonpea. However, Singh *et al*. (2003) demonstrated the inhibitory effect of seed coat on morphogenesis probably due to the release of phenolics that upon oxidation caused blackening of the medium and suppressed morphogenesis. On the contrary, in the present investigation, when seed explants were cultured with seed coat dominated in terms of response and release of phenolics leading to the blackening of medium as observed after a wk. Hence, it could be inferred that the influence of seed coat in morphogenesis is a genotype-dependent response and can be ruled against, by regular subcultures.

BAP was more effective for pigeonpea shoot organogenesis than Kn and TDZ in our study. Similar observations were made for cotyledons of pigeonpea by Eapen and George (1993); George and Eapen (1994); Prakash *et al*. (1994); Mohan and Krishnamurthy (1998); Geetha *et al*. (1998) and in mungbean by Gulati and Jaiwal (1990, 1994) and Tivarekar and Eapen (2001). The enhanced effect of BAP in induction of multiple shoots is correlated with starch, protein, phenol and anthocyanin content in *in vitro* cultures of chickpea (Paul *et al*., 2002). However, recently, Singh *et al*. (2003) induced both - organogenesis at low levels of TDZ, and embryogenesis at higher levels of TDZ by continuous exposure of cotyledons explants.

Problems with elongation and proliferation of shoots were overcome by supplementation of NAA and GA_3 at definite concentrations in the MMS medium. Sustained shoot recovery was observed in BAP (2.5 mg/l), NAA (1.5 mg/l) and GA_3 (0.5 mg/l) supplemented MMS medium. This is in accordance with Gulati and Jaiwal (1990) in mungbean and Geetha *et al*. (1998) in pigeonpea. However, Prakash *et al*. (1994) reported the proliferation of multiple shoot initials only in the presence of IAA suggesting that the cytokinin: auxin ratio was crucial for this response. This result leads to the conclusion that media with lower concentrations of PGRs favor the elongation of shoot buds.

Gradual decline in shoot recovery was observed after two subcultures in BAP and GA_3 supplemented MMS medium. Reasons for the loss of regenerative ability are still uncertain and may vary under different circumstances. George (1993) advanced three theories to explain the loss of regenerative ability (i) genetic variation, (ii) hormone promoting organogenesis and (iii) epigenetic changes in cultured cells. Similarly, shoots in culture may lose their totipotency by way of a neoplastic progression involving hyperhydricity and habituation (Gaspar *et al*., 2000; Kevers *et al*., 2004). Recently, Chandra *et al*. (2003) examined the pattern formation of morphogenesis and reported that incubation in cytokinin-containing media for a longer period could disturb the polarity of the differentiating structures in cotyledon cultures of *Cajanus cajan*. In the absence of a correct gradient, differentiation of the apical meristem was poor, and shoots were recovered at very low frequencies. This could explain the morphological aberrations induced in long term cultures containing higher levels of BAP. BAP often leads to the loss of apical dominance in shoots. Such changes could explain, at least in part, the range of variability found in plant cells, tissues and organs in culture and in microplants, namely, recalcitrance including loss of cell competence (Hagege, 1995; Lambe *et al*., 1997), hyperhydricity (Olmos *et al*., 1997) and somaclonal variation including epigenetic and genetic variation (Jain *et al*., 1998).

Thus, BAP appears the most effective plant growth regulator in all these reports indicating dose-dependent cytokinin specificity for multiple shoot induction. The procedure described here recorded an average recovery of 53 shoots per explant which lasted for 5 wks. Eapen *et al*. (1998) reported high frequency shoot regeneration from primary leaf segments, but the shoot buds appeared only after 45–50 d of induction. In cotyledonary segments, although shoot bud formation was observed in 83 per cent of cultured segments, only 56 per cent of these shoot buds further developed resulting in only 18 per cent fully developed shoots (Mohan and Krishnamurthy 1998). Cotyledonary nodes have been used to produce multiple shoot buds that may not only have been contaminated with axillary shoots but also failed to elongate further (Prakash *et al*. 1994).

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Table 1 Effect of different growth regulators on development and elongation of multiple shoots

Growth hormones (mg/l)			After 15-20 d of culture initiation		
BAP	**NAA**	**GA_3**	**Micro shoots per seedling**	**Elongated shoots**	**Shoot length (cm)**
Control			-	-	-
2	1	0.5	9.12 ± 1.15	-	-
		1	9.31 ± 0.91	-	-
	1.5	0.5	11.33 ± 1.07	6.31 ± 1.31	3.52 ± 1.25
		1	14.61 ± 1.24	4.08 ± 0.82	4.05 ± 0.91
2.5	1	0.5	15.37 ± 0.81	9.44 ± 0.34	3.92 ± 1.15
		1	19.21 ± 1.23	11.13 ± 0.13	4.31 ± 1.30
	1.5	0.5	25.35 ± 1.08	13.81 ± 0.99	8.68 ± 0.38
		1	28.91 ± 0.88	19.07 ± 0.88	5.31 ± 0.81
3	1	0.5	19.63 ± 1.34	8.34 ± 0.55	5.11 ± 1.07
		1	20.71 ± 1.51	9.34 ± 1.16	5.31 ± 1.11
	1.5	0.5	22.34 ± 1.16	10.72 ± 1.17	5.07 ± 1.34
		1	30.15 ± 0.97	12.08 ± 1.22	8.51 ± 0.88
3.5	1	0.5	21.72 ± 1.43	15.34 ± 1.43	6.99 ± 0.91
		1	28.34 ± 1.31	17.33 ± 1.05	5.72 ± 1.31
	1.5	0.5	26.15 ± 0.52	17.80 ± 1.26	5.14 ± 1.51
		1	26.01 ± 0.68	11.04 ± 1.34	5.40 ± 0.83

Mean data ± SE, presented here taken after 2 wks and are based on five replicates containing 10 explants per culture vessel.

Table 2. Effect of different PGRs on proliferation and recovery of multiple shoots during subcultures

Number of subcultures	Shoot proliferation medium		
	Micro shoots per explant	**Elongated shoots**	**Shoot length (cm)**
	BAP 2.5 + NAA 1.5 + GA_3 0.5		
S1	36.71 ± 1.17	18.91 ± 1.16	8.13 ± 0.77
S2	20.09 ± 0.96	22.52 ± 1.07	8.71 ± 1.18
S3	10.16 ± 1.37	11.14 ± 0.18	8.12 ± 1.09
S4	9.08 ± 1.18	7.09 ± 0.91	7.86 ± 0.94
	BAP 3 + NAA 1.5 + GA_3 1.0		
S1	24.81 ± 0.96	19.31 ± 0.74	5.13 ± 0.73
S2	11.18 ± 1.17	9.18 ± 0.82	5.07 ± 0.46
S3	9.34 ± 1.08	-	-
S4	-	-	-
	BAP 3.5 + NAA 1.0 + GA_3 0.5		
S1	23.71 ± 0.84	23.50 ± 1.34	5.82 ± 1.08
S2	10.09 ± 0.72	14.71 ± 1.08	5.01 ± 1.37
S3	8.15 ± 0.61	9.31 ± 0.71	5.63 ± 0.71
S4	-	-	-
	BAP 3.5 + NAA 1.5 + GA_3 0.5		
S1	30.81 ± 0.19	21.38 ± 0.98	6.08 ± 1.16
S2	16.34 ± 1.17	25.34 ± 0.88	6.20 ± 0.91
S3	11.14 ± 0.43	10.18 ± 1.67	6.24 ± 0.73
S4	-	7.82 ± 0.81	5.05 ± 0.88

Mean data ± SE, presented here taken after 2 wks and based on five replicates containing 10 explants per culture vessel. S1, S2, S3 and S4: Represent regular duration of subculturing, at two-wks intervals.

Table 3. Effect of auxins on *in vitro* rooting of elongated shoots

Auxin concentration (mg/l)	Rooting (%)	Number of roots per shoot	Root length (cm)
Half MMS basal	50.00***	4.61 ± 1.18	3.08 ± 1.28
Half MMS + IBA 1.0 + NAA 0.5	63.63**	4.37 ± 1.37	3.15 ± 1.06
Half MMS + IBA 1.0 + NAA 1.0	64.81**	4.78 ± 1.28	3.46 ± 1.14
Half MMS + IBA 2.0 + NAA 0.5	76.92	5.19 ± 1.09	4.67 ± 1.08
Half MMS + IBA 2.0 + NAA 1.0	78.57	5.84 ± 1.37	4.70 ± 0.99
Half MMS + IBA 2.5 + NAA 0.5	92.50	8.67 ± 1.46	6.14 ± 0.38
Half MMS + IBA 2.5 + NAA 1.0	89.68	8.34 ± 1.85	5.34 ± 1.37
Liquid Half MMS + IBA 5.0 + NAA 0.5	86.47	10.24 ± 0.94	4.18 ± 1.19

Mean data ± SE, presented here taken after one wk and based on five replicates containing 10 explants per culture vessel. * = Amount of basal callus.

FIRST REPORT OF *NEUROSPORA INTERMEDIA* IN BITTER GOURD (*Momordica charantia. l*) IN INDIA

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Abstract:
Bitter gourd (*Momordica charantia. L*) is a major fruit crop that has high nutritive values and profitability in India and other countries. Bitter gourd cultivation can potentially increase the economic status of agricultural sector. Leaf samples of bitter gourd plants exhibiting fungal mycelia, collected from an organic field in Pondicherry, India revealed *Neurospora intermedia* attack on the plants. The phytopathogen was identified by isolating the genomic DNA from the fungus by CTAB extraction method and further by sequencing methods. TherDNA ITS sequence was amplified by PCR (Polymerase Chain Reaction) utilizing fungi universal primer pairs (ITS 1/ ITS 4).blast analysis showed that the isolate belonged to Neurospora intermedia. This is the first record of *Neurospora intermedia* invading Bitter gourd plants from India in our perspective.

Keywords: Bitter gourd, *Neurospora intermedia*, CTAB extraction, ITS sequence, Polymerase Chain Reaction, blastn analysis

Introduction

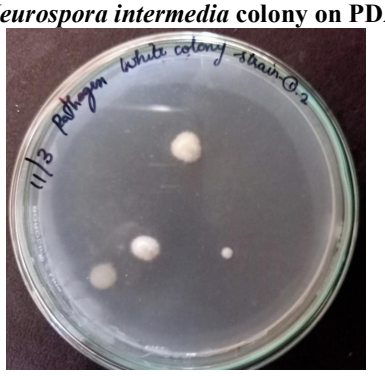
Bitter gourd (*Momordica charantia. L*) is one of the most popular leading vegetable crops of India and is grown especially in tropical and sub-tropical countries (Naveen Kumar, et al., 2012). *Momordica* belongs to Cucurbitaceae family and is an annual, climber vine plant. The flowers of *Momordica charantia. L* are monoecious and highly cross pollinated due to a high degree of heterozygosity (Singh et al., 2013). This crop is often cultivated by farmers during warm seasons mainly in sub-tropical and hot-arid regions. Bitter gourd has various reported health benefits and medicinal properties such as antidiabetic, antiulcerogenic, antiviral, antimicrobial, antioxidant, analgesic and antilipolytic activities present in the fruit (Miniraj, N., Prasanna, K. Pey et al.,1993) (Sikder B.,2004).

Materials and Methods:

The leaves of *Momordica charantia. L* showed typical symptoms of a fungal disease such assmall, circular, leaf spot, brown spots in an organic field in Pondicherry. The infected leaves were collected from the field in order to determine the morphological characterization of the pathogen. These symptomatic bitter gourd leaf samples were washed with running tap water, surface sterilized with 70% ethanol for 1 minute, washed 5 times with sterile distilled water and finally air-dried. Then the air-dried samples were placed on Potato Dextrose Agar (PDA-Difco) medium and the plates were incubated at 25℃ in dark. Similar fungal colonies with white aerial mycelia were observed on the plates after 3-4 days of incubation period. The genomic DNA was extracted from the hyphae attached to the leaves using CTAB method described by (Dhingra & Sinclair (1985)). to identify the fungal pathogen.

Figure 1: Infected leaf collected from the field

Figure 2: *Neurospora intermedia* colony on PDA medium



Using a sterile toothpick, a small amount of the fungal mycelia was transferred into a microfuge tube containing 100 µL of sterile distilled water. The mycelia were well grinded using glass rods to break the cell walls. Then, CTAB extraction buffer was added to the mycelia and incubated at 65℃ for 1 hour. Further purification of the genomic DNA was done using Phenol: Chloroform: Isoamyl alcohol mixture in the ratio 25:24:1. To separate the genomic DNA, isopropanol and the entire mixture was spun at a maximum speed in a microcentrifuge for 2 minutes. The solution was transferred to another sterile microfuge tube containing an equal volume of chloroform and spun at a maximum speed in a microcentrifuge for 2 minutes. The upper aqueous layer was carefully transferred to a sterile microfuge tube containing 600 µL of 2-Propanol, vortexed well and again centrifuged for 2 minutes. The supernatant was discarded and the pellet was washed by adding 1.8 ml of 70% ethanol. Vortex and centrifuge the mixture at a maximum speed in a microcentrifuge. The supernatant was discarded and the pellet was dissolved immediately in 300 µL of 1x TE Buffer. The pure DNA culture was deposited at

Bhagat priority private limited, Hyderabad. To confirm the identities of the isolate, rDNA ITS sequences were amplified by PCR (Polymerase Chain Reaction) utilizing fungi a universal primer pair (ITS 1/ITS 4).

The Internal Transcribed Spacer (ITS) regions have become important molecular targets for taxonomy and identification of species and strains.Due to greater sequence variation, the ITS 1 domain is more suited for species and strain identification than the 18s region (small subunit), the 5.8s region and the 28s region (large subunit) (Lafontaine, D.L et al.,2001). However, most reports identify a limited range of fungi within a genus, and use GenBank ITS sequences that are incomplete or use GenBank records that have been derived from a non-referenced culture. This approach is for rapid and accurate identification of a variety of clinically important fungi in routine diagnostic microbiology.Internal transcribed spacer (ITS) is the spacer DNA, which is situated between the small-subunit ribosomal RNA (rRNA) and large-subunit rRNA genes in the chromosome of the corresponding transcribed region in the polycistronic rRNA precursor transcript. The sequencing of the Polymerase Chain Reaction (PCR)-amplified DNA is highly sensitive in ITS and specific for fungal species identification.

Table 1: Primer sequences of ITS 1 and ITS 4

Name	Primer sequence	TM (°C)
ITS1	TCCGTAGGTGAACCTGCGG	57
ITS4	TCCTCCGCTTATTGATATGC	53

Results

The resulting sequence of 623 base pairs(bp) from ITS 1/ITS 4 were deposited in GenBank. blastn analysis revealed that was 99% identical to *Neurospora intermedia*with (*Accession number: MF319887.1*) 585 bp. The results suggested that this fungus belongs to the organism*Neurospora intermedia* and hence the identified sample was confirmed to be a fungus that invaded *Momordica charantia. L* in the field.

Table 2: blastn result of isolate sequences

Sequence	blastn Hit	Percentage of Identity	E Value	Accession Number
Isolate Sequence 1	Neurospora intermedia isolate BAB-6403 small subunit ribosomal RNA gene	99.29%	0.0	MF319987.1
Isolate Sequence 2	Neurospora intermedia isolate BAB-6403 small subunit ribosomal RNA gene	98.80%	0.0	MF319987.1

Discussion

The effects, that vegetation can have on any soil, can be both beneficial or destructive. Planting a field with a crop of particular plant provides food source for ruminants and plant itself locks bunches of nitrogen inside of it, in the stems and roots. This paves way for potential nitrogen fixation and also as plant matter decays, it adds wonderful humus and organic matter to enrich the soil. This can also be the part of infection to the plant. A fungal spore that may be air-borne or water-borne can also easily find its host and infect them within a short span of time. So, early identification of the pathogen and disease management should be considered.

Conclusion

The phytopathogen that affected *Momordica charantia. L* was identified to be *Neurospora intermedia*. The fungal lifestyles are not stable by dynamic and are likely to be influenced by genetic make up of the fungal species, host factors and changing environments. Hence, it is a crucial period to identify the appropriate pathogen affecting the hosts to manage further spread of the disease among the plant population for better yield and profit.

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PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF METHANOLIC BARK EXTRACT OF *ACACIA LEUCOPHLOEA*.

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Abstract:

*Acacia leucophloea*commonly known as 'White-bark acacia' is reported to have various health benefits and medicinal properties. Most of the natural compounds are now being confirmed for their curative properties through in vitro assays. In order to expand the spectrum of using natural resources to treat deadly diseases, *Acacia leucophloea* has been chosen. In this study, we have demonstrated the presence of several phytochemicals along with antibacterial and antimycotic activities exhibited by the plant extract. *Streptococcus aureus* and *Proteus mirabilis* bacterial strains were found to be susceptible to the extract. *Aspergillus flavus* and *Aspergillus niger* fungal strains also showed similar results to the *Acacia leucophloea* bark extract.

Keywords- *Acacia leucophloea*, Plant extract, Phytochemicals, Antibacterial activity, Antimycotic activity.

Introduction

Acacia leucophloea belongs to Fabaceae family which grows well on alluvial soil and in poor regions, it remains as a shrub or a small plant (Purohit et al., 2003). The trunks of *Acacia* are stout with many branches dividing on the top. It contains thorns that are about 2-5mm long and the flowers are actinomorphic, hermaphrodite and generally pollinated by insects. The barks of *Acacia leucophloea* have been reported to have various medicinal properties that can be used in the treatment of various diseases and disorders(Gupta et al., 2010). Traditional medicine has a promising effect on our body. Medicinal properties of *Acacia* include anti-inflammatory, in skin diseases, in leukoderma, in diarrhea, in dental caries, vomiting, hemorrhages, cough and ulcers (Anjaneyulu et al.,2010). In addition, it also has an adverse antimicrobial activity against a wide range of bacterial strains. Human race has shifted towards the discovery of drugs from natural resources.

Materials and Methods

The plant samples were collected from Perundurai, Tiruppur district and methanol extract was made as described by (Rajesh et al.,2010). The bark was separated and washed under running tap water and further several times with distilled water and shade-dried for about 2 days. The dried bark was pulverized into coarse powder; 2g of the powder was grinded with 20 ml of methanol. This methanolic bark extract was then transferred to a conical flask; incubated in a shaker for 24 hours and filtered using Whatman filter paper. The filtrate was used for analysis.

1. Phytochemical screening

Phytochemical screening was performed by means of standard procedures described by (Sofowora 1993), (Trease and Evas 1989), and (Harborne 1973). The methanolic bark extract was subjected to various phytochemical tests to detect the presence of secondary metabolites (Victor et al., 2009).

a. Test for Alkaloids

2 ml of the methanolextract was mixed with 1 ml of Wagner's Reagent and checked for the formation of reddish precipitate which indicates the presence of alkaloids.

b. Test for Terpenoids

To 1 ml of plant extract, 10 µL of chloroform and equal volume of sulphuric acid was added. Formation of reddish-brown color confirms the presence of terpenoids.

c. Test for Fatty acids

To 0.5 ml of plant extract, 5 ml of ether was added; evaporation of the mixture in filter paper is an indication of the presence of Fatty acid.

d. Test for Tannins

To 1 ml of the extract, 1% Gelatin was dissolved in NaCl solution and white precipitate formation indicates the presence of Tannins in the sample.

e. Test for Saponins

To 2 ml of the extract, about 3 ml of distilled water was added and the tube was agitated vigorously for 10-15 minutes. Foaming inside the tube confirms the presence of Saponins.

f. Test for Phylobatanins

2 ml of methanol extract was mixed with 2% HCl and boiled in a water bath and observed for the formation of red precipitate to show the presence of phylobatanins.

g. Test for Carbohydrates

A few drops of Benedict's Reagent were added to 2 ml of the extract and boiled in a water bath. Formation of brown or green precipitate indicates carbohydrate is present in the sample.

h. Test for Proteins

To the extract, a few drops of Concentrated HNO3 was added and observed for the formation of yellow color indicating the presence of proteins in the plant material.

i. Test for Phenols

The methanol extract was treated with 3-4 drops of ferric chloride. Bluish-black color development indicates that phenol is present.

j. Test for Glycosides

A few drops of glacial acetic acid were mixed with 2 ml of plant extract and ferric chloride. The contents were mixed thoroughly and finally concentrated sulphuric acid was added. Formation of blue-green color in the upper layer and reddish-brown color in the lower layer indicates the presence of glycosides.

k. Test for Coumarins

To 2 ml of methanol extract, 3 µL of 10% NaOH was added and checked for the formation of yellow color in the sample which indicates the presence of coumarins.

l. Test for Steroids

To 5 ml of the extract, 10 ml of chloroform and an equal volume of concentrated sulphuric acid was added. Formation of red and yellow color indicates the presence of steroids.

m. Test for Flavonoids

5 ml of methanol extract was mixed with NaOH solution. Yellow color is formed which disappeared after the addition of HCl confirms the presence of flavonoids.

n. Test for Quinones

To 1 ml of extract, a few drops of NaOH solution was added and development of blue green/red color indicates that quinones are present in the plant extract.

2. Antimicrobial Properties

a. Antibacterial Activity

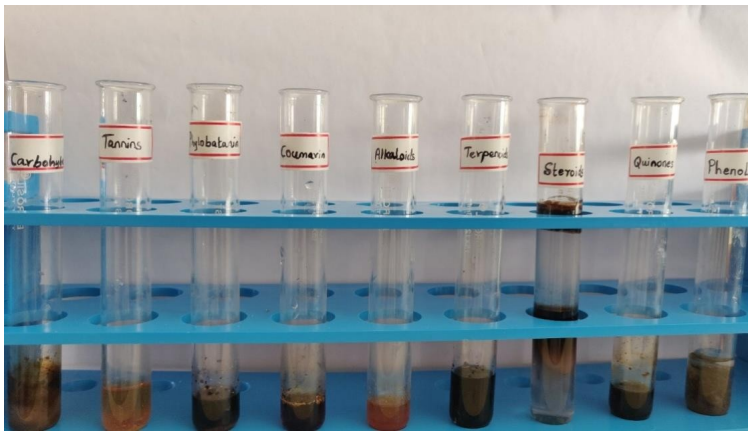
Antibacterial activity of the plant extract was carried out by well diffusion method. Two petri plates were taken in which one of the plates was streaked continuously with *Streptococcus aureus* and another plate was streaked with *Proteus mirabilis* in a similar manner under aseptic conditions. Three wells were created in each plate using sterile micropipette tips. 50 µL, 100 µL and 150 µL of methanol extract was added to the 1st, 2nd and 3rd wells respectively. The plates were incubated for 24 hours at 37°C in an incubator and Zone of Inhibition (ZI) for each concentration of the plant extract for each organism was measured. Similarly, a control plate was created in which methanol at varying concentrations such as 50, 100 and 150 µL was added to the wells and incubated.

b. AntimycoticActivity

Antifungal activity of the plant extract was carried out by well diffusion method. Two petri plates were taken in which one of the plates was streaked with *Aspergillus flavus* and the other plate was streaked with *Aspergillus niger* using continuous streak method under aseptic conditions. Three wells were made in each plate using sterile micropipette tips. 50 µL, 100µL and 150 µL of the plant extract was added to 1st, 2nd and 3rd wells respectively in each plate streaked with two different organisms. Similarly, a control plate with 50, 100 and 150 µL of methanol was added to the wells respectively. The plates were incubated for 3-4 days and Zone of Inhibition (ZI) was recorded for each plate.

Results

Figure 1: Screening of *Acacia leucophloea*methanolic bark extract to detect the presence of secondary metabolites



1. Phytochemical Screening:

Table 1. Qualitative test for the presence of secondary metabolites in the methanolic bark extract

S.no	Secondary metabolites	Presence (++) Or Absence (--)	Significance
1.	Alkaloids	++	Reddish precipitate was formed
2.	Terpenoids	++	Red color formation was observed
3.	Fatty acids	--	The mixture did not evaporate in the filter paper
4.	Tannins	++	White precipitate was formed
5.	Saponin	--	No foam was observed
6.	Phylobatanins	++	Red precipitate was formed
7.	Carbohydrates	++	Brown precipitate was formed
8.	Proteins	--	No yellow color was formed
9.	Phenols	++	Bluish black color was observed
10.	Glycosides	--	Upper bluish green and lower reddish- brown color was notobserved
11.	Coumarins	++	Yellow color was formed
12.	Steroids	++	Layers with red and yellow color was observed
13.	Flavonoids	--	The extract did not become colorless
14.	Quinones	++	Blue green color was formed

The procedure for phytochemical screening was followed as explained above and it was found that our sample contained alkaloids, terpenoids, tannins, phylobatanins, carbohydrates, phenol, coumarins, steroids and quinones. Other secondary metabolites were found to be absent in the plant extract.

2. Antimicrobial Properties

a. Antibacterial Activity

The bark extract of *Acacia leucophloea* showed antibacterial activity towards *Streptococcus aureus* and *Proteus mirabilis* as well. The Zone of Inhibition (ZI) was found to increase along with increase in concentration of the plant extract. Methanol added to the control plate did not show any antibacterial activity and hence, no ZI was formed in the plate.

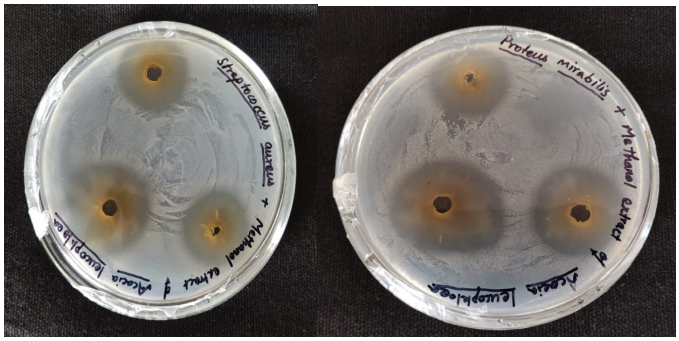
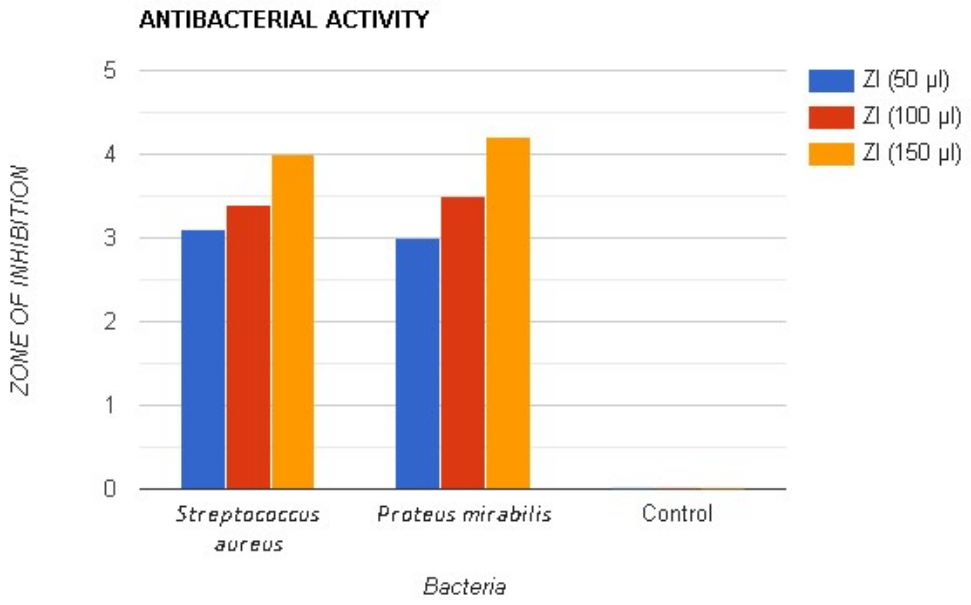


Fig. 2. Antibacterial activity exhibited by the plant extract on *Streptococcus aureus* (left) and *Proteus mirabilis* (right). Control plate not shown

Table 2. Diameters of Zone of Inhibition of the extract at varying concentrations on bacteria

Bacteria	ZI (50µL) cm	ZI (100µL) cm	ZI (150µL)
Control	0	0	0
Streptococcus aureus	3.1	3.4	4
Proteus mirabilis	3	3.5	4.2

Fig. 3. Antibacterial activity graph



b. AntimycoticActivity:

The methanol extract of *Acacia leucophloea* showed significant antifungal activity towards both the fungal strains used in the experiment. Both *Aspergillus flavus* and *Aspergillus niger* were susceptible to the extract. The Zone of Inhibition (ZI) was found to increase along with increase in concentration of the plant extract in a similar fashion. . Methanol added to the control plate did not show any antibacterial activity and hence, no ZI was formed in the plate.

Fig.4. Antifungal activity exhibited by the plant extract on *Aspergillus flavus*(Control and test) (left) and *Aspergillus niger*(Control and test)(right)

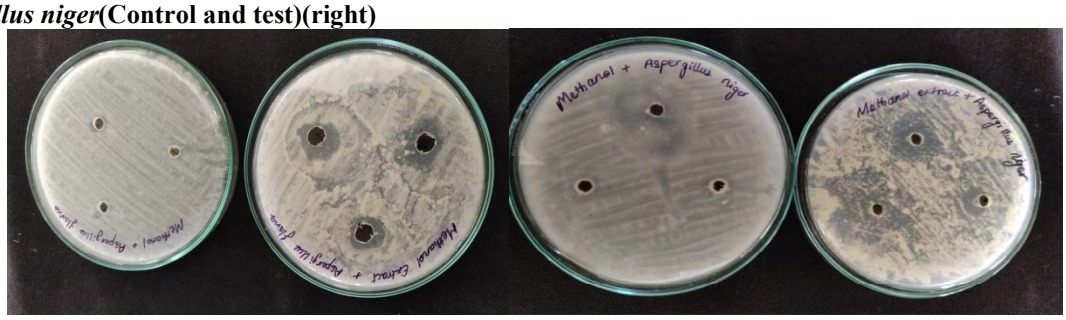


Table 3: Diameters of Zone of Inhibition of the extract at varying concentrations on fungi

Fungi	ZI (50µL) cm	ZI (100µL) cm	ZI (150µL)
Control	0	1	0
Aspergillus flavus	0.9	1.3	2
Aspergillus niger	0.7	1	1.7

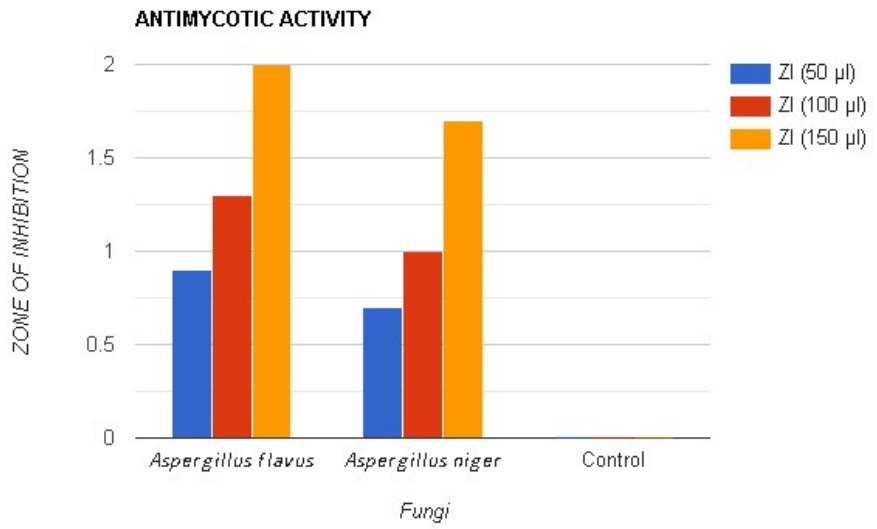


Fig. 5: Antimycoticactivity graph

Discussion

Drugs derived from natural resources play significant roles in prevention and curation of human diseases. In many countries, traditional medicine is one of the primary health care systems (Mariaet al., 2013). Herbs and plants are widely exploited in the traditional medicine and their curative potentials are documented, since they have a large contribution towards human health and wellbeing.

Conclusion

Various medicinal properties have been identified in the bark sample of *Acacia leucophloea*. About nine phytochemicals such as alkaloids, terpenoids, tannins, phylobatanins, carbohydrates, phenol, coumarins, steroids and quinones are present which can be extracted and used to treat most of the clinical conditions of our body. In addition, the extract also exhibited antimicrobial activities against bacteria and fungi.

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A STUDY ON TWIN TOWN: WHERE TRUTH IS STRANGER THAN FICTION

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Abstract

Formation of twin babies is a fascinating and unique phenomenon which when decoded could open doors to endless revelations and discoveries about genetic information transfer and population growth. My work detailed here is about one such unique occurrence, i.e... the birth of a vast number of twin babies in a small village named Kodinhi.

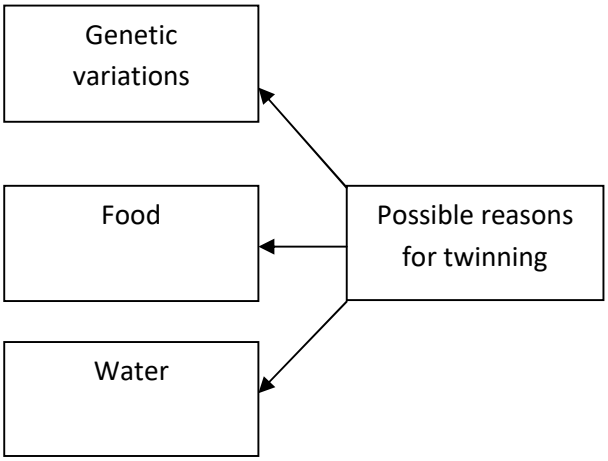
Keywords: Monozygotic twins, Dizygotic twins, FSH, FSHB gene, SMAD3 gene.

Introduction

Twin babies are formed when a single fertilised egg undergoes a cleavage or two eggs undergo fertilisation by two separate sperms in the same menstrual cycle. In the first case the so formed babies are highly similar to one another and are called identical twins (monozygotic twins). In the second case the babies formed are genetically unique or dissimilar (dizygotic twins) (*Raising Children Network Australia Limited, 2006-2021)[1].

Global rate of twinning is 12 per 1000 but some places show a slightly different data. The case of Kodinhi in Malappuram is one such fascinating one. Here the rate of twinning was 45 per 1000 (Bhupindher Singh, 2021)[2].

- Three major aspects where suspected as the base reason for high twinning rate.



Facts and Findings

Hyderabad-based CSIR-Centre for Cellular and Modular Biology, Kerala Universities of Fisheries and Ocean Studies (KUFOS) and the University of London conducted various studies in Kodinhi to find out the reason behind this unusually high twin rate. After intensive research they were able to come to the following conclusions (Megha Varier, 2017).

1. The study of genetic traits and possible gene variations pointed out no significant (or serious) mutations in the genetic assembly of the current generation of inhabitants there and their predecessors. The genetic set up of the neighbouring towns was also studied and no anomalies were observed[3].

Inference: Hence genetic variations as a possibility of this unusual phenomenon were ruled out.

2. A detailed analysis of the food and fodder grown there and nutrition patterns of the people of Kodinhi was conducted. No unusualities were observed[4].

Inference: Food habits were not the reason behind the unusual phenomenon

3. Water samples of the nearby water bodies and groundwater samples were tested in laboratories. The samples did not contain any foreign element that could have influenced the births there. The neighbouring towns and villages which used the same water source did not show such high twinning rate as Kodinhi.

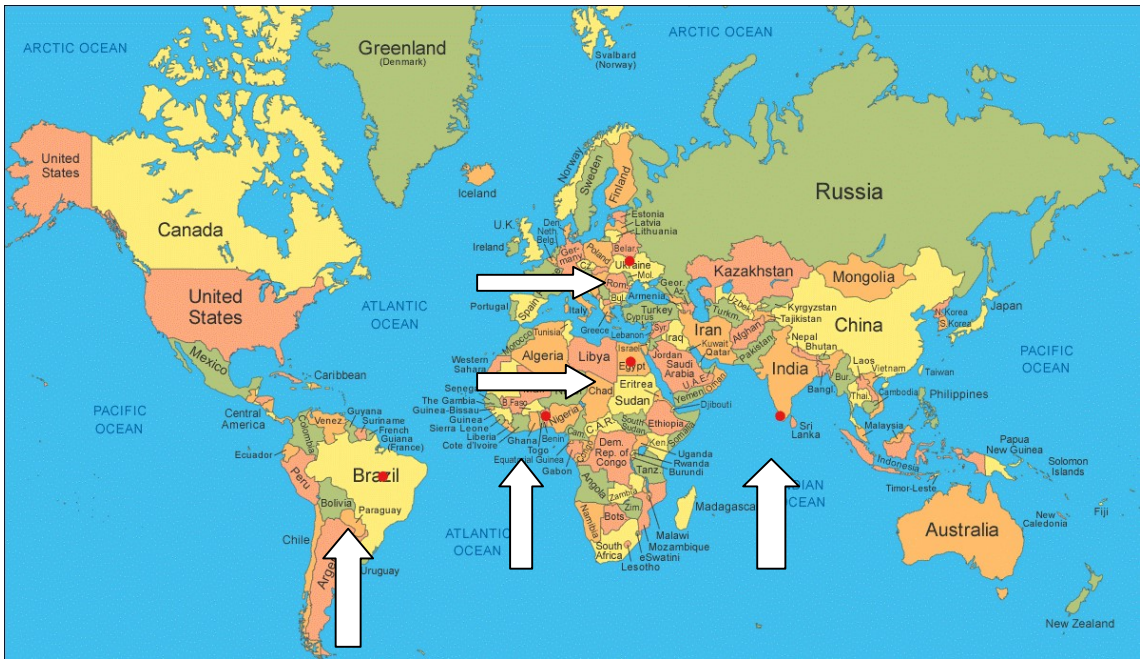
Inference: Water was not the factor responsible for the mysterious phenomenon.

Assumptions

As factors like water, food and genetic variations were not the reason behind this mysterious development we need to look at other possible explanations for the same.

FSHB gene studies of the population in Kodinhi may help us crack the mystery behind this unusually high twinning rate.FSH or follicle-stimulating hormone is the hormone responsible for aiding the ovaries in production of ova and hence aiding in fertility. When there is an alteration in the level of FSH it will also affect the production of eggs by ovary which in turn may result in phenomenon like twinning[5].

An altered FSHB gene and $SMAD_3$ gene can directly affect reproduction so we can very well conclude that a genome-wide association study (Study of DNA markers and genes like FSH) can help in obtaining some breakthrough in Kodinhi case.[6]



(Image courtesy: https://geology.com/world/world-map.shtml)

There are similar places in the world where the rate of birth of twins is very high as compared to global average.

Benin (Charles Q.Choi, 2011), Igbo-Ora (Nigeria) and Abu-Atwa (Egypt) in Africa, Cândido Godói in Brazil, and Velikaya Kopanya in Ukraine and Kodinhi village in Kerala India are all places with such high twin rate. Like our study these places are also mysterious as the exact reason for the twinning rate is unknown there[7].

Conclusion

We can conclude that even though the exact reason for the unusual phenomenon occurring in Kodinhi is still a question mark we were able to make a few useful guesses regarding the twinning rate there. A wide genome study for anomaly in FSHB gene in the population there is one such measure which can help us gain valuable insights into these biological mysteries. Study of twin population can help us greatly in developing healthcare and also preventing infant deaths. A detailed study of Twin Town can be a doorway for many milestone discoveries in the field of modern healthcare and reproduction. It can also help us know more about the Human Dna and the great mysteries it holds.

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CLOVE BASED PHYTOCOMPOUNDS AS ANTAGONIST TARGETING SPIKE GLYCOPROTEIN OF ZIKA VIRUS

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Abstract

The aim of the performed work is to virtually screen for clove based phytocompounds with antagonist property against three viral proteins namely: NS3(PDB ID –5VI7), Zika envelope DIII (PDB ID : 5KVD) and NS3 helicase(PDB ID: 6L50) by docking and simulation studies. Proposed ligand structures from clove were downloaded from IMPPAT: Indian Medicinal Plants, Phytochemistry and Therapeutics, a curated database and Zinc Database. The structure of proteins are retrieved from RCSB PDB and binding sites for placing the grid was identified. Ligands where validated for pharmacokinetic properties following with docking studies performed using autodock tools. Best performing complexes are driven into Simulation studies and free energy of binding was calculated to find the feasibility of reaction. None of the compounds found toxic and upon Docking studies interaction of Eugenol with2I8B and 5VI7, Biflorin with 6l50, 2,3digalloylglucose with 5KVd and 5VI7 and syzygininb with 5VI7 and 6l50 was found significant. Most notable result was interaction of eugenol with 5VI7, biflorin with 6L50 and 2,3digalloylglucose with 5KVD which was subjected for simulation studies and identified as potential target for future application as potential lead to combat viral infection.

Key words: Zika virus; Molecular Dynamic Simulation; pharmacokinetics; Envelope DIII protein; NS3.

Introduction

Zika virus belongs to *Flaviviridae* family, the family that homes several human disease causing viruses like Dengue, West Nile, Yellow fever, Japanese encephalitis and Tick-borne encephalitis. Predominantly this virus transmitted through arthropod vectors causing central nervous system injury and hemorrhagic fevers [1]. None of medical breakthrough has occurred with respect to treatment of Zika virus infection, but the rapid transmission and expansion to every geographic location and its clinical severity in neurological complications and dreadful effect on fetus, increased the competition to race out the drug discovery. This virus has now become focus of medical community as being categorized as threatening emerging diseases. Zika has marked its name on history since 1947, when it was first isolated in the Zika forest Uganda from a rhesus macaque, unfortunately it was found already infecting humans which was identified by undertaking serological test to local communities same year among the local community [2]. Further studies revealed that the infection has already enrooted to major African and Asian countries. Until the outbreak in Pacific island and South American countries, the virus was categorized under infection of limited geographical distribution. In current strand various therapeutics has been suggested to block the viral entry into cells, like blocking entry be receptor binding agent such as nanchangmycin has been proposed, other approaches are inhibition of endosomal fusion with help of endolysosomal vesicles like chloroquine and using squalamine for disrupting the electrostatic interaction [3].

Three proteins namely Zika NS2B-NS3(PDB ID –5VI7), Zika envelope DIII (PDB ID: 5KVD) and NS3 helicase (PDB ID: 6L50) are all identified as potential target for its crucial role in zika viral proliferation and pathogenesis. NS3 helicase has structural homology to RNA helicase of Dengue virus expressing an typical variability in the conformation of loops that are involved in ATP and RNA binding [4]. This identified segment plays an significant role in viral replication by unwinding RNA after hydrolyzing ATP. NS3 is most predominantly studied antiviral target, the binding site of ATP is located amidst two domains and this non structural protein 3 (NS3) helices play important role in viral RNA replication [5]. Zika envelope III are critical in combating viral proliferation by acting as target for action of neutralizing antibodies like anti-E antibodies that protects individuals form subsequent infection [6]. The NS2B-NS3 structure is essential for the maturation of viral protein and hence is validated as potential anti-zika viral target, the negatively charged active site hinders the development and action of orthosteric ligand based inhibitors. Fragment based drug discovery has emerged as promising tool to identify potential drug compound against this target [7].

Herbal-based new pharmaceutical compounds are finding their significance in recent years [8]. Especially compounds like glycosides, saponins, flavonoids, steroids, tannins, terpenes, alkaloids, etc., of diverse herbal origin, are being reported as efficient against various microbial species [9]. Botanically termed as *Syzygium aromaticum*, clove. The phytoconstituents are traditionally used as a food preservative and naturopathy medical ingredient comprises a rich source of a phenolic derivative like Eugenol, Gallic acid, and Eugenol acetate [10]. Recent technological advancement with respect to extraction of highly pure phytocompounds has increased reliability towards the usage of these highly pure phyto-extracts as potential therapeutics. A high throughput computational study has become robust tool for rapid screening and validation of numerous possible drug compounds against the provided target with advent of bioinformatics has become. The current work is based on identification of pontential drug target against zika viral protein by exploiting structure based drug discovery process. In this work, clove phytocompounds were assigned to act against three proteins: NS3(PDB ID –5VI7), Zika envelope DIII (PDB ID : 5KVD) and NS3 helicase(PDB ID: 6L50)

Materials and Methods

Protein preparation

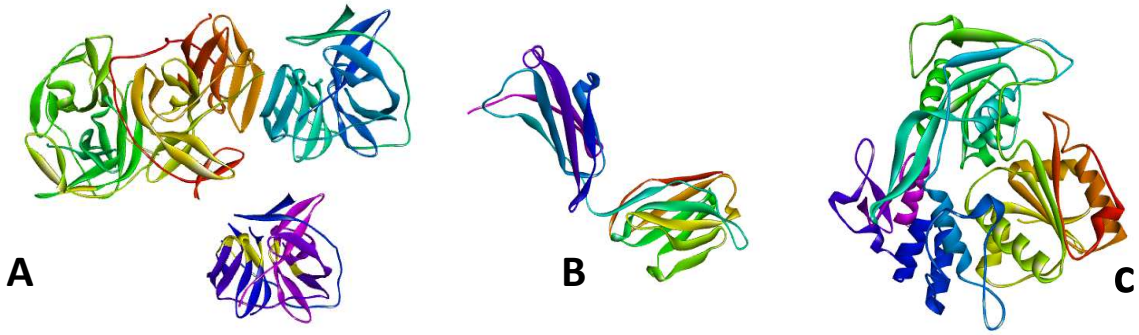
A total of thirty-two ligand compounds where made to interact with the three main targets namely 5VI7, 5KVD and 6L50. Attached ligands are deleted, water deleted and non polar hydrogen were added, homology modeling was performed using SwissModel and any further analysis of the protein was carried in the modeled protein structure [11]. The Discovery Studio 2021 (Dassault System BIOVIA) visualization tool performed the deletion of nucleic acid. Energy of proteins was minimized using Swiss PDB viewer software. Image of proteins are provided in figure 1.

Ligand preparation

Data of active phytochemicals present in clove was acquired from IMPPAT: Indian Medicinal Plants, PhytochemistryAnd Therapeutics, a curated database [12]. This structure subsequently was retrieved from Zinc Database. For further exploitation of ligand, these were energy minimized and optimized using Avogadro software and saved as PDB. The retrieved 3D structures in the format of PDB were further used for the docking studies.

Fig. 1: Representation of Three target protein:

A) NS3(PDB ID –5VI7), B) Zika envelope DIII (PDB ID: 5KVD) and C) NS3 helicase (PDB ID: 6L50)



Molecular docking

Molecular docking studies were carried out using AutoDockTools (ADT) (Scripps Research US) with the extension suite to the Python Molecular Viewer of MGL tools with perl program. Energy minimization of protein was carried out in SPDBV (Swiss PDB viewer aka deep view) while the ligand energy minimization was carried out in Avagadromodule. Protein was processed by the deletion of water, addition of polar hydrogen and merging of non-polar hydrogen. Later the Gasteiger and Kollman charges added to ligand and protein prior to the preparation of the grid parameter file, respectively [13]. The docking studies performed using the Lamarckian genetic algorithm (LMA) and empirical free energy function with a standard protocol. The protein and ligand interactions were analyzed for various bonds like hydrogen, hydrophobic, 2D structure interaction in the Discovery Studio tool (Biovia, 2021 client).

2.4 Pharmacokinetics profiling:

ADME (Adsorption, Distribution, Metabolism and Excretion) analysis vouchsafes the pharmacokinetic properties that a ligand must boat to establish their function in dministered body. This property analysis was executed using DruLiTosoftwarefor ADME analysis [14].

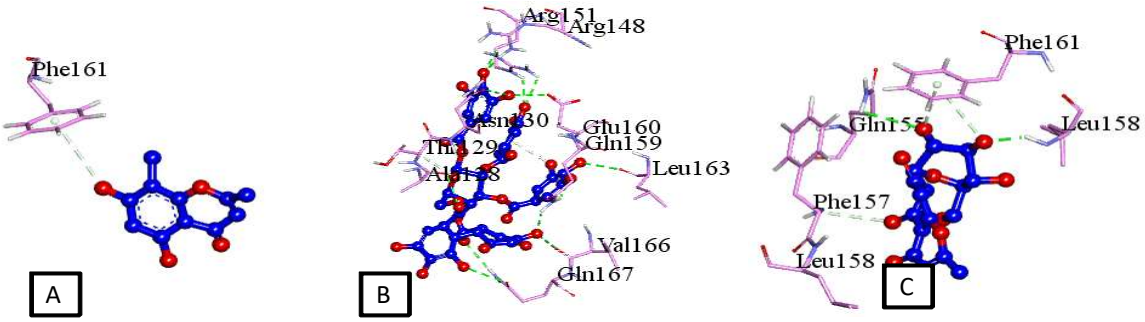
2.5 Molecular dynamics simulation and Free Energy Calculation

Molecular dynamics studies were carried out using Gromacs (Berendsen, et al. 1995) [15]. The forcefield used to generate the parameterization of proteins, phospholipid membrane and ligands was CHARMM36 (https://www.charmm.org/archive/charmm/resources/charmm-force-fields/).The simulations were performed by building a cubic box of 819.74 Nm^3around thesoluble proteincomplexes and of 1048.98 Nm^3 around the membrane protein complexes.The thermal balancing was set to 500 ps, to bring the temperature at 303.15 K, useful for subsequent "*in vitro*" studies. Writing occurred every 50 ps (10 writes). The pressure balance was set to 1 atmosphere (sea level pressure) [16]. The total pressure simulation was 3 ns, and writes occurred every 300 ps (10 writes). Finally, the molecular dynamics simulation was 200 ns, with a dt of 2 fs. Writes were performed every 2 ns (100 frames). Software MMPBSA / MMGBSA (Wang, C., Nguyen, et al.2016) was used to calculate absolute binding affinity. The binding energy was analyzed taking into consideration Decomposition energy of amino acid residues, van der Waal energy, Electrostatic energy, Polar solvation energy, SASA energy, SAV energy and WCA energy.

3. Results

Docking of three receptors was performed against 33 ligands, while the interacting species are analyzed using biovia drug discovery studio. The complex demonstrating the least energy and high docking score upon interaction with all three proteins discussed in detail along with interacting bond type (Hydrogen and Hydrophobic). Docking and interaction study reveals the best binding molecules that could interact efficiently against the target. Biflorin, Eugeniin, digalloylglucose and SyzygininB expressed highest binding energy during docking. From ADMET studies none of the identified compounds expressed toxic nature, harmful secondary compounds and found stable in the protox servers target prediction server. Docking results of best performed compounds are provided in Figure 2.

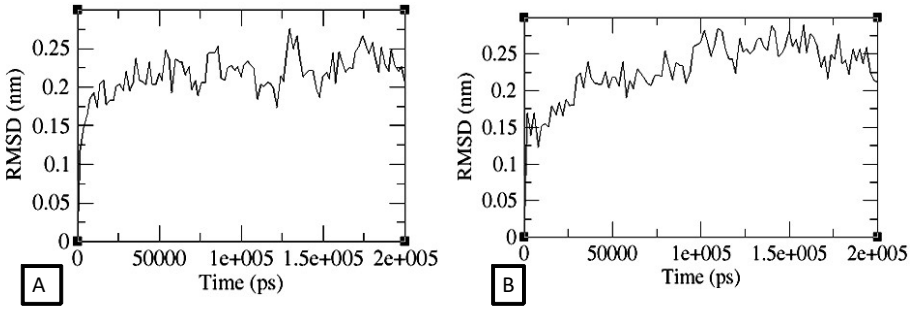
Fig. 2: Interaction of A. eugenol with 5VI7, B. biflorin with 6L50 and C. 2,3digalloylglucose with 5KVD



Molecular Dynamic Simulation of protein ligand complex:

Among 11 complexes subjected for simulation and free energy calculation three complexes are ruled out due to release of positive energy, while the rest being spontaneous are taken for further analysis. The negative value signifies spontaneous interaction with release of energy on the course of interaction, while the positive interaction signifies the need for external input of energy for complex formation while they may spontaneously undergo reverse reaction. As eugenol with 5VI7 complex and biflorin with 6 L50 and 2,3digalloylglucose with 5KVD express a large positive energy of 1457.85 KJ/mol, 1.972 KJ/mol and 1.97 KJ/mol respectively and these were taken for simulation studies. Eugenol with 5VI7 has RMSD fluctuation within range 0.2 to 0.25nm after the stabilization at 1000ps, while Biflorin with 6L50 express an RMSD fluctuation within 0.125 -0.2nm until little over 25000 ps, and a surge post 25000 ps to 0.2ps carried the RMSD fluctuation between the values 0.2 to 0.25 till the period of simulation. MD Simulation results are provided in figure 3.

Fig. 3. A. Eugenol with 5VI7, B. Biflorin with 6L50.



Conclusion

Interaction of Eugenol with 5VI7, Biflorin with 6L50 and 2,3digalloylglucose with 5KVD where identified as feasible and significant form doking and Simulation studies. Prior to molecular dynamics studies the validation of compounds by Docking studies, ADMET screening, target prediction was done. The pharmacokinetic studies were evaluated for supporting our compound's characteristics where Biflorin and 2, 3 Digalloylglucose exhibited stronger drug-like character with no fluctuation from the rule of five and rule of three, while the rest though exhibit a notable fluctuation still the current trends in research aforementioned supports their use as potential lead compounds. All the compounds were not known to express any toxic characters and no dreadful off-target interactions. The work strongly bids the further *invitro* and *invivo* analysis of screened compounds to effectively combat the infection.

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EFFECT OF NANO PARTICLES SEED TREATMENT ON GERMINATION AND SEELING VIGOUR IN PIGEONPEA (*Cajanus Cajan (*L)*.)*

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Abstract

Pigeonpea (*Cajanus cajan (*L.*)* is an important legume crop grown in tropical and sub-tropical environments. Among legumes, pigeonpea occupies an important place in rainfed agriculture. An experiment was conducted at Seed Centre, TNAU, Coimbatore to enhance the seed germination in pigeonpea. The seeds were treated with nano particles and bulk formulations of Zinc oxide, Titanium dioxide, Silicon dioxide each with 100 mg/kg, 250 mg/kg, 500 mg/kg, 750 mg/kg and 1000 mg/kg. After treatment, seed quality parameters were assessed along with control. The results revealed that among the nano chemicals zinc oxide recorded better results for all the seed quality parameters than the other chemicals irrespective of varieties BRG 2 and BRG 4. Seeds treated with 500 mg/kg zinc oxide of nano particle recorded higher germination (92% & 94% respectively), field emergence (87% & 91%, respectively),dehydrogenase activity (0.316 & 0.381 OD) than control (83% & 84% , 79% & 81%, 0.246 & 0.312 OD, respectively). Seed treatment with bulk formulation with zinc oxide was also effective than other chemicals and control but the effect was lesser than the nano formulation.

Introduction

The pigeon pea (*Cajanus cajan* L.) is a perennial legume from the family Fabaceae. Since its domestication in India at least 3,500 years ago, its seeds have become a common food grain in Asia, Africa, and Latin America. Pulses provide a cheaper source of nutrients/ proteins as they generally contain nearly twice as much as protein as that of cereals and hence correctly called poor man's meat (Hariprasanna and Bhatt, 2002). India is producing 14.76 million tons of pulses from an area of 23.63 million hectares, which is one of the largest pulses producing countries in the world. However, about 2-3 million tons of pulses are imported annually to meet the domestic consumption requirement. Thus, there is a need to increase production and productivity of pulses in the country by more intensive interventions. Modern agriculture with its bias for technology and precision, demands that each and every seed should readily germinate and produce a vigorous seedling ensuring higher yield.

Nanotechnology, the science of working with smallest possible particles, raises hopes for the future to overcome the difficulties encountered in agriculture. Nanoparticles (NPs) by virtue of their nano size (10-9m) possess larger surface area resulting in increased catalytic activity and are highly reactive. Zinc, Silicon and Titanium are being micronutrients, required for the normal plant growth and development and they are important components of various enzymes that are responsible for driving many metabolic reactions in all crops (Raju and Kumar, 2017). Recently use of these elements in the form of nanoparticles gaining importance especially for enhancing seed quality in few crops. In this context, an effort was made in the present investigation to find out the effect of seed treatment with Zn, SiO_2 and TiO_2 nanoparticles on seed germination and seedling vigour of pegionpea.

Materials and Methods

The experiment was conducted at Seed Centre, TNAU, Coimbatore during 2019-21. Seeds of Pigeonpea cv. BRG 2 and BRG4 were collected from GKVK, Bangalore and treated with nano particles and bulk formulations of Zinc oxide, Titanium dioxide, Silicon dioxide each with 100 mg/kg, 250 mg/kg, 500 mg/kg, 750 mg/kg and 1000 mg/kg along with control. Immediately after treatment, germination test was carried out in paper medium in quadruplicate using 100 seeds for each treatment with four sub replicates of 25 seeds (ISTA, 1999) in a germination room maintained at a temperature of 25 ± 1°C and RH 96 ± 2 percent with diffused light (approx. 10 h) during the day. Germination, field emergence, vigour index, seedling dryweight and dehydrogenase enzyme activity (Kittock and Law, 1968) were estimated. The data were subjected to an Analysis of Variance and treatment differences tested (test) for significance ($P \geq 0.05$) (Gomez and Gomez ,1984). Wherever necessary, the percentage values were transformed to arc sine values.

Result and Discussion

The results revealed that among the nano chemicals, zinc oxide recorded better results for all the seed quality parameters than the other chemicals irrespective of varieties. Pigeonpea BRG 2 and BRG 4 seeds treated with 500 mg/kg zinc oxide of nano particle recorded significantly higher values for germination (92% & 94 %, respectively), field emergence (87 % & 91 %, respectively) than control (83% & 84 % , 79 % & 81 %, respectively) (Table 1 & 2). Among the different concentrations, 500 mg/kg in all nano particles recorded significantly higher values of vigour index I (2796 & 3412), dehydrogenase activity (0.316 & 0.381 OD) than control (2133 & 2662, 0.246 & 0.312 OD, respectively) (Table 3.)

In bulk formulation treatment also zinc oxide was effective than other chemicals and control but the effect was lesser than the nano formulation. BRG 2 and BRG 4 seeds treated with 750 mg/kg zinc oxide recorded significantly higher values for germination (88 % & 90 % , respectively) and field emergence (83 % & 85 %, respectively) than control (83 % & 84 % ; 79 % & 81 %, respectively) (Table 4&5). Among the different chemicals, zinc oxide recorded significantly higher values of vigour index I (3766 & 4331, respectively) than control (3635 & 3923). The dehydrogenase activity was maximum with 750 ppm zinc oxide (0.290 and 0.360 OD) than control (0.246 & 0.312, respectively).

The beneficial effect of these NPs in improving the germination and production of essential biomolecules as well as essential nutrients required for plant growth are important components of various enzymes which are responsible for driving many metabolic reaction (Senthilkumar, 2011). Prasad et al. (2012) observed that ZnO nanoparticles improved the germination, root growth, shoot growth dry weight in groundnut, significantly as compared to chelated ZnSO4. This could be ascribed to the increased synthesis and activity of hydrolytic enzymes during the early phases of germination and effective mobilization of the available food reserves in the seeds resulted in the early emergence and growth of the seedlings. In proportional to increase in seedling growth, dry matter production was also increased. These results are in agreement with findings of in *Cicer arietinum i.e*. ZnO NPs increased the level of IAA in the roots (sprouts) and thereby resulted in increase in the growth rate of the seedlings. It can be concluded that seed treatment with zinc oxide 500mg /kg nano particle can be used to enhance the seed germination and seedling vigour in pigeonpea.

Table 1. Effect of nano particle treatment on germination (%) in pigeonpea

	BRG2			Mean	BRG4			Mean
	ZnO	TiO	SiO_2	(VxC)	ZnO	TiO	SiO_2	(VxC)
T_1-Control	83	83	83	83	84	84	84	84
T_2-100 mg/kg	85	82	84	84	87	86	87	87
T_3-250 mg/kg	89	84	86	86	88	86	87	87
T_4-500 mg/kg	92	84	88	88	94	89	88	90
T_5-750 mg/kg	90	83	87	87	93	89	90	91
T_6-1000 mg/kg	86	82	85	84	88	87	86	87
Mean	88	83	86	85	89	87	87	88
	C	NNP	V	CxNNP	CxV	NNPxV	CxNNPxV	
SEd	0.71	0.83	NS	0.92	NS	1.23	NS	
CD (p=0.05)	1.43	1.62	NS	1.84	NS	2.46	NS	

Table 2. Effect of nano particle treatment on field emergence (%) in pigeonpea

	BRG2			Mean	BRG4			Mean
	ZnO	TiO	SiO_2	(VxC)	ZnO	TiO	SiO_2	(VxC)
T_1-Control	79	79	79	79	81	81	81	81
T_2-100 mg/kg	81	78	79	79	84	83	82	83
T_3-250 mg/kg	84	80	82	82	87	85	82	85
T_4-500 mg/kg	87	82	85	85	91	88	85	88
T_5-750 mg/kg	85	82	84	84	86	88	89	88
T_6-1000 mg/kg	82	80	84	82	86	84	86	85
Mean	83	80	82	82	86	85	84	85
	C	NNP	V	CxNNP	CxV	NNPxV	CxNNPxV	
SEd	0.32	0.51	0.63	0.73	NS	NS	NS	
CD (p=0.05)	0.64	1.02	1.25	1.47	NS	NS	NS	

Table 3. Effect of nano particle treatment on dehydrogenase activity (OD) in pigeonpea

	BRG2			Mean	BRG4			Mean
	ZnO	TiO	SiO_2	(VxC)	ZnO	TiO	SiO_2	(VxC)
T_1-Control	0.246	0.246	0.246	0.246	0.312	0.312	0.312	0.312
T_2-100 mg/kg	0.267	0.266	0.254	0.262	0.354	0.341	0.338	0.344
T_3-250 mg/kg	0.282	0.253	0.267	0.267	0.362	0.336	0.357	0.351
T_4-500 mg/kg	0.316	0.242	0.281	0.279	0.381	0.340	0.362	0.361
T_5-750 mg/kg	0.307	0.240	0.294	0.280	0.377	0.347	0.370	0.364
T_6-1000 mg/kg	0.392	0.251	0.276	0.306	0.336	0.341	0.350	0.342
Mean	0.301	0.249	0.269	0.273	0.353	0.336	0.348	0.346
	C	NNP	V	CxNNP	CxV	NNPxV	CxNNPxV	
SEd	0.003	0.005	0.007	0.011	0.016	0.019	0.024	
CD (p=0.05)	0.006	0.010	0.014	0.022	NS	NS	NS	

Table 4. Effect of bulk formulation treatment on germination (%) in pigeonpea varieties

	BRG2			Mean	BRG4			Mean
	ZnO	TiO	SiO_2	(VxC)	ZnO	TiO	SiO_2	(VxC)
T1-Control	83	83	83	83	84	84	84	84
T2-100 ppm	82	83	85	83	85	84	85	85
T3-250 ppm	86	85	84	85	87	86	86	86
T4-500 ppm	88	87	86	87	89	89	86	88
T5-750 ppm	88	86	85	86	90	87	88	88
T6-1000 ppm	85	86	85	85	86	87	85	86
Mean	85	85	85	85	87	86	86	86
	C	NNP	V	CxNNP	CxV	NNPxV	CxNNPxV	
SEd	0.62	0.77	0.92	1.12	1.42	1.75	2.14	
CD (p=0.05)	1.24	1.54	NS	2.25	NS	NS	NS	

Table 5. Effect of bulk formulation treatment on field emergence (%) in pigeonpea

	BRG2			Mean	BRG4			Mean
	ZnO	TiO	SiO_2	(VxC)	ZnO	TiO	SiO_2	(VxC)
T1-Control	79	79	79	79	81	81	81	81
T2-100 ppm	78	80	81	80	83	80	83	82
T3-250 ppm	78	78	81	79	85	82	82	83
T4-500 ppm	83	81	83	82	85	83	87	85
T5-750 ppm	81	80	81	81	83	86	88	86
T6-1000 ppm	80	80	82	81	83	86	85	85
Mean	80	80	81	80	83	83	84	84
	C	NNP	V	CxNNP	CxV	NNPxV	CxNNPxV	
SEd	0.21	0.40	0.68	0.91	1.23	1.84	2.25	
CD (p=0.05)	0.42	0.82	1.26	1.82	2.46	NS	NS	

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THERAPEUTIC LIFESTYLE MODIFICATIONS AMONG YOUNG WOMEN WITH RISK OF CARDIOVASCULAR DISEASE

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Abstract

Women with her multiple role in the family, career and society faces tremendous challenge in her everyday life. Increase in lifestyle standards, more of convenience food consumption and recreation with reduced physical activity has made women the victims for cardiovascular disease. Young women in the age group of 21 to 40 years are highly prone to risk of developing cardiovascular disease in this modern day lifestyle and hence were selected as study participants. Intervention strategies like a combined approach of diet, weight and stress management by the young age groups will help in the primary prevention of cardiovascular disease. The present research was undertaken with the objective to promote suitable intervention strategies and evaluate the impact in reducing the risk for cardiovascular disease. From the 1000 young women screened for risk assessment of cardiovascular diseases, a total of 168 young women with risk for cardiovascular diseases were divided into four group with 42 women in each group. Combination intervention methods with use of two or three interventions such as diet, weight and stress management were adopted for a period of four months namely diet and weight management, diet and stress management and diet, weight and stress management and one group of women as control group with no interventions respectively. In Combination approach method, diet and weight management group showed that all the parameters had significance at one per cent level revealing the positive impact of improving the health of the women. The mean weight reduction showed a reduction of 5.5 kilograms over the intervention period of four months. Simple dietary and lifestyle changes are highly important in today's modern lifestyle and adoption of these will help to keep young women healthy.

Key Words: Lifestyle modifications, Intervention, productive years.

Introduction

India is experiencing a rapid health transition, with a rising burden of chronic diseases. The epidemic of infectious diseases is rapidly being overtaken by non-communicable diseases. In recent years, non-communicable diseases (NCDs), such as cardiovascular diseases (CVD), diabetes, chronic obstructive pulmonary diseases (COPD) and cancers have become an merging pandemic globally with disproportionately higher rates in developing countries (Ghafoorunisa and Krishnaswamy, 2014). Seven out of every ten deaths will be from chronic non-communicable diseases by 2020. Cardiovascular mortality in Asian Indian population is likely to climb up 90 percent in women by 2015. It has been estimated that by 2020, Cardiovascular disease will be the largest cause of disability and death in India with 2.6 million

Indians predicted to die due to Cardiovascular disease (Mag and Ghosh, 2015). While many women apparently still do not know it, heart disease is the number one killer of women. About a half million women die of heart disease in the United States, indeed, more women than men die from cardiovascular disease. It is because women often end up with microvascular disease, blockage of smaller arteries instead of larger arteries that is revealed in the tests. A diet rich in high carotenoid (an antioxidant) is associated with a reduced risk of heart disease. Beta-carotene is an economically available form of antioxidant from all green leafy vegetables (Darren et al., 2013)). Hence the study was undertaken with the objective to

- promote suitable combination intervention strategies with diet, physical activity and stress management and evaluate the impact in reducing the risk for cardiovascular disease among young women

Methodology

Selection of Women with risk for cardiovascular disease

Women, especially young women have a greater risk for cardiovascular disease in the modern day lifestyle and hence the risk among healthy young women for heart diseases was assessed. Women in the age group of 21 to 40 years were selected as the target group to assess the heart health risk. The inclusion criteria for sample selection was women with a known history of cardiovascular disease and newly detected patients with cardiovascular disease. Women who reported of congenital disease and patients who came for repeated visits were excluded from the study. This age group was selected because this is the prime age and women get affected by various physical and psychological problems. From the total of 100 young women, who were screened for risk of cardiovascular disease. The high risk women with a Body Mass Index above 23.5 and Waist to Hip Ratio above 0.8 were grouped into five groups with each group consisting of 42 women making up a total of 168 were selected for the intervention for a period of four months. An informed consent was obtained from all the women to know their willingness to participate in the study.

Therapeutic Lifestyle Changes (TLC) are the first and possibly the most important therapy to treat many chronic health problems. Therapeutic Lifestyle changes are recommended as the first line of therapy for reducing the risk of serious health conditions, such as heart disease, stroke, diabetes, arthritis, osteoporosis and obesity Adoption of therapeutic lifestyle intervention in the early stages of life can postpone or delay the onset of the disease and bring behavioural modifications and hence intervention methods were adopted. A combination approach method with two or more interventions were adopted. Diet and weight management, diet and stress management and all the three interventions together namely diet, weight and stress management were adopted for the combination approach methods.

A total of 168 women four groups of women with three groups treated as experimental groups and one group served as the control. Combination intervention method was adopted namely group 1 with diet and weight management, group 2 with diet and stress management and group 3 with diet, weight and stress management and in group 4, no intervention was given and was monitored as a control group.

Intervention with diet, weight and stress management

Diet management

A fibre and antioxidant rich supplement was developed for the diet intervention. Whole grains and millets rich in fibre were selected. Italian millet (*Setaria itallica*), whole wheat (*Triticum aestivum*), bajra (*Pennisetum typhoideum*) and soya flours (*Glycine max*), carrots, curry leaves and spices such as turmeric, pepper, cinnamon and garlic, almonds, groundnut oil and salt were the ingredients selected. The whole grains and millets were selected for the rich source of fibre, almonds and groundnut oil for their mono unsaturated fatty acid content, carrots and curry leaves for the rich source of beta carotene and spices were included for their essential compounds. Consumption of nuts is associated with decreased risk of cardiovascular disease reported Murray *et al.*, 2013. It is because women often end up with microvascular disease, blockage of smaller arteries instead of larger arteries that is revealed in the tests (Jahangeer *et al*., 2010). A diet rich in high carotenoid (an antioxidant) is associated with a reduced risk of heart disease. Beta-carotene is an economically available form of antioxidant from all green leafy vegetables (Mishra et al., 2009).

Spices such as garlic, pepper, turmeric and cinnamon contain a variety of natural compounds that act as antioxidants, protecting cells from invasive damage caused by free radicals and hence these advantages were used for the development of the supplement. Three recipes namely porridges, soups and cookies were developed. The recipes were standardised and evaluated for acceptability by a 30 members panel. Organoleptic qualities such as appearance, texture, flavor and taste were evaluated with five point rating scales (Scores five to one with 5 – Extremely, 4 – Very Good, 3- Good, 2- Fair, 1- Poor). The product which was rated with the highest overall acceptability was finally selected for the diet intervention.

Cookies with Italian millet flour, wheat flour, and soya flour in the ratio of 1:1:0.5 obtained the highest overall acceptability and hence was selected for supplementation. Nutrient analysis was carried out for protein, fat, carbohydrate, total fibre, iron, sodium, potassium, calcium, mono unsaturated fatty acids, total antioxidant activity and beta carotene was also estimated using standard procedure The microbial safety of the product was tested through standard plate count method and the tests were conducted on the initial, fifth,tenth and fifteenth day of storage. The total viable count for microbial safety was determined by the aerobic colony count by HBP method for the target micro organisms. Four Cookies weighing 25 grams each was given for a period of four months, two as midmorning and two as evening snack to group I women. Biscuits were prepared once in five days and were packed in polyethylene covers and were distributed in person to the target group.

A counselling session was conducted prior to supplementation and the women were advised to maintain a food diary to record their daily intake. The diary was intended to be as a self assessment aid and the investigator periodically monitored the consumption. In addition to this, emphasis on balanced diet, role of fibre, antioxidants, type and quantity of fat to be consumed was imparted through diet counselling to the women using power point presentation and pamphlets through individual and group counseling. The booklet with guidelines for healthy heart was distributed to all the women. A twenty-four-hour recall method for three consecutive days was recorded to determine the nutrient intake of the women prior to the intervention. The body weight and twenty-four-hour recall was recorded for all the women every month.

Weight management

Physical inactivity increases the risk of developing heart disease by 1.5 times and doubles the risk of developing type II diabetes and significantly raises the risk of high blood pressure. A direct relationship between physical inactivity and cardiovascular mortality for the development of coronary artery disease is well established. Persons who remain sedentary have the highest risk for cardiovascular disease mortality, and hence weight management was taken up as an intervention strategy and group II women were given aerobic exercises for four months. Women were linked with a fitness centre at Coimbatore and were advised to carry out work outs at the gym. Aerobic exercises for 30 minutes every day was given for weight management. Aerobic exercise is any form of exercise that can be sustained for few minutes while the heart, lungs, and muscles work higher. The women were advised to maintain an activity diary and were periodically monitored by the investigator to check continuity among the women in the weight management.

Table 1 Details of Equipment and Physical Activity

EQUIPMENT	BENEFIT
Tread mill	Lower body and cardio
Elliptical fitness exerciser	Calf and thighs
Pedelar	Calf and thighs
Rowing	Arms
Cycle	Lower body
Twister	Hip muscles
Strength routine	Muscle group – biceps, triceps, gluteous and abdomen
Stretch machines	Stretching muscles

Women worked out with the equipment such as tread mill for lower body and cardiac strength for four minutes. In the elliptical fitness exerciser, the work out was for three minutes and pedelar for four minutes to strengthen calf and thigh muscles. Rowing to strengthen arms, cycle to relax lower body, twister to hip muscles, strength routine for muscle group namely biceps, triceps, spinal region and abdomen stretching machines for stretching and relaxing muscles for 20 minutes with four minutes each. Enas, 2009 quoted that the work out of 30 minutes every day would burn 100 kilocalories everyday and a reduction of two kilograms every month. Weight was recorded every month for the women and body mass index was calculated.

Stress Management

American Psychiatric Association refers stress to mental tension and highly associated with negative effects on the heart and other parts of the body. Acute and chronic stress lead to other risk factors and behaviours, such as high blood pressure and cholesterol levels, physical inactivity and overeating. Hence stress management was given in two modules with yoga as module I and positive therapy as module II for group III women for a period of four months.

Module I comprising yoga classes were conducted by a yoga expert. Women performed yoga for 30 minutes every day for four months. Module II, was conducted twice a month with the components of positive therapy including deep breathing exercises, auto suggestions for positive thoughts, counseling, tension releasing exercises including smile therapy and laughter therapy. The details of the various stress management namely yoga, positive therapy is presented in Table 2

Table 2. Yoga And Its Benefit

Yoga	Benefit
Suryanamaskar (sun salutation)	Whole body, blood circulation
Ardh halasana	Abdomen,legs,spine,reproductive organs
Sarbangasana	Nerves, thyroid, circulation
Halasana	Spine,nervous system,lungs
Bhujangasana	Legs,hips,digestion
Padahastasana	Digestion,spine, legs
Shavasana	Physical and mental relaxation
Positive therapy	Relaxation therapy
	Tension releasing exercise Counselling

The interventions with focus to stress management involved yoga, and positive therapy for 30 minutes every day. The yogas performed were suryanamaskar, ardh halsana, sarbangasana, halasana, bhujangasana, padahastasana, shavasana for three minutes each and for a total of 21 minutes and positive therapy for 10 minutes.

Combination approach Method

The group 1 women were given diet and weight management intervention. This included diet counselling, supplementation of cookies for four months and regular aerobic exercises for 30 minutes everyday. Women in the diet and stress management group (Group 2) were given supplementation with the cookies for four months, diet counselling and yoga for 30 minutes every day along with positive therapy. Women in the diet, weight and stress management group (Group 3) had all the three approaches with diet counselling, supplementation, regular physical activity, yoga and positive therapy. The fourth group, Group 4 with no intervention was monitored for a period of four months.

Impact of Therapeutic Lifestyle Modification

The impact of the therapeutic lifestyle changes were evaluated by testing for changes in BMI, WHR, blood pressure and lipid profile comprising Triglycerides, Total Cholesterol, HDL, LDl and VLDL at pre and post intervention for all the 168 women in four groups. Grundy *et al*. (2000) reported that the newer predictive factors for cardiovascular disease may significantly increase the numbers benefiting from twenty first century diagnostics and treatment. Comparision of parameters to study the differences within and between groups were statistically analysed using ANOVA to interpret the levels of significance in the assessment parameters at pre and post intervention.

Results and Discussion

The nutritional contribution of the cookies was 401 Kilo calories meeting 21 per cent of the day's total calorie requirement. The protein content was 11.31 grams meeting 22.6 per cent of the total requirement for a day. The biscuit provided 3.0 milligrams of iron and ten per cent of the RDA for fibre was noted. A high Beta carotene with 2158.6 micro grams was present as it contained carrots and curry leaves. Micronutrients such as beta carotene, iron and calcium was good and the total antioxidant activity was 0.52 per cent.The nutritive value of the biscuits proved to be effective to be given as a nutritional supplement.

diet and weight management

The comparison of parameters for women in group 1 with diet and weight management is given in Table 3

Table 3 Diet And Weight Management (Pre And Post Intervention)

Parameters	Pre intervention (n=24	Post intervention (n=24)	t value	Significance
BMI	27.71 ± 4.85	25.00 ± 3.64	7.474	**
WHR	1.03 ± 0.05	0.92 ± 0.05	4.551	**
BP – Systolic	125.5 ± 31.90	118.0 ± 27.10	3.540	**
BP – Diastolic	88.0 ± 25.50	85.0 ± 10.01	2.998	**
TGL	93.36 ± 32.54	85.93 ± 10.59	3.440	**
Total cholesterol	163.96 ± 26.19	156.99 ± 20.36	9.114	**
HDL	45.83 ± 8.15	51.08 ± 8.72	-6.879	**
LDL	99.58 ± 26.55	91.63 ± 18.42	6.629	**
VLDL	17.89 ± 7.13	13.78 ± 3.28	4.293	**

All the parameters namely body mass index, waist to hip ratio, triglycerides, total cholesterol, high density lipoproteins, low density lipo proteins and very low density lipoproteins had significance at one per cent level revealing the positive impact on the health of the women. The mean weight reduction showed a reduction of 69 kilograms to 63.5 kilograms.

Diet and Stress Management

Comparison of parameters for women with diet and stress management (Group 2) at pre and post intervention is given in Table 4

Table 4 Diet and Stress Management (Pre And Post Intervention)

Parameters	Pre intervention (n=24)	Post intervention (n=24)	t value	Significance
	Mean ± S.D	Mean ± S.D		
BMI	28.75 ± 4.75	26.23 ± 3.78	5.186	**
WHR	0.96 ± 0.03	0.89 ± 0.04	2.251	*
BP – Systolic	115.5 ± 48.12	108.0 ± 43.20	3.450	**
BP – Diastolic	89 ± 15.16	87 ± 14.76	3.230	**
TGL	120.38 ± 56.23	116.46 ± 50.37	2.359	*
Total cholesterol	167.69 ± 26.70	165.62 ± 26.03	6.848	**
HDL	42.86 ± 7.08	42.55 ± 6.99	-1.947	Ns
LDL	103.11 ± 25.09	103.50 ± 24.45	-0.583	Ns
VLDL	19.73 ± 10.71	14.13 ± 2.69	2.720	*

** - Significant at one percent level, * - Significant at five per cent, NS - Not Significant

The combination interventions, diet and stress management had significance at one per cent level for body mass index and waist to hip ratio. There was a reduction in the weight of the women with four kilograms. Waist to Hip Ratio, high density lipo proteins, low density lipo proteins levels showed insignificant values but the mean values showed a gradual improvement

Diet, Weight and Stress Management

Comparison of parameters for women with diet, weight and stress management (Group 3) at pre and post intervention is given in Table 5.

Table 5 Diet, Weight And Stress Management (Pre And Post Intervention)

Parameters	Pre intervention (n=24)	Post intervention (n=24)	t value	Significance
	Mean ± S.D	Mean ± S.D		
BMI	28.16 ± 4.7	26.11 ± 2.98	7.274	**
WHR	1.05 ± 0.08	0.93 ± 0.09	3.256	**
BP – Systolic	138.5 ± 26.50	121.0 ± 23.20	2.350	**
BP – Diastolic	87.25 ± 10.10	84.50 ± 09.56	2.190	**
TGL	116.46 ± 35.82	113.34 ± 30.45	0.688	**
Total cholesterol	192.71 ± 20.56	184.67 ± 11.57	6.236	**
HDL	43.42 ± 4.001	44.54 ± 0.88	-1.446	Ns
LDL	125.0 4 ± 16.35	116.04 ± 10.17	6.845	**
VLDL	24.16 ± 9.15	24.12 ± 7.35	0.017	Ns

** - Significant at one percent level, * - Significant at five per cent, NS - Not Significant

The intervention group of women with all three strategies namely diet, weight and stress management showed a better impact. The lipoproteins high density lipoproteins, very low density lipoproteins did not show any significant difference at pre and post intervention but there was a mean difference noted between pre and post intervention for the women in this group. The mean reduction of weight of the women was five kilograms during the intervention period.

Details on control group

Comparison of parameters for women with no intervention (Group 4) at pre and post intervention is given in Table 6.

Table 6 Control Group - Pre And Post Intervention

Parameters	Pre intervention (n=24) Mean ± S.D	Post intervention (n=24) Mean ± S.D	t value	Significance
BMI	26.52 ± 5.03	26.30 ± 4.88	2.588	*
WHR	0.96 ± 0.07	0.95 ± 0.07	0.188	Ns
BP – Systolic	126.5 ± 17.12	120.0 ± 19.56	2.980	**
BP – Diastolic	92.0 ± 11.98	88.5 ± 09.96	2.879	**
TGL	80.13 ± 9.36	82.63 ± 8.20	-1.976	Ns
Total cholesterol	170.77 ± 15.29	172.9 ± 13.02	-2.578	*
HDL	43.95 ± 1.41	43.84 ± 1.25	0.717	Ns
LDL	110.71 ± 15.23	112.36 ± 13.70	-2.484	*
VLDL	16.07 ± 2.04	16.49 ± 2.37	-2.095	*

** - Significant at one percent level, * - Significant at five per cent, NS - Not Significant

Though there was a meager difference in body mass index, waist hip ratio, the lipoproteins namely triglycerides, total cholesterol, very low density lipoproteins increased during the intervention period of four months revealing the importance of lifestyle modifications needed for healthy living for the women of young age.

STATISTICAL INTERPRETATION OF PARAMETERS BETWEEN AND WITHIN GROUPS

The results of the various parameters were compared within and between groups and is given in the following tables

COMPARISON OF GROUPS USING ANOVA

The values of the various criteria when compared using ANOVA is given in Table 7

Table 7 Comparison of Groups Using Anova

Criteria	Between Groups		Within Groups		f	Significance
	Sum of squares	Diff.	Sum of squares	Diff.		
BMI	120.343	6	3269.89	161	0.99	NS
WHR	4.461	6	0.507	161	235.9	**
TGL	40977.58	6	225526	161	235.97	**
Total Cholesterol	17830.85	6	82387.03	161	5.81	**
HDL – C	1665.21	6	6182.63	161	7.23	**
LDL- C	10378.53	6	76339.72	161	474.16	**
VLDL- C	1736.54	6	10517.44	161	4.43	**

** - Significant at one percent level, NS - Not Significant

The above table gives the ANOVA results comparing the mean values of the criterion namely Body Mass Index, Waist to Hip Ratio, triglycerides, total cholesterol, high density lipoproteins, low density lipoproteins, very low density lipoproteins of the different groups to assess whether the initial values of the groups are randomised. The ANOVA results showed that mean body mass index values do not differ significantly among the groups and longer period of intervention may be needed to note a statistical significance. The other criteria showed a significant difference at one per cent level indicating the benefits of the intervention among the young women.

Conclusion

The prevalence of cardiovascular disease among young women and the Heart Health Risk Assessment Index among healthy young women population showed alarming results. Intervention strategies like a combined approach of diet and weight management by the young age groups will help in the primary prevention of cardiovascular disease.

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Fig 1. Experimental setup for measurements of dielectric properties

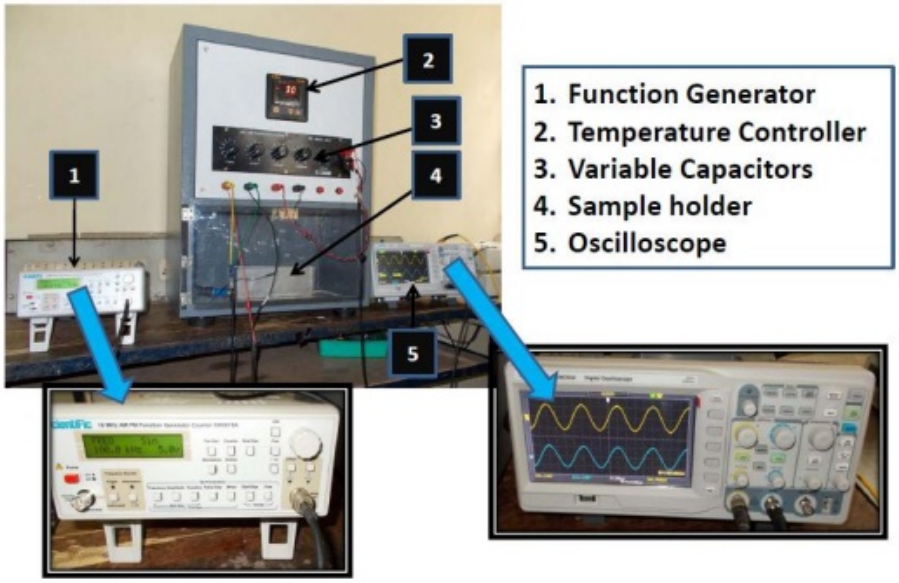


Fig 2. Block diagram indicates hardware's of capacitive sensor

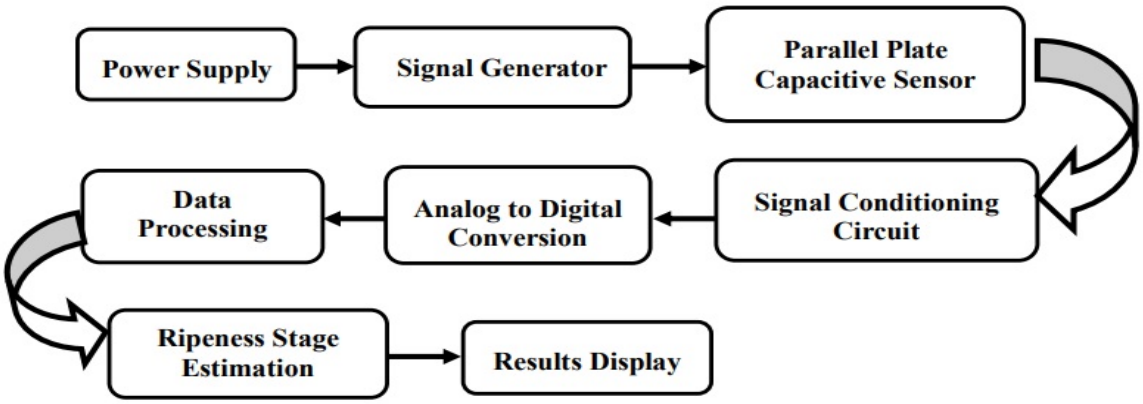


Fig 3. Developed capacitive sensor for banana quality measurement

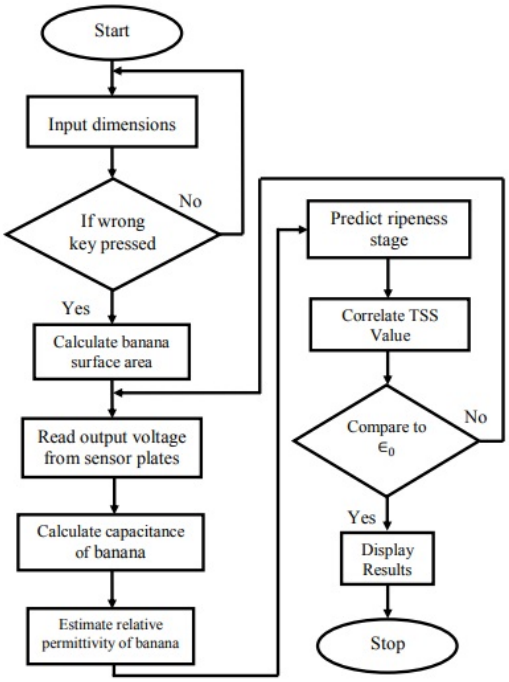


Fig. 5 Linear curve estimation to predict the TSS of Robusta cultivar

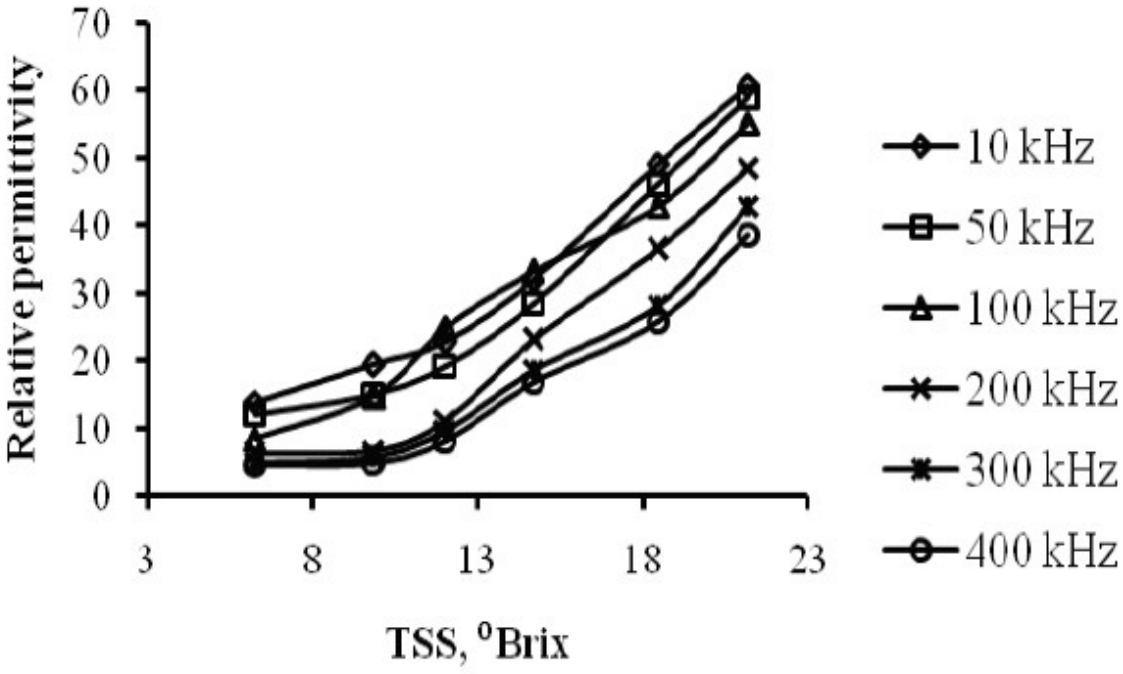
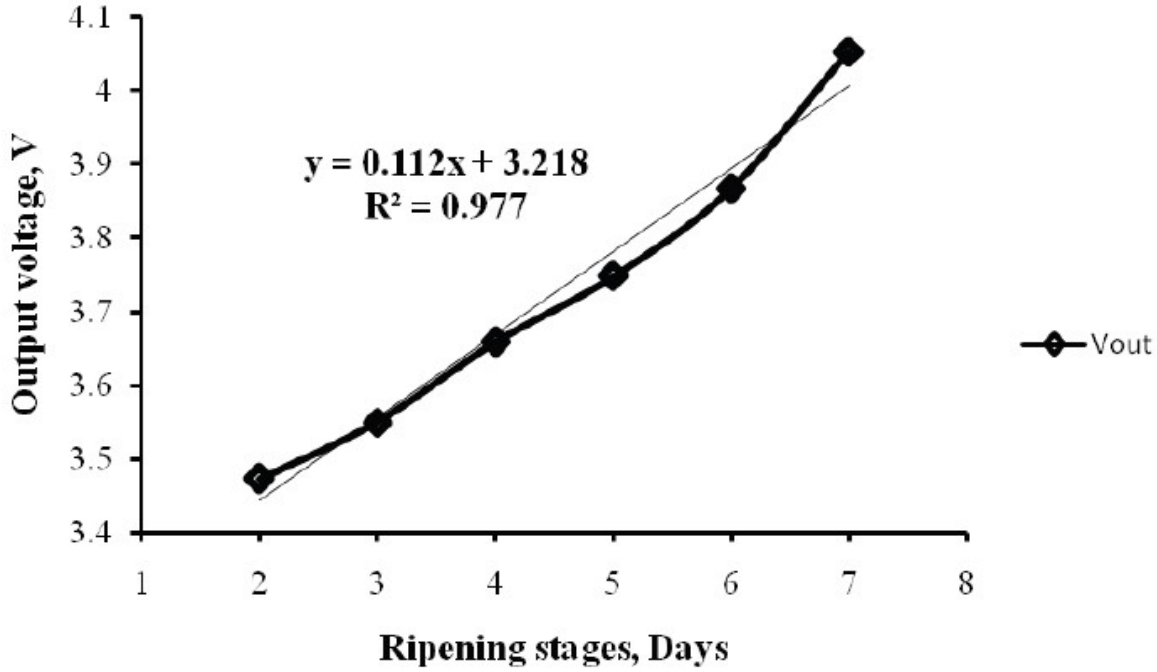


Fig 6 Output voltage responses obtained from portable device during ripening



EFFECT OF VARIOUS DRYING METHODS ON THE PROXIMATE, PHYSICO-CHEMICAL, AND ANTIOXIDANT PROPERTIES OF *Ziziphusmauritiana.*

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Abstract

This study investigated the changes in physicochemical properties, proximate contents, total phenolic content (TPC), total flavonoid content (TFC), and DPPH activity of unripe *Z. mauritiana* fruits after four drying treatments (solar tunnel drying, tray drying, fluidized bed drying, and freeze drying). The proximate analysis was carried out by the AOAC method. The TPC content was observed by the FolinCiocalteu method and expressed in gallic acid equivalents. The proximate content such as protein, moisture,ash and crude fiber composition of freeze dried sample (FZD) had significantly higher values than the other dried samples except for the fat content (0.32%) where the tray dried sample stood first (0.68%). The physical properties such as water activity (0.48aw) and particle size (2.816 µm) of the fluidized bed dried samples were significantly higher than other samples. The solar tunnel dried samples (SD) had higher content of TPC, TFC, and DPPH activity followed by tray dried samples(TD). The antioxidant profile indicates that solar drying can be chosen as the best method for processing *Z. mauritiana* fruits.

Keywords: Jujube, drying techniques, phytochemicals, and antioxidant activities.

Introduction

Ziziphusmauritiana plant belongs to the Rhamnaceae family which is widely seen in the tropical and subtropical regions of South Asia. In recent years, it is also cultivated in southeastern European countries (Wojdyło et al., 2016). Among the Rhamnaceae family, *Ziziphusmauritiana* and *Ziziphusjujuba* are having commercial importance due to their distribution and economic significance. Jujube is a drupaceous fruit, produced from the regular flowers which are found to be originated from China. China contributes approximately 98% of the total production of jujube in the world, making it the largest producer. As per the studies conducted, there are about 17 varieties produced in India and cultivated in the drier regions (Sebastein et al., 1990).

Consumption of jujube fruit is greatly preferred throughout the world due to its taste, health benefits, and their ability to prevent diseases. They are rich sources of nutrients such as vitamins, minerals, fibre, and other bioactive compounds that nourish the body and keep it fit against diseases. The fruit is green in its initial stage and then slowly turns to reddish-brown. Jujube fruit is widely known for its bioflavonoid and phenolic compounds. Its dry form is better used extensively as a flavoring, food additive over years for its high nutritional value. The high nutrient content is present in peels and seeds rather than the pulp of fruits. Moisture content in Z. mauritiana fruit and seed are 88.32% and 29.42 % respectively. There is no significant difference in crude protein amount found in fruit (6.67%) and seed (6.64%). The crude fibre content in seed (48.12%) was found to be significantly higher than fruit (4.76%). Carbohydrate in fruit (1.97%) was the lowest compared to the leaves (19.39%) and seed (63.24%). The fat content of the seed is 1.89% and the fruit is 0.45% which has a lower content of fat compared to the other parts (Mohd Jailani et al., 2020). The problem of concern is the generation of the volume of wastes in excess mainly peels and seeds

Z. mauritiana fruits are used for making paste, puree, syrup, and confectionery. They are proved to have beneficial effects in improving digestion and maintaining health. In many Chinese medicines, it is the main ingredient and is used for curing anorexia, fatigue, and diarrhea in spleen disorders and hysteria in women (Gao et al., 2013). They have a sweet flavor and a pleasant smell and is a little sour. Compared to other fruits, they are endowed with sugar, vitamins, bioactive compounds, edible fibre, and minerals. Chemicals of importance are produced from the output of various fruits such as seed and peel.

The *Z .mauritiana* is a seasonal fruit and available from November to March. Peels contain a large amount of water in them and hence have high perishability. Since fresh fruits are perishable and difficult to store due to their high moisture content they are subjected to drying which is the most common preservation technique by reducing the moisture content to prevent the growth of microbes. To reduce perishability, various drying methods namely oven drying, freeze-drying, air circulation are done. Very few studies have been done on the physicochemical and functional properties of the unripe fruit. The main objective of this study is to ponder the nutritional factors like proximate composition, physical properties, and antioxidant properties of *Z. mauritiana*in unripe fruit samples with peel and to find out which drying method is suitable for the preservations of wholesomeness of the fruit.

Materials and methods

The unripe jujube fruits of good quality were procured in bulk quantity from the local market of Pondicherry for the further described studies. All the chemicals and reagents are of analytical grade unless mentioned specially, procured from the HiMedia Co. Ltd Mumbai.

Different drying and sample preparation

The jujube fruits were subjected to cleaning and washing with water to remove the impurities, dust, and dirt. The cleaned fruits are grated with peel using the steel grater and separated the seeds from the unripe fruit. The grated pulp of the unripe fruit along with the peel weighed in total and it was equally divided into four shares for the processing of the sample by four different drying methods. These were stored in separate polythene Ziploc pouches and placed in aluminiumsilver foil plastic pouches as per Caparino et al., (2012). Before subjecting for drying, the

sample pouches were sealed and placed at -30°C to prevent oxidation. The samples were thawed at room temperature just before the initiation of the drying process.

Fig.1. Flow chart of the process carried out for the *Z. mauritiana*

Freeze drying

The frozen samples were transferred to the freeze dryer trays with a thickness range of about 5mm. The condenser set temperature was -40°C and the shelf temperature is set to 28°C. The initial freezing was given for 2 hours and subjected to vacuum for 36 hours for drying. After drying, the vacuum is released and the driedsamples were again collected in the polythene Ziploc pouches and placed in aluminium silver foil plastic pouches then sealed. Once the sample packages are sealed it is named and placed in an airtight container to store in the deep freezer at -30°C for further processing.

Fluidized bed drying

The frozen samples were thawed before placing them in the product container of the fluidized bed drier. The inlet temperature is fixed at 70°C and the outlet temperature is also fixed at 70°C to attain the maximum efficiency of the dryer in a short span of time. This drying process took about 4 hours to dry the sample. After drying the samples from the product container of the dryer were collected using a stainless steel spoon without damaging the mesh. The dried unripe pulp is taken in polythene Ziploc pouches, placed in the aluminium silver foil plastic pouches, sealed, named, and placed in an airtight container. This container is stored along with the freeze dried samples at -30°C.

Tray drying

After thawing the frozen samples at room temperature the samples were loaded to the trays of tray dryer with a maximum thickness of 5mm as in the freeze drying trays for even drying. The temperature of the dryer was set to 60°C and the drying time took around 10 hours for complete uniform drying of the sample. Once the drying is done, the dried unripe samples were collected in the polythene Ziploc pouches then placed in the aluminium silver foil plastic pouches then sealed and named to keep in the airtight container. The airtight container is stored at-30°C to prevent oxidation and moisture absorption until next use.

Solar tunnel drying

The thawed samples were distributed over the drying chamber of the solar tunnel dryer. The temperature of the drying chamber varies according to the weather of the day and the maximum temperature of the hot air inside the chamber was observed to be 54°C and the average would be 48°C to 50°C. This took about a week to show unvarying drying in the unripe fruit sample. The evenly dried samples were collected in polythene Ziploc pouches then sealed in an aluminium silver foil plastic pouches then named and stored in the airtight plastic container forfurther use at -30°C in the deep freezer

Handling, grinding, sieving, and storage of the sample

All the collected and packed samples of each drying were separately subjected to the powdering process. Initially, the mortar and pestle were used to powder the samples. Unfortunately, they are hard and fibrous to make

them into powder using mortar and pistol. Then electronic mixer grinder was used to powder the samples with 3 to 4 rounds of each 5seconds run to prevent the internal heat production inside the mixer jar. The powder was then sieved with 60 ASTM mesh size to give about 250-micrometer size particle (Barbosa et al., 2005). After grinding and sieving of the dried samples, the powdered samples were separately packed into the Ziploc pouches and placed inside the aluminium foiled bags and heat sealed to prevent moisture absorption. To protect the sample from oxidation and environmental moisture they are stored at -30°C in an airtight plastic container until required for future analysis (Caparino et al., 2012).

Proximate composition

The moisture, crude protein, crude fat, and total ash was carried out with slight modification from the AOAC standard methods (AOAC, 1990). The moisture content of the powdered sample is carried out in the hot-air oven at 105°C as described in the standard methods. In the moisture dish, 5 grams of the sample is taken for drying and the final reduction in the weight of the sample after drying is reported as the moisture content of the sample. For the crude fat, 5gram of the moisture-free powdered sample is taken in the whatmann filter paper and placed in the thimble then subjected to the soxhlet extraction unit with the 200ml of 60°C-80°C petroleum ether as extraction solvent. The extraction is carried out at 70°C for 3hours and the excess solvent is being removed by the evaporation technique. The remaining residue is measured, calculated, and expressed as crude fat. The crude protein of the powdered sample has proceeded with the Kjeldahl method. Initially, 0.2g of the powdered sample was taken in the Kjeldahl tube along with the 3g of digestion mixture (1:3 ratio of copper sulphate and potassium sulphate) and 7ml of concentrated sulfuric acid (98%) for the digestion process. The digestion takes place at 350°C for 3hours and the tubes are cooled before the distillation process. The distillation process is carried out with the 40% sodium hydroxide solution and the ammonium gas released is been dissolved is the conical flask containing boric acid with methyl red indicator (boric acid is neutral to this indicator so it will not disturb the titration) and forms alkaline ammonium borate turning the solution pink to blue. The sample from the conical flask is titrated against 0.1N hydrochloric acid to get a pale pink color. The titer value is found and executed in the percentage of nitrogen in the sample formula and further multiplied with the conversion factor, for fruits it is 6.25 to get the crude protein of the sample. To determine the total ash content of the sample, 5g is taken in a dried weighed crucible burnt with the help of a Bunsen burner until the sample completely turns black and the smoke disappears. The crucible is then shifted to a muffle furnace then ignited at 550°C for six hours (until the sample color turns from black to greyish white) to make it free from carbon. Once removed from the muffle furnace place in a desiccator until cool and note the weight of the sample. Express the obtained values in the total ash percentage formula to get the % of ash in the sample. The crude fibre estimation is carried out by the procedure followed by Yerima et al., (2011) with slight modification.

Physical properties

Color analysis

The color of the unpeeled unripe jujube pulp powders of different drying methods was studied using the Hunter Lab Colorimeter (D-25, Hunter Associates Laboratory, Ruston, USA) after the calibration is done as per the previously used method (Surya et al., 2016).

pH measurement

The pH of the unpeeled unripe jujube pulp powders of different drying methods was measured using the electronic pH meter which is calibrated with acidic, basic, and neutral pH solution at room temperature using the procedure given by Surya et al., (2017). The pH probe was washed with distilled water and wiped to remove excess water using clean tissues before testing for the next sample. Once the measurements of pH of the samples are done then the probe is to be placed in the 4M potassium chloride solution to keep the probe moist.

Water activity

The water activity meter (Aqualab Series 4TE, Decagon Devices, Inc., Pullman, Washington, USA) was used to obtain the water activity of the unpeeled unripe jujube pulp powders of different drying methods at room temperature to give the relative humidity around the sample that is the water activity of the sample.

Particle size analyzer

The particle size distribution of the powdered sample was studied using the Malvern Mastersizer using ethylene glycol as a dispersant (S Markovic et al., 2012).

Fourier transform infrared (FTIR) spectroscopy

Using Fourier transform infrared (FT-IR) Spectrophotometer (Thermo Nicolet Model: 6700, UK) at room temperature, the FT-IR spectra of the four differently dried jujube samples were obtained. A tiny quantity of the sample is taken along with the IR spectroscopy grade potassium bromide (KBr), mixed well using motor & pistol then made into small pellets for the spectroscopy examination. Before reading the samples, the instrument is standardized with the KBr pellet as a blank and the spectrum of the samples were obtained between the range of 400-4000cm^{-1}(Bashir M et al., 2016).

Scanning electron microscopy

Scanning electron microscope (HITACHI, S-3400N, Tokyo, Japan) was used to capture the microstructure of the dried jujube fruit powder and the acceleration voltage used during micrography is about 15kV. The sampleswere sprinkled and placed on an adhesive tape which is mounted on aluminiumstubs, then coated with carbon under vacuum at room temperature and observed for the morphological structure (Bashir M et al., 2016).

In-vitro antioxidant properties

The in-vitro antioxidant analysis for the four differently dried jujube unripe fruit powder was determined by performing the DPPH IC-50, total phenolic content, and total flavonoid content using the Shimadzu UV-Vis 1800 - Spectrophotometer, Japan.

Ultrasonication assisted sample extraction for antioxidant assays

The solvent extraction method with the ultrasonication assistance is been used here. The ultrasonication was carried out at the pre-set temperature of 60°C of the ultrasonicator for 30 minutes. Methanol was used as the organic solvent in the ratio 40:10 % (v/v) and the ratio weight of the sample to the volume of the solvent is 1g/50ml. Once the ultrasonication-assisted solvent extraction is done the solvent mixture is centrifuged at 3000rpm for 15 minutes. Then followed by filtration using whatmann grade 1 filter paper in a conical flask. The collected filtrate is then stored in Tarson centrifuge tubes with tight caps to prevent solvent evaporation and refrigerated for future analysis (Cho et al., 2006).

Total phenolic content

Using Folin-Ciocalteu reagent the total phenolic content of 80% methanolic extract of each sample is determined calorimetrically using the previous study of Kamilogluet at., (2009) with some modifications. To 200µl of the 80% methanolic extract of the unripe jujube fruit sample, 1ml of the Folin-Ciocalteu reagent (diluted in 1:10 ratio FC reagent: water) is added. A few minutes later 1ml of 10% sodium carbonate was added and the volume is made up to 5ml using distilled water. The mixture is vortexed and allowed to stand for 2 hours in dark at room temperature. The absorbance was observed at 765nm using a UV-1800 spectrophotometer, Shimadzu, Japan. The crude amount of phenolic content was measured from the standard curve of gallic acid, and expressed as µg gallic acid equivalent (GAE)/ gram of dried unripe fruit powder.

Total flavonoid content

The methanolic extracts of the unpeeled unripe jujube fruit powders of different drying methods were tested to estimate the total flavonoid content using the method discussed by Meera et al., (2019). 250µl of the 80% methanolic extracts were taken in test tubes and further diluted with 1.25ml of distilled water. Further 75µl of 5% sodium nitrite is been added. The assay mixture is left for 6 minutes at room temperature and continued with the addition of 150µl of 10% aluminium chloride and allowed to stand for 5 minutes. Then 0.5 ml of 1M sodium hydroxide is added. The absorbance of the following assay mixture is read at 510 nm using UV-1800 spectrophotometer, Shimadzu, Japan. Here in the catechin was used as the standard and the results are expressed in catechin equivalents to per gram of dry weight of the sample.

DPPH IC-50

The DPPH-IC50 value was determined with the help of the detailed procedure given by Shimamura et al., (2014) with some modification. The IC-50 values of the dried unripe fruit samples of 80% methanolic extracts were expressed as the equivalents of the ascorbic acid.

Statistical assay

All analysis was done in triplicate. The data were subjected to one-way ANOVA to analyze the significance of the difference in all data and Duncan's Multiple Range Test (DMRT) ($P \leq 0.05$) to analyze the significance of the difference between mean values of samples using SPSS 18 software (SPSS Institute Inc., Cary, NC, USA).

Result and discussion

Proximate composition

From table-1, it is known that the moisture content was higher in the freeze dried sample followed by solar dried sample. There is no significant changes were observed in the tray dried and fluidized bed dried sample. The amount of moisture content present in any of the food or food component is the measuring unit of water activity. This moisture content is used as one of the parameters to detect the food's stability and its susceptibility to microbial contamination. The high moisture content is highly responsible for the rapid deterioration of the jujube powder. One of the most important properties of powder is moisture because the moisture content of the powder is related to the drying efficiency (Santhalakshmy et al., 2015).

The ash content of the four differently dried-unripe jujube falls between 2.62g/100g DW to 4.25g/100g DW and the values are in accordance with the Chinese jujube varieties as per Sunil Pareek et al., (2013). The highest is recorded with the solar tunnel drying and the least is the fluidized bed drier. The prior studies on the fruits of *Z.jujube* showed a range between 0.78-1.10 g/100g (K Chen et al., 2019, J Zhou et al., 2013). The increased ash content observed here in the unripe shows that the reduction in the ash content occurs as the fruit gets matured. S Mahoodi et al., (2016) shows the highest ash content in the initial mature state with 2.82% and the least at fully ripened state with 1.6%. The difference in the ash content of the same unripe *Z .jujube* may be due to the difference in the drying technique used as the moisture loss has a connection with the ash content. The loss of moisture increases the nutritional bulk and thereby causesan increase in the ash content (T Delgado et al., 2016).

The protein content of the pulp was low in general for all the fruits. The test fruit samples had their protein value in the range of 5.68% to 6.348%. The protein content of the freeze dried sample was comparatively higher followed by tray

Table 1. Proximate composition of the differently dried unripe with peel *Z. mauritiana*

Samples	Protein	Moisture	FAT	ASH	Crude fibre
FZD	6.348±0.657[a]	31.637±0.272[c]	.32 ± 0.02[a]	3.37±.07[b]	7.25±.01[d]
FBD	5.686±0.002[a]	12.877±0.071[a]	.56 ± .00[b]	2.62±.12[a]	6.65 ±.75[c]
TD	6.020±0.330[a]	13.330±0.644[a]	.68±.02[c]	3.24±.06[b]	4.90±.10[b]
SD	5.909±0.219[a]	15.649±0.680[b]	.32±.00[a]	4.25±.01[c]	3.20 ± .33[a]

dried sample. The protein content of the fruit may contribute to its high nutritive content which may be due to the presence of protein-compounds such as amino acids and enzymes (Afroz et al., 2014). The high amount in the peel may be due to the effect of fertilizers (nitrogen-containing) and the soil variety in which it is cultivated.

The crude fat determined from the unripe *Z. jujube*by different drying techniques showed no significant difference as the fat has not get affected by the thermal and non-thermal process of drying (T Delgado et al., 2016). The fat content of the tray dried samples shows a higher value than the other differently dried samples. The fat content ranges between 0.32- 0.68 % and these values are in accordance with the prior findings of Sunil Pareek et al., (2013) for Chinese jujube varieties and FNA MohdJailani et al., (2019) for the fruits of Malaysian *Z. mauritiana* (0.45%) and Kongkachuichai et al., (2015) for the vegetables of Thailand (0.08-4.36%).

The crude fibre ranges from 3.20-7.25% for the differently dried unripe *Z. jujube* while the crude fibre content of solar tunnel drying is undetermined even after several trials. The unripe crude fibre values met with the values of the fruits of Malaysian *Z. mauritiana*-4.76% (FNA MohdJailani et al., 2019). In this study, the freeze dried samples showed higher values than the other three drying methods. The thermally treated samples showed a reduction in the crude fibre than the freeze dried samples which might be due to the damage caused to the cellulose, hemicelluloses, and the lignins which are the measure of crude fibre (SitiMahirah et al., 2018).

Physical properties

Color analysis

One of the most important sensory quality indices of the dried powder after drying is color. If the color of the dried powder is darker it's quality and market values will be affected (Fang et al., 2009). Color values observed for the samples were depicted in table-2. Freeze dried sample exhibited higher L* value followed by FBD. Since no heat is involved the whiteness of the freeze dried sample was increased. The solar dried sample exhibited the lower values of lightness and higher redness this may be due to the increased exposure to temperature. It was observed that a solar dried sample has less visual appealing because of its lower hue angle and chroma value.

Table 2. Color values of the differently dried unripe with peel *Z. mauritiana*

Sample	L*	a*	b*	Hue angle	Chroma
FZD	67.690±0.034[d]	6.776±0.028[a]	27.413±0.051[c]	76.114±0.034[d]	28.571±0.565[c]
FBD	59.276±0.030[c]	7.853±0.032[b]	28.370±0.087[d]	74.525±0.104[c]	29.436±0.075[d]
TD	57.446±0.128[b]	8.296±0.035[c]	26.610±0.095[b]	72.668±0.072[b]	27.869±0.095[b]
SD	25.663±0.243[a]	10.440±0.160[d]	14.393±0.332[a]	54.039±0.614[a]	17.781±0.317[a]

pH is the measurement of H+ ion activity, it measures active acidity. Ph may be determined by measuring the electrode potential between the glass and reference electrodes. Low Ph enhances microbiological and physiochemical stability (Magaia et al.,2013). The solar dried sample showed a lower ph value of 3.24, which means it is more acidic than the other 3 samples. So it is clear from the report that as the fruit starts to ripen its acidic level increases. Ph is also described as the measure of the strength of acid present in the sample suspension. pH value was higher for FBD and TD. Unripe with peel had high pH than semi-ripe with the peel. The report of Ara et al., 2014) was closely related to this study. The high value in the unripe jujube sample is related to the acidic content of unripe fruits than ripened ones.

Water activity

Water activity indicates the free water available for any kind of reactions such as microbiological or biochemical reactions (Santhalakshmy et al., 2015). The higher the amount of free water present in the sample lower will be its shelf-life. The water activity of the given samples ranged from 0.334 – 0.48. So, the jujube powder can be considered to be moderately stable, when compared to the average water activity of powders having good stability ranged from 0.18 to 0.25 (Quek et al., 2007).The hike in water activity is attributed to the fructose sugar in it causing absorption of water (Bahrasemani, M., & Mohammad, 2018). The water activity of the sample lies below 0.6, this value is sufficient to make the fruit powder microbiologically safe. Water activity is also affected by the inlet temperature (75˚C) of the fluidized bed drier, which has an influence drying rate of the sample (I.E, 2018).

Particle size distribution

Table 3. Physical properties of the differently dried unripe with peel *Z. mauritiana*

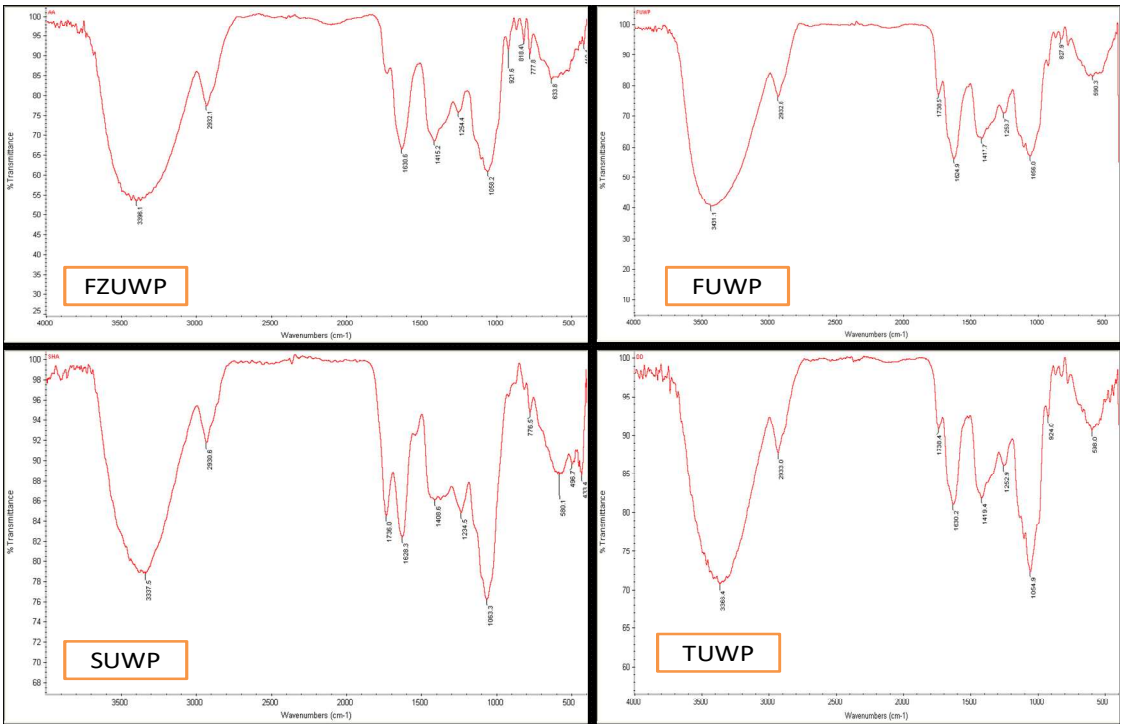
Samples	Water activity	pH	Particle size (d.mm)
FZD	0.334 ± 0.001^{a}	4.650 ± 0.010^{b}	1.341 ± 0.72^{b}
FBD	0.482 ± 0.002^{d}	5.665 ± 0.055^{c}	2.816 ± 1.00^{c}
TD	0.339 ± 0.000^{b}	5.795 ± 0.175	1.133 ± 0.56^{a}
SD	0.408 ± 0.001^{c}	3.245 ± 0.015^{a}	1.306 ± 0.73^{b}

The particle size of the powder sample remains critically important in many fields especially in the food industry as it plays a major role in the processing, handling, and quality of food products. This also has a direct connection with the water absorption capacity andflowability of the powdered samples. The flowability increases with the increase in the particle size and thereby also reduces the internal friction angle of the powdered sample (L X Liu et al., 2008), while the water absorption and holding capacity of the powdered sample increases with the decrease in the particle size which might be due to the increase in the surface area and energy (Y Tao et al., 2018). The average particle size of the four differently dried samples of unpeeled unripe jujube fruit was in the range from 1.10 to 2.80mm. The sample of FBD had the highest particle size of 2.816±1.00 mm while the FZD, TD, SD had 1.341±0.72, 1.133±0.56, 1306±0.73mm respectively is seen from table-3. The larger difference for FBD sample from the other sample may be due to the poor sample preparation or the samples would not be properly dispersed in the liquid due to the swelling or dissolution and reactivity of the sample nature (S Markovic et al., 2012).

Fourier transform infrared spectroscopy

FT-IR spectroscopy is used as a tool for monitoring the structural characteristics of the sample. FT-IR spectrum of the dried samples are shown in Fig 2. The FT-IR spectra of all the dried samples had a broad O-H spectrum and C-H stretching at 3396.1-2930.6 cm^{-1}. The strong peaks at the region 2933.0-2930.6 cm^{-1} indicates C-H stretching. It was observed a weak peak of bending of CH_2 groups at a spectrum of 1252.9-1234.5 cm^{-1}. The weak peaks assigned at a spectrum of 1419.4-1415.2 cm^{-1} assigns rocking of =C-H bending. Small peaks assigned at a spectrum of 818.4-496.7cm^{-1} depicts the presence of –C-OH bending vibrations (Falade & Christopher, 2015). The presence of hydroxyl compounds attributes the WAC and OAC of the powdered samples(Sangeethapriya & Siddhuraju, 2014).

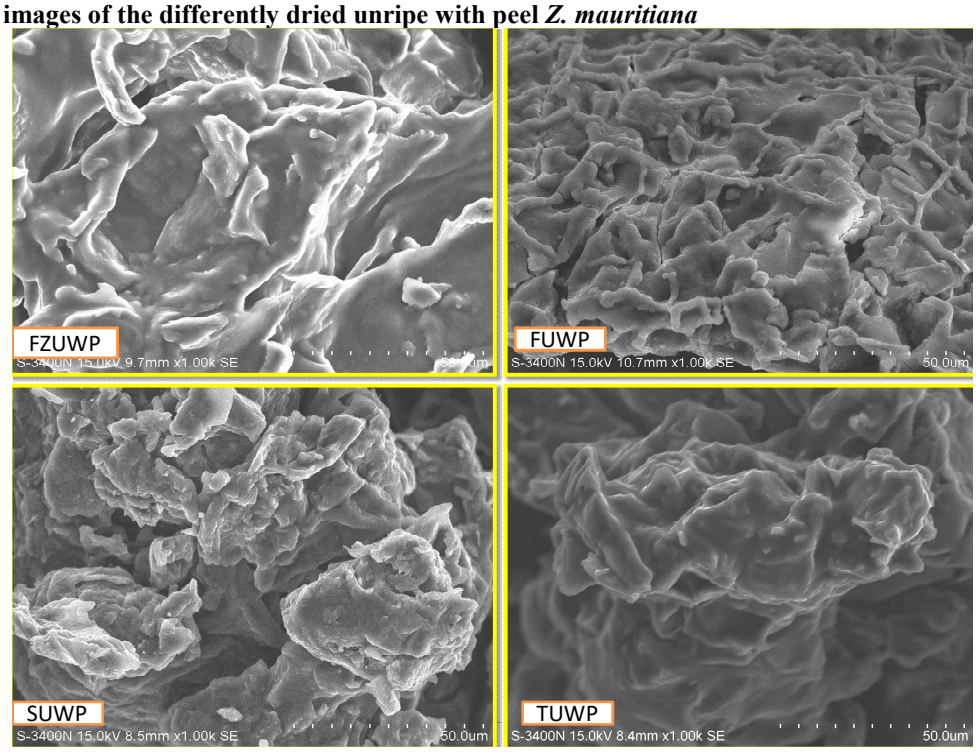
Fig. 2. FT-IR images of the differently dried unripe with peel *Z. mauritiana*



Scanning electron microscopy

The morphological study of differently dried jujube powders wereanalyzed using Scanning electronic microscopy and depicted in Fig .2. It was observed that the dried *Z.mauritiana* powders had amorphous structures.This may be attributed by milling during the powder preparation which causes the formation of amorphous biomaterials (Kim et al., 2012). The images showed more irregular and uneven surfaces with cracks, these available surface might help in the adsorption and binding properties(Sangeethapriya & Siddhuraju, 2014)

Fig.3. SEM images of the differently dried unripe with peel *Z. mauritiana*



Antioxidant properties

Table 4. Antioxidant properties of the differently dried unripe with peel *Z. mauritiana*

Samples	TPC	TFC	DPPH-IC50
FRESH	549.77 ± 6.55^{a}	218.068 ±3.49920^{a}	18.88 ± .65^{c}
FZD	4010.83 ± 49.16^{b}	892.63 ± 38.94^{b}	6.57 ± .73^{b}
FBD	3525.83 ± 214.16^{b}	1357.89 ± 40.00^{c}	3.99 ± .10^{a}
TD	7500.83 ± 315.83^{c}	1363.73 ± 13.15^{c}	7.03 ± .55^{b}
SD	29468.33 ± 315.83^{d}	2528.42 ± 109.47^{d}	3.26 ± .00^{a}

Total phenolic content

The TPC of the four differently dried unpeeled unripe jujube fruit powder samples and the fresh unpeel unripe jujube fruit sample of 80% methanolic extract showed a huge difference in the TPC that is ranging from 549µg GAE/g DW to 29468µg GAE/g DW (0.5-29.4 mg GAE/g DW). Here the least one is the fresh sample, next comes to the FBD, FZD, TD and the highest is the SD sample with 0.5, 3.5, 4.01, 7.5, and 29.4 mg GAE/g DW respectively. The reason for the higher TPC in the dried sample might be due to the prevention of phenolic compound loss by deactivation of the hydrolytic and oxidative enzymes. This result also goes in accordance with the study that says the TPC increases with the ambient temperature along with the vitamin C loss (S Sahin et al., 2018). These results somehow match with the prior findings of jujube fruit of different varieties by Li et al., (2005) and Kamilogu O et al., (2009) of range 5.18 - 8.53 mg GAE/g DW and 2 to 42 mg GAE/g DW respectively.

Total Flavonoid content

The TFC content in ascending order comes as FRESH<FZD<FBD<TD<SD with 0.2<0.8<1.35<1.36<2.5 mg catechin equivalents /g DW (0.2 to 2.5 mg or 218 to 2528 µg/g DW). The findings of Krishna H et al., (2013) show the TFC range of jujube fruit of various Indian varieties from 0.6 to 1.7 mg/g DW similar to that of our result. And this range also matches with other fruits like grapes and orange (Goulas et al., 2012) and lemon (Del R et al., 2004). The results show that both the thermal and non-thermal process of drying influence the increase in the TFC than the fresh sample and this is in accordance to the study of Chang C.H. et al., (2006) and the reason for the increase can be biochemically explained as many reactions happen internally in the peel and the pulp of the jujube fruit during the drying process like the breakdown of the bounded flavonoid compounds resulting in higher TFC.

DPPH IC-50

The most common method to determine the antioxidant capacity is by the stable free radical DPPH. The intensity of the free radical decreases as the scavenging takes place by the antioxidant present providing reduced absorption at 517nm (AS Upadhye et al., 2016). The DPPH-IC50 of the fresh and differently dried unpeeled unripe jujube fruit methanolic extract sample shows a range from 3.26 to 18.88 µg/ml. and the order is SD<FBD<FZD<<TD<FRESH with 3.26<3.99<6.57<7.03<18.88 µg/ml. The values are expressed in µg ascorbic acid equivalent/g DW of the sample. The lower DPPH-IC50 value indicates the higher scavenging ability of the compound. A previous study also says that unripe green jujube varieties provide better scavenging activity than the riped ones (AC LINN et al., 2012). Though the IC50 values of this study don't match with the previous studies of jujube, it's the scavenging activity of the SD methanolic extract sample was found higher than the other samples. The IC50 value of green *Z.mauritiana* was found as 7.5mg/ml (S Das, AC LINN et al., 2012) which much low when compared to the obtained result. This increase in the antioxidant capacity may be due to the drying process and extraction solvent and technique used.

Conclusion

The *Z.jujuba*is well known for its health benefits and it is also used to cure various health problems in the traditional method. This study also reveals that jujube has nutritionally important compounds along with higher antioxidant activity. The freeze dried sample preserved the color, flavor, and aroma of the unripe fruit paving way for its improved application in the food industry. The solar tunnel dried sample came up with increased antioxidant activities in multiple folds to the fresh sample. Drying techniques have influenced the phenolic compounds in the fruit with the difference in the temperature used. Considering the higher phenolic content in the green jujube it is much likely to expect an increase in the use of jujube in the pharmaceutical industries in the form of supplements or functional foods in the upcoming years. Further studies are required in the areas of the phenolic breakdown during the drying process with respect to the temperatures.

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STANDARDIZATION OF A METHOD FOR IMMOBILIZATION OF MICROORGANISM IN CHITOSAN BEADS

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Abstract
Soil acts as a reserve for various microorganism. The distribution of bacteria depends upon various factors. The number of cultural bacteria maybe even low in environmentally stressed conditions. The interaction between soil bacteria and plant may be beneficial, neutral, harmful. The (PGPB) are the plant growth promoting bacteria and these play a huge role in the production of biofertilizers. Through encapsulation technique the enzymes are immobilized. These are used in bioremediation, biodegradation, bio fertilizer production.

Key Words : PGPB strains, Immobilization

Introduction

Soil is the main source of innumerable microorganisms like bacteria fungi, algae and actinomycetes. The class bacteria arethe most common microorganism which accounts nearly 95%. Aeration, temperature, moisture, salts and other chemicals influence the type of bacteria present in the environment. Theconcentration of bacteria is usually high around the rhizosphere region as the nutrients such as sugars and amino acids are present there. The bacteria that enhancesthe plant growth are called Plant growth promoting bacteria (PGPB)[1]. These involve in regulating hormone level and they act as biocontrol agents by inhibiting plant pathogens. They also involve in phytoremediation where the plants take up environmental pollutants and degrade them. Chemical fertilizers used have hazardous nature and bio fertilizers are suggested as an alternative. PGPBacts as a Basic Components of a Bio Fertilizer.The strains such as *Burkholderia, Azospirillium, Enterobacter, Azotobacter, Rhizobium* and *flavobacterium* are some of the PGPB strains. These plant growth promoters increase the production of phytostimulators and the availability of nutrients such as Nitrogen, Phosphrous, Potassium, Copper, Iron and Zinc. Biofertilizers are made by the combination of one or more microorganisms. Encapsulation or particle immobilization is a technique used to entrap cells or biomolecules like enzyme within polymers[2]. The polymers that are commonly used are agarose, alginate, carrageenan, polyacrylamide[3].

Materials and Methods

Chitosan (100% deacetylated) and penta-sodium tripolyphosphate (TPP) (molecular weight: 367.86) are the chemicals required[4]. soil sample was collected from an organic farm land. soil sample was taken within 5 cm surrounding the rhizosphere region of focus carioca l. (fig tree) in clean bags and stored at 4°c before processing [5]

Procedure

Direct spreading method was performed to isolate the bacteria present in the sample. for which, 10g of the soil sample was first mixed in 100ml of sterile distilled water. The mixed solution was serially diluted from 10-1 to 10-8. each dilution was spread directly onto the surface of Luria berating agar (lab) medium (ph. 7.0). samples were incubated at 37°c overnight. after incubation, the colonies obtained were streaked onto lab plates for establishing individual colonies. The effective single isolates were transferred to 50% glycerol stock vials and deep frozen until next use. Bacterial cultures revived from glycerol stock were enriched in sterile lb medium (ph. 7.08). enriched cultures were streaked directly onto freshly prepared lab plates and incubated at 37°c overnight.

Morphology of the bacterial colonies was studied by direct observation. The colonies were consecutively sub-cultured 3 times onto sterile lab plates to obtain pure colonies. Molecular identification pure cultures of the isolates were sent for sequencing of the 16srna gene region. bacterial dana is to be extracted using extraction kit and par amplification of the gene is to be performed using the universal primers 27-f and 1492-r. sequence analysis is to be performed with blast and to be run against registered sequences in the embank database. formation of chitosan beads formation of beads was evaluated in sterile distilled water and lb medium. briefly, 0.3g of chitosan (3% w/v) was dissolved in 10 ml each of distilled water and lb medium separately. about 750 µl of the completely dissolved chitosan gels (chitosan in distilled water and chitosan in lb medium) were added dropwise onto 1% (w/v) TPP solution (ph. 9.02) and left undisturbed for several hours Immobilization in chitosan beads the bacterial cultures from lab plates were transferred to tubes containing 50 ml of lb medium and vortexed to ensure complete suspension. simultaneously, 3g of chitosan was dissolved in 50 ml sterile distilled water. the prepared chitosan solution was slowly poured onto the culture and mixed until no lumps were seen. Beads were formed by dropping the chitosan culture mixture onto TPP solution[67]. the beads were left undisturbed for 3 hours in TPP solution for hardening. the hardened beads were then washed twice in 0.15m dipotassium hydrogen orthophosphate (k2hpo4) buffer of ph. 8.0. the encapsulated chitosan beads were stored at 4°c. evaluation of immobilized bacterial isolates in chitosan beads to determine the viability of bacterial cells inside the chitosan beads, they were crushed manually and placed on nutrient agar (an) plate. also, the beads crushed and vortexed in 0.9% saline were spread plated onto a medium. all the plates were incubated at 37°c for 24 hours to check the bacterial growth[8].

Results

Generally, the plant growth promoting bacteria can be formulated into efficient bio-inoculants and can be used as biofertilizers, the green alternatives to chemical fertilizers and pesticides. Different soil bacteria isolated so far, also play crucial roles as bio-remediators addressing the environmental degradation. In this study, a bacteria were isolated from the rhizosphere of Ficus carica L. and 16S rRNA sequencing was carried out to identify the bacterial species. Encapsulation of the bacterial isolates was successful in 3% chitosan-TPP beads. Also, the immobilized cells were found to be viable[9]. Hence, chitosan TPP blends can be effectively used to immobilize the isolated bacteria and can be further used for bio-remediation and bio inoculant application.

Fig. 1. Plating of enriched culture: a) Strain 2 LBA b) Strain 3 LBA

Isolation and culture of bacterial colonies

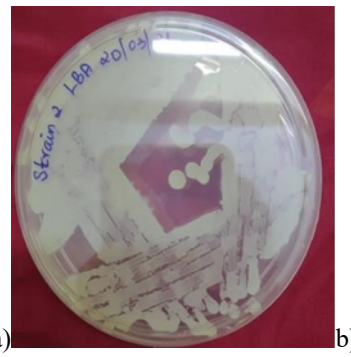
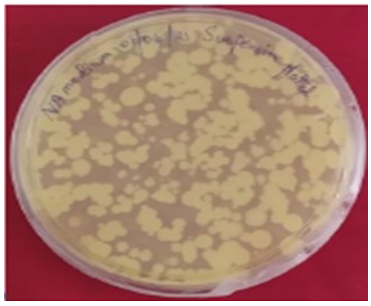
a)  b) 

Fig. 2. The Growth of Colonies from the immobilized bacterial isolates in chitosan beads

Evaluation of immobilized bacterial isolates in chitosan beads



Discussion

The primary purpose of the study was isolate and immobilize the bacteria in chitosan-TTP beads. The use of bacterial inoculants has contributed to increased usage of bio fertilizers[10]. The basic technique applied is the cell immobilization of the isolated bacteria in a biodegradable polymer[8]. Previous studies such as Angelim *et al*. (2013) developed a bioremediation strategy, both bio-augmentation and bio-stimulation for oil contaminated mangrove sediments using chitosan beads and chanratana *et al*. (2018) evaluated the plant growth promoting activity of chitosan[611]. Usage of chitosan beads can be considered as a potential delivery system for plant growth promoting bacteria[512].

Conclusion

The use of bacterial inoculants has contributed to increased agronomic efficacy by reducingproduction cost and use of chemical fertilizers. Such inoculants can also be used in bioremediationof pesticides applications. Isolation of such bacteria types from the commonresources of soil are crucial. Cell immobilization of the isolated bacteria in a biodegradable polymermatrix is an important process for the proper functioning of formulations in field conditions'. Formulations based on chitosan and tripolyphosphate were preparedusing a bacteria obtained from the root regions of Ficus carica L.

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FORMULATION AND QUALITY EVALUATION OF VALUE ADDED PICKLE FROM PLANTAIN *Musa paradisiaca L.* (FLOWER, STEM AND UNRIPE BANANA)

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Abstract
The study was conducted to develop the pickle from plantains (flower, stem and unripe banana) and analysed for physico- chemical properties, microbial status, sensory attributes, overall storability and HACCP plan forthe pickles. Pickle was prepared with salt, oil and spices in 3 variations. The physico- chemical analysis of pickles showed that moisture content, total solids, pH, acidity, peroxide value of variation 3 (plantain flower + unripe banana) was highly acceptable. The microbiological studies revealed that a total bacterial count was less in the pickles prepared without any preservatives. The 25 sensory panellists marked for colour, flavour, texture and overall acceptability and analysed statistically. Storage studies were carried out up to one month at room temperature plantain flower + unripe banana pickle was best and acceptable.

Key wprds: *Musa paradisiaca,* Value addition

Introduction

Plantains play an important role in income generation for both large scale and small scale holders in the country, especially for those who produce them within their homestead or gardens (Falade *et.al,* 2010). Plantain is a plant producing fruits that are starchy at maturity.

Banana inflorescences are popularly known as 'navels' (Catharina Fingalo *et. al,* 2012). Banana flower, also known as banana blossom or heart or bud, is the splendid looking male, sterile flower of the banana plant (Khin Nann Nyunt Swe 2012).Musa flowers have been traditionally used to alleviate menorrhagia, dysentery, diabetes mellitus, heart pain, diarrhoea, stomach cramps and infantile mal nutrition. It acts against cancer cells and also reduce cardiovascular diseases (Debabandhya Mohapatra *et.al,* 2010).

The inner part of tender pseudo-stem is edible, fibrous and it is highly beneficial. After the fruit production, the trunk of the banana plant that is the pseudo-stem is thrown as agricultural waste to great extent. Out of the 14-18 sheaths available in stem, the outermost 4-6 sheaths yield course fibre (Vigneswaran *et.al,* 2015). Banana stem is high in fiber & can aid in the treatment of ulcers or an acidic stomach (Namrata Sutur *et.al,* 2010). It has cooling properties, which are beneficial in hot environments. Banana stem is also a diuretic diet & can help prevent kidney stone (Sabyasachi Mishra *et. al,* 2014).

In the unripe stage, banana stands out for having high starch content, deserving industrial interest for developing new products. When it is in the unripe stage, banana is considered to be a functional food of the prebiotic type. A few species, such as "Monthan" (*Musa paradisiacal*), are less sweet to taste and people to consume unripe or green (Vasso Apostolopoulos *et.al,* 2017 and Abiodun- Solanke *et. al,* 2010)

Pickle is a vegetable based convenience food. Pickle is one of the oldest and most successful methods of food preservation known to human. Pickle is defined as an edible product that has been preserved and flavoured in a solution of brine and edible acid such as vinegar (Arzu Akpinar Bayizit *et. al,* 2007). Therefore, the overall aim of the research is to process and preserve plantain pickles are as follows: To formulation, sensory acceptability, yield and cost, physico- chemical properties, statistical data and shelf-life characteristics of plantain pickle

Materials and Methods

Plantain flower was taken discard the pink outer most layer of the banana flower, remove the 2 inedible portion (stamen and flap) from the flower and it was cleaned. Cut into small pieces, soak the pieces into buttermilk. Plantain stem was taken, discard the porous outer layer of stem. Peel to scrape inedible portion in inner layer. Slice stem into circles and remove threads like fibers cleaned and the peeled, cut into small pieces, soaked the pieces into buttermilk. Unripe banana was taken remove the peel and it was cleaned, soak the pieces into buttermilk. (40ml) gingelly oil was taken in a sauce pan and heated it with (5g) of Mustard with (6) Curry leaves, add (15g) of ginger garlic paste, now based on variations plantain flower pieces, plantain stem pieces and unripe banana respectively added to it and fried continuously by adding (5g) of Chilli Powder, (2g) of Asafoetida, and (3g) of fenugreek seeds. Finally, the Pickle was coated with the remaining (10ml) of gingelly Oil.

Ingredients	Variation 1 (FS)	Variation 2(SU)	Variation 3(FU)
Plantain flower	100g	-	100g
Plantain stem	100g	100g	-
Unripe banana	-	100g	100g
Salt	10g	10g	10g
Chilli powder	5g	5g	5g
Asafoetida	2g	2g	2g
Gingelly oil	50ml	50ml	50ml
Curry leaves	6 leaves	6 leaves	6 leaves

Mustard Seeds	5g	5g	5g
Ginger	8g	8g	8g
Garlic	8g	8g	8g
Fenugreek seeds	3g	3g	3g

Sensory evaluation

The value added pickles were prepared using plantains and are organoleptic ally evaluated by a panel of 25 semi trained members on 5 points hedonic scale. the parameters evaluated were colour and appearance, consistency, taste, flavour and overall acceptability. The statistical inference of formulated plantain pickle was calculated based on the data from the mean sensory scores. All the data were analyzed by two-way analysis (ANOVA) of variance at 5% significant level.

Quality analysis

Formulation of value added pickle from plantains in 3 different variations (FS, SU and FU), the sensory analysis was conducted on 1st day variation 3 (FU) had excelled in all terms of sensory characteristics than variation 1 (FS) and variation 2 (SU). Thus the variation 3 was selected for quality analysis such as physic- chemical parameters and total plate count as per the standards and methods.

Nutrient content

Nutritive value such as Energy, protein, carbohydrates , fat , fiber , calcium , iron and other specific aspects are computed .The nutrient content were calculated for the selected product using "Nutritive value of Indian Food" by National Institute of Nutrition, Hyderabad, India (2010)

HACCP plan

HACCP is a management system in which food safety is addressed through the analysis and control of biological, chemical, and physical hazards from raw material production, procurement and handling, to manufacturing, distribution and consumption of the finished product. HACCP (Hazard Analysis Critical Control Point) consists of 7 principles and 5 preliminary steps. Appoint multi- disciplinary food safety team include production manager, quality assurance manager, food analyst, Maintain record of knowledge and experience of the team (Food analysis- Khetarpaul 2002). HACCP plan was implemented for plantain pickle (flower, stem and unripe banana).

Results and Discussion

Sensory evaluation

The value added pickle from plantain was formulated in three different variations (FS, SU, and FU) which the sensory analysis conducted for 1st day, 15th day and 30th day with sensory panel of 25 members. The variation 3 (FU) pickle had excellent in all terms of sensory characteristics right from the 1st day to the 30th day of storage of the pickle, due to the increased oil absorption rate and browning was lesser than variation 1 (FS) and variation 2 (SU). Hence the variation 3 (FU) pickle is overall acceptable in terms of like colour and appearance, consistency, flavour and taste than variation 1 (FS) and variation 2 (SU) and shelf-life character was better.

The statistical analysis was conducted based on the sensory scores data using ANOVA for 3 three variations (FS, SU and FU) of plantain pickle, there is no significance difference between the 3 variations at 5 % significance level (F<0.05%).

Criteria	Variation 1 (FS)			Variation 2 (SU)			Variation 3 (FU)		
	1day	15days	30days	1day	15days	30days	1day	15days	30days
Colour & appearance	3.6±0.6	3.52±0.5	3.5±0.42	3.73±0.57	3.7±0.47	3.75±0.52	4.2±0.66	4.25±0.68	4.28±0.7
Consistency	3.4±0.48	3.44±0.3	3.46±0.32	4.06±0.57	4±0.48	4.1±0.53	4.26±0.44	4.18±0.42	4.9±0.43
Flavour	3.46±0.5	3.5±0.56	3.53±0.57	4.13±0.5	4.2±0.6	4.22±0.58	4.4±0.46	4.5±0.47	4.52±0.48
Taste	3.26±0.67	3.3±0.57	3.34±0.6	4±0.63	4.12±0.56	4.18±0.6	4.46±0.5	4.38±0.46	4.44±0.49
Overall acceptabily	3.33±0.7	3.2±0.4	3.28±0.62	3.8±0.53	3.9±0.55	4±0.56	4.33±0.47	4.35±0.4	4.36±0.5

Quality analysis

Plantain flower and unripe banana pickle contains drained weight 65%, moisture content 41.6% and total solids. Sultana (2014) studied that developed mixed pickle using carrot, green chilli, brinjal contains moisture 63.7, total solids 36.2. Plantain flower and unripe banana pickle contains titrable acidity 1.28% and pH 4.9. Panda *et. al*., (2006) research was did lactic acid fermentation of sweet potato into pickles with 8 and 10% brine solutions had a pH of 2.9–3.0 and titrable acidity of 2.9–3.7 g/kg. Plantain flower and unripe banana pickle contains peroxide value 27meq/kg and total bacterial count 8 cfu/g. Balaswamy *et al.* (2010) roe pickle prepared with rose of rohu was analysed for microbial load in terms of total plate count (TPC) results revealed that an increase in TPC from 4.8 to 5.0 log cfu/gm after 6 months of storage was observed.

Physico-chemical parameters	Standard/Method	Values
Drained weight	not less than 60% (FSSAI and FPO)	65%
pH	not less than 4.8 (BIS)	4.9
Moisture content	AOAC	41.6%
Total solids	FSSAI	58.4%
Titrable acidity	1.2 % (FPO)	1.28 %
Peroxide value	FPO	27 meq /kg
Total bacterial count	10^{-6} cfu (FAO)	8 cfu /g

Nutrient content

Variation 3 (FU) pickle has higher energy value (48.52kcal) than variation 2 (FS) and variation 1(SU) (43.41kcal and 40.7kcal). Variation 1 (FS) pickle has higher fat content (3.65g) than variation 2 (SU) and variation 3 (FU) (3.48g and 3.47g). variation 3 (FU) contain high carbohydrate value (4.46), variation 1 (FS) and variation 2 (SU) have minimum carbohydrate value (2.23g and 2.33g). Plantain flower + unripe banana pickle has high protein content (35.51g) and variation 1 (FS) and variation 2 (SU) contain less protein content (0.75g and 0.37g). Variation 2 (SU) contains high crude fiber content (0.97g), variation 1 (FS) and variation 3 (FU) contains minimum crude fiber content (0.56g and 0.46g). Plantain pickles have lesser mineral content (0.49g, 0.3g and 0.13g).

The nutrient content of plantain pickle was compared to Shanta *et. al*, (2014) formulate stem and amaranth pickle contains 2.80 % proteins, 0.12 % fat, 3 % ash content and Javier Casado *et. al*, (2004) studied that proximate composition of garlic pickle contains 3.35 % of protein, 2.1 % dietary fibre,0.35 % fat and 8.40 % ash content which was closed to the plantain pickles nutrient content.

Nutrients	**Variation 1** (FS)	**Variation 2** (SU)	**Variation 3** (FU)
Energy	43.41kcal	40.7kcal	48.52kcal
Fat	3.65g	3.47g	3.48g
Carbohydrate	2.23g	2.33g	4.46g
Protein	0.75g	0.37g	35.52g
Crude fiber	0.56g	0.97g	0.46g
Minerals	0.49g	0.13g	0.3g

HACCP plan

Product Description	Plantain pickle was standardized nutritious product and best way of utilizations of plantains
Composition	Plantains- flower (25%), stem (25%), unripe banana (25%), oil (10%), salt (10%), spices (5%)
Method of Preservation	Heating with oil, salt and spices
Packaging-Primary	Glass containers
Packaging-Secondary	Shrink wrap
Storage Conditions	Room temperature
Distribution Method	Wholesale-Retail, Retail-Consumers
Shelf Life	1-2 months
Special Labelling	Blood pressure patients not consumed
Sensitive population	Hypertension
Intended Use	All age groups

	Principle 1							Principle 2	
Step/ Input	Hazard	Cause	Likelihood	Severity	Control measures	Q1	Q2	CCP	Reason for Decision
Purchasing	Physical Chemical Biological	Dust, dirt, stones, sticks, metal pieces, insects, flies	High (3)	High(3)	Proper quality tested raw material was purchased	Yes	Yes	CCP	It may affect the quality of final product and cause health benefits
Washing& cutting	Physical Chemical	Dust, dirt, stones, sticks, metal pieces	Medium (2)	Medium (2)	Hygienic condition	Yes	No	Not CCP	Prevent contamination
Soaking	Biological	Enzymatic reaction	High (3)	High(3)	Don't use old mothers cultured butter milk	Yes	Yes	CCP	Prevent browning
Cooking	Chemical	Metallic contamination	Medium(2)	Medium(2)	Hygienic condition	Yes	No	Not CCP	Prevent rancid flavor & metal contamination
Filling & storage	Physical Chemical Biological	Dust, dirt in bottle, metal pieces, Microbes	High(3)	High(3)	Cool to filling and proper sealing, Stored at room temperature	Yes	Yes	CCP	Prevent contaminations and human food safety

Step/ Input	Hazard	P1 P2 Critical control measure(6)	Hazard Type(7)	P3 Critical Limit(8)	Monitoring (9) What	Where	How	When	Who	P5 Corrective Action(10) What & Who	P6 Verification(11) What & Who	P7 Record
Raw material& processing	Physical, chemical & biological hazard	Prevent browning	Physical, chemical & biological	Enzyme reaction and moisture content	Dust, dirt, stone, sticks	Preprepation and cooking	Visual inspection	processing	Food analyst	Proper handling, don't use old mothers cultured butter milk production manager	Prevent browning & food analyst	Project report
Finished product inspection	Biological hazard	Microbial load	Biological hazard	Rancid flavour	Microbes & odour	storage	Microbial analysis	storage	Food analyst	Add excess oil Production manangr	Prevent browning & microbes Food analyst	Project report

Conclusion

Plantain pickle was standardized, physico- chemical properties, nutritive value, cost of the product and statistical analysis were calculated. The results revealed that plantain pickle was excellent in all its sensory characteristics quality analysis, statistical analysis. The pickle had good sensory characteristics and better acceptance during storage for 30 days. Thus this study indicates about acceptance of plantain pickle which could be best way of utilization of plantain.

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INSILICO ANALYSIS OF FLAVONOID PATHWAY ENZYMES OF EUPHORBIACEAE FAMILY

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Abstract

Plant species from Eurphobiacea family are taken for insilico analysis from the available protein base. 10 plant species from this family and 10 selected enzymes are taken for dry lab analaysis. The phylogenetic relationship for the compounds in flavonoid biosynthesis for the chosen ten plants with various families was investigated utilizing the KEGG pathway data set.Various flavonoid accumulate subsequently the amino corrosive arrangement was retrieved from the data set and were adjusted utilizing different succession arrangement and further broke down in Clustal omega.Different families showed fluctuation for the enzyme.

Key words: Flavonoids, Phytochemicals, Phylogenetic, Clustal Omega

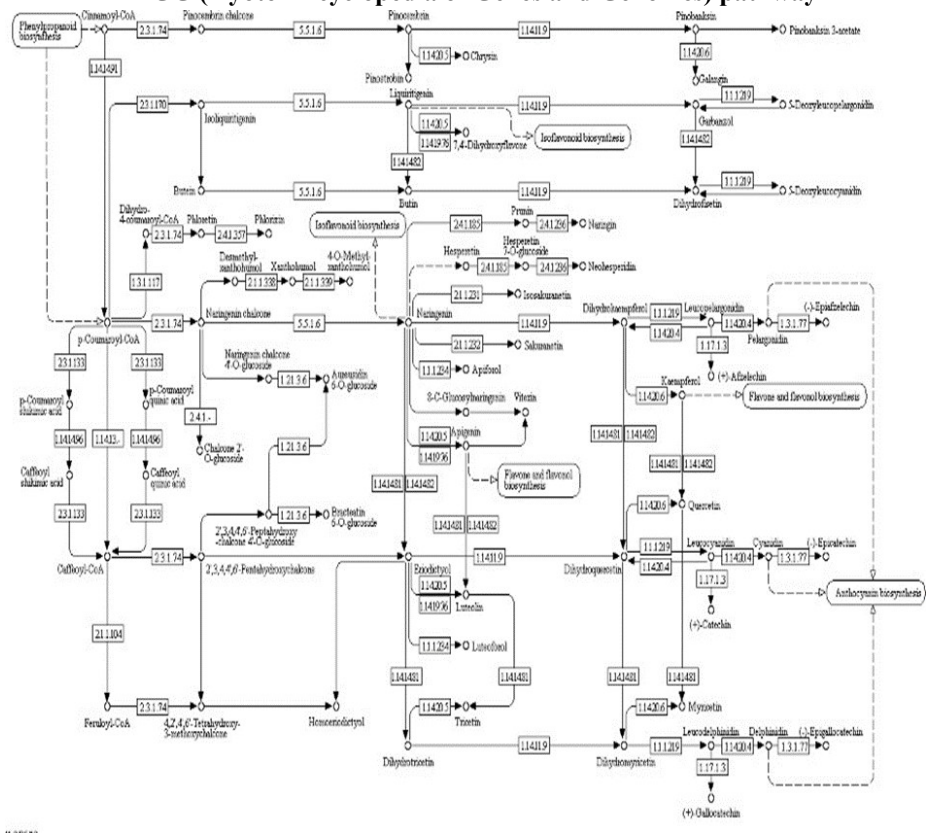
Introduction

Euphorbiaceaeor the spurge family is the largest family among flowering plants and consists of 300 genera and around 7,500 species[1].Plant species from eurphobiacea family are taken for insilico analysis from the available protein base.10 plant species from this familyand 10 selected enzymes are taken for dry labanalaysis. Euphorbiaceae has auxiliary metabolite classes like alkaloids, flavonoids, tannins, glycosides, diterpenes, and different mixtures[23].

KEGG Pathway

KEGG(Kyoto Encyclopedia of Genes and Genomes) pathway develop an organization that gathered the Alzheimer's infection pathway and showed the outcomes on bunching and determination of center pathway.KEGG pathway needs to utilize slope investigation from chief compound examination (PCA)[4]. Flavonoid content in Dendrobium is still up in the air with the standard references. In which 23139 unigenes were acquired from genome information. 10398 qualities were explained in the quality metaphysics data set, 4203 were clarified in the KEGG data set. 31 unigenes explained in the KEGG information base have been engaged with the flavonoid pathway[5]. These qualities were associated with bio-change, transportation, collection, and the guideline of flavonoid biosynthesis and inferred that figuring out the articulation level were connected with qualities.

KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway



As per the figure we can understand KEGG pathway information base is unbeatable around worldwide.

***KEGG stores** data like*

a) *classification pathways*
b) *subcategory pathways*
c) *optional pathways*

which are encoded by coupled qualities on the chromosomes and are especially helpful in expectation of quality capacity.

Sequence retrived from KEGG database:

Multiple sequence alignnment is a progressive alignment in which more no. of sequence aligned for phylogenetic analysis. Amino acid sequence areretrived from the KEGG database in the flavonoid biosynthesis pathway. Alignment done based on functional or structural similarity[8]. Aminoacid sequence are retrived in fasta format (complete sequence). Analysed using Clustal omega (ClustalΩ).Clustal omega is a software package used for analysing belonged data, either gene or protein[54].

Sample collection

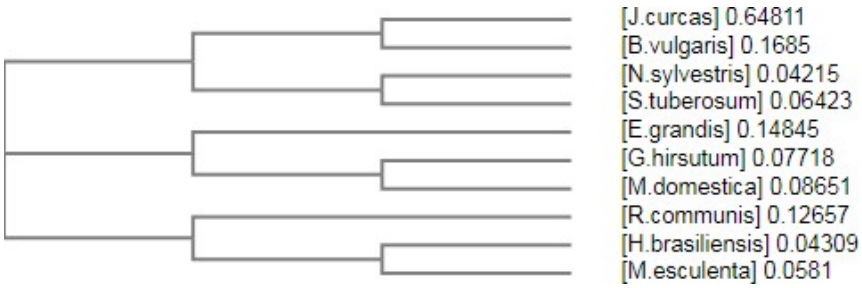
The Euphorbiaceaesamples which contain medicinal values were collected from local area of Coimbatore are considered as medicinal plants, the plant species selected for analysis from euphorbiacea family are Acalypha indica, Euphorbia hirta,Euphorbia milli,Phyllanthus emblica, Phyllanthus acidus, Jatropha gossypifolia, Jatropha curcus, Riccinus communis, Manihot esculenta ,Crotonbonplandiaum.

Table 1. Enzymes choosen from KEGG pathway for *in silico* analysis

No	Enzymes
1	Anthocyanidinreductase
2	Acyltransferase
3	Chalconeflavononeisomerase
4	Chalconesynthase
5	Dihydroflavonol4-reductase
6	Flavonoid3-monooxygenase
7	leucoanthocynidinreductase
8	Methyltransferase
9	Naringenin3-dioxygenase
10	Flavonolsynthase

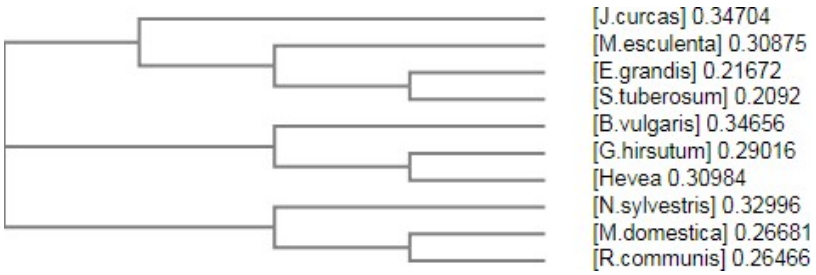
Phylogenetic analysis for enzymes in Flavonoids

1.PhylogenetictreeofAnthocyanidin reductase



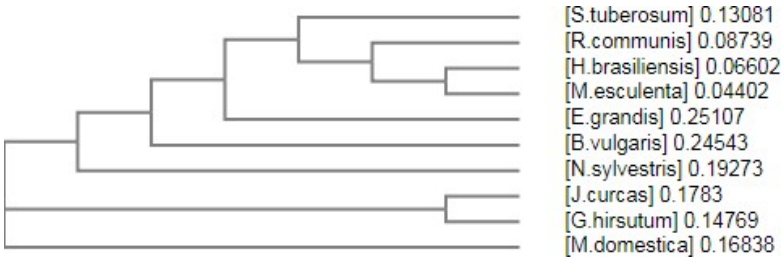
Sequences of M.esculenta(0.0581) and H. brasiliensis(0.04309) were found to besimilar and belongs to Euphorbiaceaefamily. The sequence of R.communis(0.1265) isdiverged from M.esculenta and Hevea family. M. domestica (0.0865) and G.hirsutum(0.0771)werefoundtobesimilarwiththesequence.E.grandisisvaried fromM.grandisand G. hirsutum. Sequence of N. sylvestris (0.0421) and S.tuberosum(0.0654) were foundto be similar with the sequence of J. curcas(0.6481) and B. vulgaris (0.1685) family. Allthesefamilyshows therelationship with homologoussequencebetween differenttaxa.

2.PhylogenetictreeofAcyltransferase



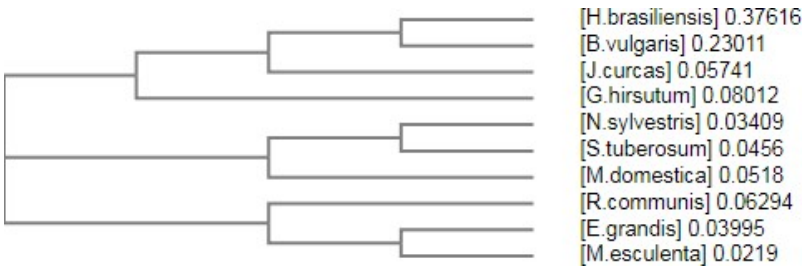
Sequence variance of *M. domestica* (0.3470) and *R.communis*(0.2646) were foundto be similar which is deviated from the sequence of *N. sylvestris* (0.3299) and it showedthe common relationship. *G. hirsutum*(0.2901) and *H. brasiliensis*(0.3098) were found tobesimilartothesequence,whichisvariedfrom*B.vulgaris*family.*E.grandis*(0.2167)and*S.tuberosum*(0.2092) were similar in variance. *M.esculenta*(0.3087) which has commonsequencesimilaritytothe*J.curcas*(0.3470)belongsto*Euphorbiaceae*family.Thehomologous sequence of enzyme acyl transferase showed phylogenetic relationship withdifferent taxonomyof plant

3.Phylogenetictree ofChalconeflavononeisomerase



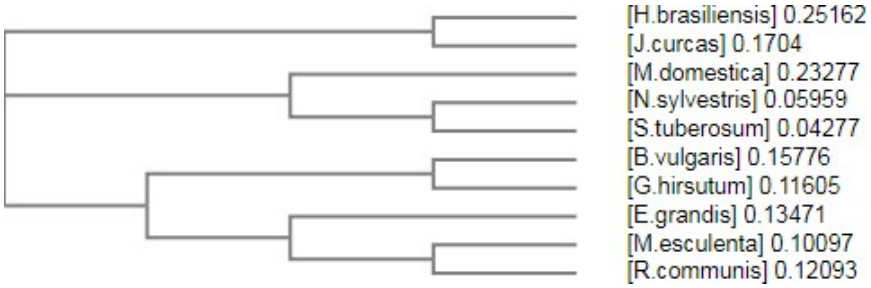
Sequenceof*M.esculenta*(0.0440)and*H.brasiliensis*(0.0660)of*Euphorbiaceae* familyweresimilartothesequencewhichisdeviatedfromthesequence*R.communis*(0.0873)and*S.tuberosum*(0.1308).Thes equenceof*E.grandis*(0.25107)showedvariancewith *B. vulgaris* family. *J. curcas*(0.1783) and *G. hirsutum*(0.1476) sequence showedcommon similarity in variance. *N.sylvestris*(0.1927) showed high level of variance. *M.domestica* (0.1683) has common sequence similarity. The sequence of enzyme chalconeflavonone has thephylogenetic relationship ofhomologous sequence with differenttaxonomy.

4.Phylogenetic treeofChalconesynthase



Sequence of *H. brasiliensis*(0.3761) and *B.vulgaris*(0.2301) showed similarity invariancewhichisdeviatedfrom*J.curcus*family.Sequenceof*J.curcus*(0.05741)issimilarto the sequence of *G.hirsutum*family. Sequence of *N. sylvestris* (0.0340) and *S. tuberosum*(0.0456)weresimilarwithsequencevariance.Sequenceof*E.grandis*(0.0399)and*M.esculenta*(0.0219)weresimilarinthevarianceofsequenceof*R.communis*(0.0629).Thehomologous sequence of enzyme chalcone synthase has common similarity with differenttaxonomyof plant byphylogeneticanalysis.

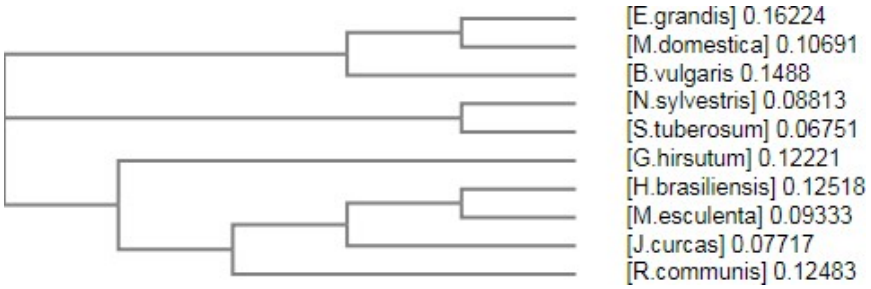
5.PhylogenetictreeofDihydroflavonol4-reductase(DHFR)



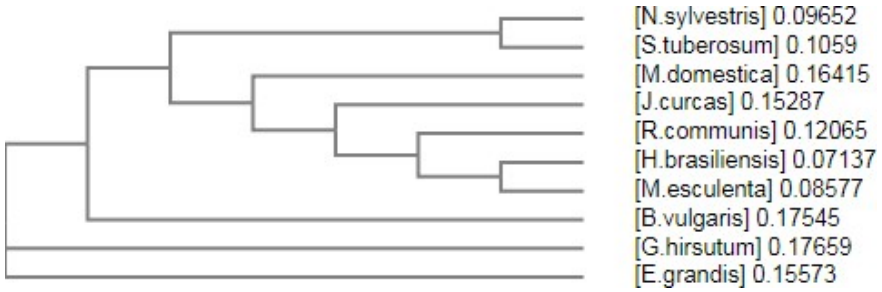
Sequence of *R.communis*(0.1209) and *M.esculenta*(0.1009) were similar whichbelongs*Euphorbiaceae.E.grandis*(0.1371)isvariedwithhighvariance.*B.vulagaris*(0.1577)and*G.hirsutum*(0.1160)wererelativelysimilarinvarianceSequenceof*N.sylvestris*(0.0596)and*S.tuberosum*(0.0427)werehighlysimilarinvarianceanddeviatedfromthe*M.domestica*(0.2327).Sequenceof*H.brasiliensis*(0.2516)and*J.*curcas(0.1704)were similar belongs to *Euphorbiaceae*. Homologous sequence for the enzymes DHFRshowed the similaritywithin thetaxanomyof plantsbyphylogeneticanalysis

6.Phylogenetictree ofFlavonoid3monooxygenase

The enzyme flavonoid 3 monooxygenase in the family *E. grandis* (0.1622) and*M.domestica*(0.1069)showedsimilaritywithhighlevelofvariance,theyaredeviatedwith*B.vulgaris*(0.1488)insequence.Sequenceof*N.sylvestris*(0.0881)and*S.tuberosum*(0.06751)showedsimilarvariance.*H.brasiliensis*(0.1251)and*M.esculenta*(0.0933)weresimilar to the sequence of *J.curcas*(0.0771) and *R.communis*(0.1248) are highly similarand has same origin *Euphorbiaceae.* All these families has common relationship to thesequenceof*G.hirsutum*(0.1221)byphylogeneticanalysis.Theenzymeflavonoid3monooxygenasehas thehomologous sequencewithin the taxonomyofthe plants



7.PhylogeneticanalysisofLeucoanthocynidin reductase

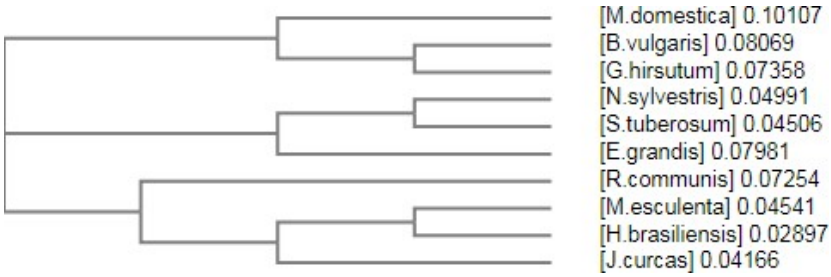


Thesequenceofenzymesleucoanthocynidinhassimilaritywithin*N.sylvestris*(0.0965)and*S.tuberosum*(0.1029).*J .curcas*(0.1528),*R.communis*(0.1206),*H.brasiliensis*(0.0713) and *M. esculenta* (0.0857) were highly similar which belongs to *Euphorbiaceae*andshowedtheirvarianceandphylogeneticrelationshipbetweenthefamilies.Sequenceof*G.hirsutum*(0.1765) and *E.grandis*(0.1557) has common similarity with all these familieswithsimilarvariance.Thusthephylogeneticrelationfortheenzymeleucoanthocynidinaresimilar within theplant taxonomyto determinetheir relationship amongthetaxa.

8.PhylogenetictreeofMethyltransferase

Thesequenceofenzymesmethyltranferaseinthefamily*G.hirsutum*(0.0632)and *N. sylvestris* (0.0663) showed similar to the sequence. *H. brasiliensis*(0.09399) werediverged with high variance. *J. curcas*(0.1402) and *M.esculenta*(0.1558) has a commonsequence similar in *Euphorbiaceae*. *M. domestica* (0.1221) and *R.communis*(0.1102) has acommon sequence similar. *S. tuberosum* (0.1664) were deviated from family and showedphylogenetic analysis. The sequence of *B. vulgaris* (0.2912) has a common similarity withallfamilies byphylogeneticanalysis within different taxonomyof plant

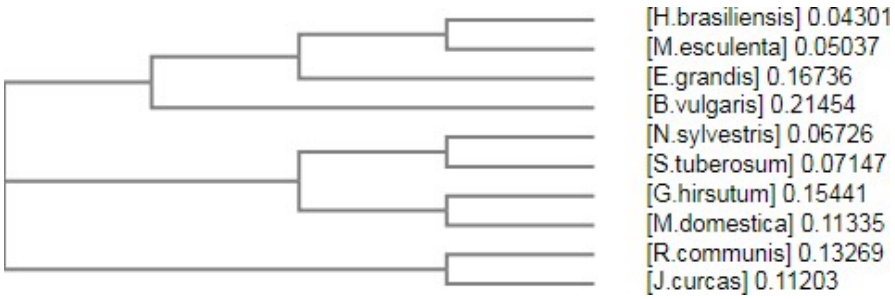
9.Phylogenetictree ofNaringenin 3-dioxygenase



The sequence of *B.vulgaris*(0.0806) and *G. hirsutum*(0.07365) has a commonsimilarity with variance. The sequence of *N. sylvestris* (0.0499) and *S.tuberosum*(0.0450)hasa commonsimilarityinsequence.The sequence of*M.esculenta*(0.0454)and*H.*brasiliensis (0.0286) has a common similarity with sequence of *Euphorbiaceae*. *J. curcas*(0.04166)sequencearedeviatedandsimilartothesequenceof*R.communis*(0.04725).Thus

thesequenceoftheenzymeNaringeninshowedphylogeneticanalysiswithinthedifferenttaxaof theplant.

10.Phylogenetic tree of Flavonolsynthase



Sequence of *H.brasiliensis*(0.0430) and *M.esculenta*(0.0503) were highly similarwhich corresponds to the family *E.grandis*(0.1673) are diverged with variance. *E.grandis*are highly similar to the family *B. vulgaris* (0.2145) *N.sylvestris*(0.0672) and *S. tuberosum*(0.0714) sequence are similar in variance value. *G.hirsutum*(0.01544) and *M.domestica*(0.1133) sequence also similar with less variance has common phylogenetic relationshipwith the sequence of *R.communis*(0.1326) and *J. curcas*(0.1120) of family.Thus theenzyme flavonol synthase were analysedphylogeneticaly with different *Euphorbiaceae*familiesof plants.

Table 2. Analysis of variance: Enzymes are Anthocyanidin reductase, Acyltransferase, Chalcone flavonone isomerase, Chalcone synthase,Dihydroflavonol 4-reductase.

Plant	Anthocyanidin	Acyltransferase	Chalcon eflavono ne	Chalcon esyntha se	Dihydroflavonol -4reductase
Jatropha curcus	0.61	0.34	0.17	0.03	0.09
Betavulgaris	0.10	0.34	0.02	0.15	0.15
Nicotiana sylvestris	0.04	0.32	0.10	0.03	0.05
Solanumtuber osum	0.06	0.20	0.05	0.04	0.04
Eukalyptus grandis	0.14	0.21	0.25	0.03	0.13
Gossypium hirsutum	0.08	0.3	0.14	0.08	0.11
Malus domestica	0.07	0.27	0.16	0.05	0.24
Riccinuscom mnis	0.12	0.26	0.08	0.06	0.12
Heveabrasilien sis	0.04	0.30	0.07	0.38	0.25
Manihot esculenta	0.06	0.31	0.05	0.02	0.10

Table 3.Analysis of variance: Enzymes are Flavonoid 3- monooxygenase, leucoanthocynidin reductase, Methyltransferase, Naringenin 3- dioxygenase, Flavonol synthase.

Plant	**Flavonoid 3 monooxygenase**	**Leuco anthocynidin**	**Methyltransferase**	**Narngenin3-dioxygenase**	**Flavonol synthase**
Jatropha curcus	0.04	0.13	0.14	0.01	0.11
Beta vulgaris	0.07	0.17	0.29	0.08	0.21
Nicotiana sylvestris	0.09	0.09	0.07	0.04	0.06
Solanum tuberosum	0.06	0.10	0.16	0.04	0.07
Eukalyptus grandis	0.16	0.15	0.19	0.07	0.16
Gossypium hirsutum	0.12	0.01	0.06	0.07	0.15
Malus domestica	0.10	0.16	0.12	0.10	0.11
Riccinus commnis	0.12	0.04	0.11	0.07	0.13
Heveabrasiliensis	0.12	0.07	0.09	0.28	0.04
Manihotesculenta	0.09	0.08	0.15	0.04	0.05

Conclusion

Phytochemical screening was performed to decide the subjective examination for the presence of optional metabolites[9]. Among ten plants, two were chosen to decide the amount of flavonoid compound. The phylogenetic relationship for the compounds in flavonoid biosynthesis for the chosen ten plants with various families was investigated utilizing the KEGG pathway data set. Various flavonoid accumulate subsequently the amino corrosive arrangement was retrieved from the data set and were adjusted utilizing different succession arrangement and further broke down in Clustal omega. In which the four Euphorbiaceae plants *M.esculenta, R.communis, J.curcus,* and *H. brasiliensis* showed more comparativeand it addresses the normal beginning. Other families showed variance for the enzymes. Thusthe analysis showed that the enzymes taken for study possess more similarity for the Euphorbiaceae plant

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AN OPEN COLLABORATIVE DRUG DISCOVERY MODEL

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Abstract

Drug discovery is a process which aims at identifying a compound therapeutically useful in curing and treating disease. This process involves the identification of candidate's synthesis characterization, validation, optimization, screening and assays for therapeutic efficacy. Once a compound has shown its significance in these investigations, it will initiate the process of drug development earlier to clinical trials. New drug development process must continue through several stages in order to make a medicine that is safe, effective, and has approved all regulatory requirements. One overall theme of our article is that the process is sufficiently long, complex, and expensive so that many biological targets must be considered for every new medicine ultimately approved for clinical use and new research tools may be needed to investigate each new target. From initial discovery to a marketable medicine is a long, challenging task. It takes about 12 – 15 years from discovery to the approved medicine and requires an investment of about US $ 1 billion. On an average, a million molecules screened but only a single is explored in late-stage clinical trials and is finally made obtainable for patients. This article provides a brief outline of the processes of new drug discovery and development.

Keywords: Drug discovery, clinical trials, biological target.

Introduction

Drug disclosure in clinical preliminary is an extremely extensive undertaking and takes around 10-12years for execution.With late advances in AI and further work that has been done in this field has given us extension to accomplish more experimentation.the get here is to observe a virtual compound which assists us with requiring some investment for superfluous walkthrough for the cycle. Past work in this field incorporates a nontrivial AI model imitate normal synthetic variety for wanted particlesinthe intel open VINO toolbox depends on two-venture process where initial step is making the streamlining that creates.Early drug discovery is essential part of pharmaceutical sector. The entire drug discovery process during clinical trials takes lot of time because there are multiple phases of testing namely phase 1, phase 2 and phase 3 trials. A large portion of the times drugs compound bombs testing at stage 2 and stage 3. The conventional interaction includes essential examination to reveal focuses on that might be vulnerable to assault, for example, an illness related protein receptor on the outer layer of specific cells.Then, various techniques for natural and substance testing are utilized to adjust the design or test different highlights, for example, a compound's capacity to arrive at the objective in a life form.

Why is it usefulWe are utilizing the profound learning, PC vision and AI techniques to characterize and recognize virtual atom which assists us with choosing and decrease time for pointless walkthrough for the whole clinical preliminary cycle as portrayed previously.The interaction connected with technique for logical trial and error particularly utilizes in the medication disclosure and pertinent to the areas of science and chemistryusing data handling/control programming,fluid taking care of devicesand delicate detectors, throughput screening hangman's tree analyst can rapidly lead a large number of synthetic, hereditary or pharmacological tests.Here is the pleasant part, marking the ideal articles in each picture.labelling is a valuable device for this purposepoint naming to the ideal registry and afterward attract a container around the item each picture.

Characteristics and architectural details

A. Architecture, Back -end framework, Dataset:

The model is having two variations one worked in quicker RCNN and the other SSD versatile net.final one is in the SSD Mobile netas SSD Mobile net model is all around upheld by both intel conveyance of open VINO toolbox and Tensor FlowLite .

B. Model optimization and inference:

Model improvement and surmising on equipment running intel engineering is being completed by intel dispersion large open VINO toolbox.

Drug discovery

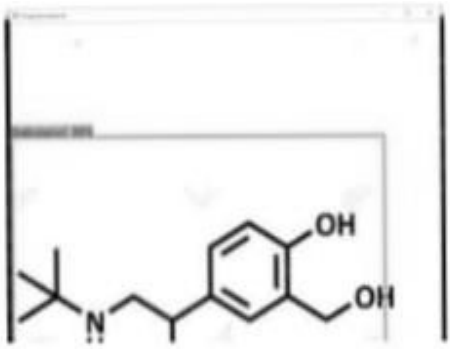
We will also use the main approach which is known as objective Reinforced -GAN.

ORGAN3 allows us to

- Generate samples that are both diverse and interesting.
- To direct the generative process towards certain objectives as an approach to Reinforcement learning.

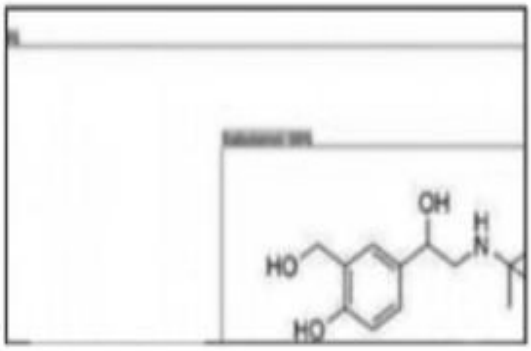
The sample images from test inference shows how model has successfully identified the different classes of drugs.

A. Salbutamol



Drug identifier in action.

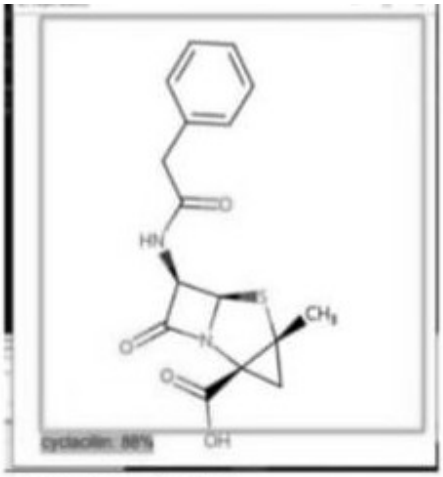
The trial shows that the model effectively distinguishes the medication salbutamol given the atomic construction as information. It makes a jumping box and shows the name of the medication alongside the exactness in percentagethe above drug is salbutamol and is accurately recognized by the model. It likewise shows the exactness of discovery in percentage; in



2.Identifying Salbutamol.

this case, the acknowledgment precision is 89%.
B.Cyclacillin

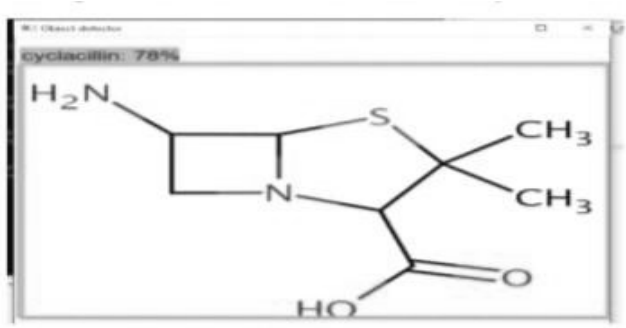
The model distinguishes the other class of medication accurately and precisely as well.the above drug is cyclacillin, it is generally utilized for treatment of bacterial contaminations brought about by the model as displayed on the above picture.



lentifying Cyclacillin with 88% accuracy.

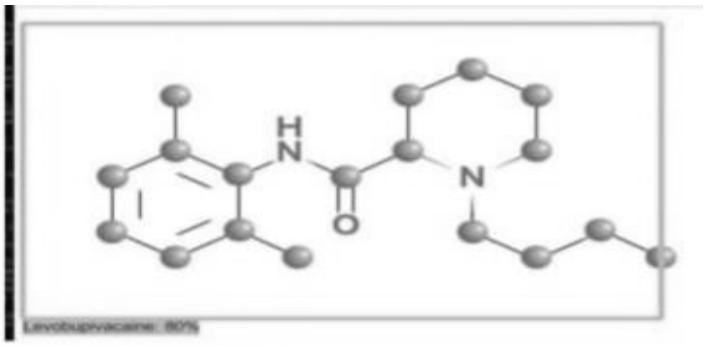
The exactness of this experiment is 78%.
C .Levobupivacaine

The moel recognizes the third medication accurately and precisely also. The above structure takes after the medication



.Identifying Cyclacillin with 78% accuracy.

levobupivacaine.it is a nearby or local sedation or absence of pain for surgery, for oral medical procedure techniques, for demonstrative and remedial procedures, and for obstetrical procedures.it is accurately distinguished by the model as displayed on the above image. The exactness of this experiment is again 80%.



.Identifying Levobupivacaine.

Conclusion

Distinguishing drugs was the premise of this paper and we have accepted sub-atomic constructions as the main element. These sub-atomic designs are firmly taken after and look basically the same as each other.thus, removing highlights and isolating them into various classes is very hard for a PC. Our model is effective in doing this hard task. The above delineates the significance of medication recognizable proof and medication revelation in the advanced world medical services and practice and how this arrangement shows precise outcomes in distinguishing the medications correctly,given the sub-atomic structures. The model gives a decent use instance of profound learning and PC vision presenting a PC vision formula utilizing the model optimiser and surmising motor of the intel appropriation of open VINO toolkit.model improvement helps us in getting great edges each second while doing deduction on live video feed.

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GREEN SYNTHESIS OF SILVER NANOPARTICLESUSING VARIOUS PLANT EXTRACTS

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Abstract

Nanoparticles produced from biological sources are gaining a lot of attention these days and they have a wide spectrum of uses. The fact that it is both environmentally benign is the key reason for its widespread popularity. Our current study uses a green approach to describe the biological production and characterisation of silver nanoparticles made from a conventional leaf extract. The antibacterial and antifungal activity of silver nanoparticles prepared with plant extracts such as *Allium fistulosum* and *Tabernaemontana divaricate*is also assessed in this study. Scanning Electron Microscopy and Transmission Electron Microscope was utilized to characterise the shape and morphology of produced silver nanoparticles. Silver nanoparticles with sizes of 57 and 40 nm were observed to have a solid block-like, rod-like structure. AgNPs show bactericidal action towards both gram-positive and -negative microbes, according to in vitro investigations. The findings are highly positive, demonstrating a significant increase in the activity of the undamaged fractions. The use of biological sources to synthesise NPs adds a new dimension to all application areas.

Keywords: *Allium fistulosum, Tabernaemontanadivaricate*,Green synthesis, Antimicrobial activity, Antifungal activity and Silver nanoparticles

Introduction

Metallic nanoparticles (NPs) were of major attention due to its exceptional physico-chemical properties and possible biological benefits. Metallic nanoparticles (MNPs) have distinct properties that are determined by the ways of fabrication and the composition of the precursors (Venkatesh *et al.,*2018). Physical methods for preparing AgNPs have been tried, however they are not cost-effective, waste more energy, and require the use of specialised instruments. However, because of toxicity issues, they have limited biological applicability. External stabilisers, some of which are hazardous, are routinely used to improve their stability. The use of biological resources to produce nanoparticles, particularly plants, can eradicate the toxic issue. Floras were widely available, non-toxic, and simple to manage. Plants also have phytochemicals, might be reducing and capping substances, making the production method simple. Silver (Ag) NPs have gained a lot of attention among all the metallic nanoparticles.

Chemical-based reduction, micro-emulsions, radiation, hybrid-based approaches, photo-chemical reduction and sono-electrochemical, microwave-based systems, and now a green production route have all been developed for the production of AgNPs (Yaqoob *et al.,* 2020; Shanmuganathan*et al.,* 2019; Syafiuddin*et al.,* 2017). However, despite the fact that some of these physiochemical procedures are long-lasting and technically viable, their usage on a broad scale is limited owing to its usage of dangerous chemicals, higher costs, higher energy and time requirements, and strain in wastage purification. As a result, there was an improving claim for cost-effective, ecologically friendly, and green nanosilver manufacturing pathways that utilize non-toxin chemicals. Green production of AgNPs employing a variety of microbes, plants, and algae, on the other hand, is a natural, biocompatible, and ecologically friendly process (Loo *et al.,*2018; Sanchooli*et al.,*2018; Rajeshkumar, 2014; Bakht Dalir*et al.,*2020).

Plant-based materials may be extra advantageous for nanosilver production than microbial and chemical approaches since they pose no risk of microbial and harmful chemical infection, need lesser energy, have broader consequences, and are easier to utilise (Yadi*et al.,* 2018; Ahmad *et al.,*2019). Furthermore, the inclusion of functional substances like phenol, ketones, aldehydes, and so on in the green production of AgNPs based on a plant-based extract mode of actions improves metal ions (Rafique *et al.,*2017; Masum *et al.,*2019). AgNPs were produced using a number of organic plants, including Emblicafficinalis fruit extract, Citrus limon leaves extract, green tea (Camellia sinensis) (Nakhjavani*et al.,* 2017), Coffea Arabica (Dhand*et al.,* 2016), and neem (Nakhjavani*et al.,* 2017).

Silver nanoparticles were synthesised using medicinal herbs such as *Tabernaemontana divaricateand Allium fistulosum* in this study. Pinwheel flower, *Tabernaemontanadivaricata*, belongs to the Apocynaceae family and is a wonderfully shaped evergreen shrub that blooms in spring. Plant extract has antinociceptive, antioxidant, anti-inflammatory, and reversible acetylcholinesterase inhibitory properties, according to studies (Kalaimagal*et al.,*2019).It is said to have originated in India or Indonesia and is exceptionally heat tolerant. It has thick, semi-succulent leaves that are heart-like and it has a moderate flavour and mucilaginous texture. It has antinociceptive, antioxidant, and antibacterial properties, and it's also used to treat diarrhoea (Mani *et al,*2021*).A. fistulosum*is a traditional medicine that is considered a rich source of nutrients. Several research have revealed the anti-oxidant (Zuo*et al.,* 2018), antimicrobial (Chang *et al.,* 2016), anticancer (Pan, Zheng & Ho, 2018), antihypercholesterolemic (Choi *et al.,* 2017), anti-obesity, and anti-inflammatory activity of *A. fistulosum* for human health (Zhao *et al.,*2021). *A. fistulosum's* active chemicals help it perform a variety of biological functions. The anti-oxidant size of *A. fistulosum*, for example, is closely associated by its total phenolic composition, while allicin is responsible for its antibacterial activity (Chang *et al.,* 2016). Because of the active components contained in most

plants, they exhibit a wide spectrum of activities. As a result, the goal of our study was to look at the biological nature of Ag NPs made fromtwo distinct extracts.

Materials and methods

Preparation of plant extract

The leaf extracts were properly cleaned to eliminate dirt and fungal-based spores, and then shade desiccated to eliminate humidity. About 10g of sample leaves were placed in a 250ml beaker with 200ml distilled water and cooked on the heating mantle for 45 minutes. The extract was then chilled to room temperature (T) before being sieved. This procedure was used to prepare both plant extracts (*Tabernaemontana divaricate*and *Allium fistulosum*).

*Synthesis of Ag*NPs

For AgNPs production, an Ag nitrate solution was equipped. *Allium fistulosum*and *Tabernaemontana divaricate* aqueous solutions are introduced in varying amounts to test tubes containing 2mM aqueous silver nitrate solution. The studies were performed at different Ts to find the best conditions for AgNPs production.

Characterisation of Pd NPs

UV- Vis spectral analysis

Using a UV-visible spectrophotometer (Systronics, India Model: 2202) by a slit breadth of 2nm and a 10-mm cell at room T, a UV-visible spectrophotometer by a slit breadth of 2nm was used to analyse the extract. For proximate analysis, the material was studied in visible and UV light with wavelengths varying from 300 to 800nm. Within an hour of starting the reaction, silver ions were reduced and silver nanoparticles were formed. $AgNO_3$ was used to maintain control.

Fourier transform infrared spectroscopy (FTIR)

Ag NP colloid solution (50 mL) were created by ideal conditions like EFE(5%), 1 milli Molar of silver nitrate, and centrifuged at 20,000 revolutions per minute for 20 minutes for Fourier transform infrared (FTIR) spectroscopy measurement. The pellets were then resuspended and lyophilized for 16 hours. To determine the distinctive functional groups in the produced Ag nanoparticles, FTIR analysis can be performed using Bruker, Alpha T, Germany. It gives information about a molecule's structure, which may often be gleaned from an absorption spectra.

Scanning electron microscopy (SEM) and Transmission electron microscopy (TEM)

The shape and morphology properties of AgNPs are measured by employing a SEM. After centrifuging Ag NPs at 15000 rpm for 10 mins, the pellets are collected and deposited in a dehydration oven at 50˚C to remove any remaining water. An FEI Nova Nanolab 200 SEM was used to examine the prepared sample (FEI company Hillsboro, OR, USA). For both imaging and EDX study, the electron beam's energy was fixed at 15 keV. Moreover, TEM is employed to examine the morphological characteristics of the synthesized NPs (TEM). One drop of materials was kept on a Cu grid for TEM investigation, and then dried by employing the dry vacuum. This instrument (Tecnai G -10, Philips) was also used to image the dried nanoemulsion, as well as an 80 kV TEM by a W-sourcing as well as an ultrahigh-resolution pole piece by 1.9 resolution.

Antimicrobial study

Bacterial culture

Themicrobial culture was acquired from Microbial Type Culture Collection and Gene Bank (MTCC, India). The obtained bacterial culture was confirmed using biochemical system supplied via Bergey's Manual of Systematic Bacteriology (Vol 2, Second Edition).

Well diffusion study

The agar-based well diffusion procedure was employed to examine the anti-microbial action of produced AgNPs. In the petri dishes, 20 mL semi-solid mueller-hinton agar (MHA) medium was transferred. The microbes are cultivated in NB for 24 hours and then cultured with 1.5 106 CFU/mL suspensions of test bacteria on an exterior of solid-based medium MHA using a sterile brush (Staphylococcus aureus, Enterococcus faecalis, Escherichia coli and, Salmonella typhi). A variety of Ag NP concentrations (varying from 0.32 to 10 mg/mL) were saturated onto wells by a diameter of 6 mm and kept on the surface of inoculated plates. For above 4 mentioned bacteria, petriplates were placed for 24 hours at 37°C. Ciprofloxacin is thought to be a positive control. The diameter of inhibition zone in mm was employed to quantify antimicrobial actionand all antibacterial tests are done in triplicate (Akintelu*et al.,*2019).

Antifungal activity

To test the antifungal impact of Ag NPs, *Aspergillus niger* and *Candida albicans* are cultured in PDA fluid media for 2 days at 35°C, then plated on new PDA solid-based medium comprising Ag NPs and incubated for 5 days at 35°C. Controls were Ag-free PDA plates cultivated below the similar circumstances. The colonies are measured in millimetres. Ketoconazole is utilized as a positive control in our research (Rajeshkumar*et al.,*2019).

Results and Discussion

Ag NP was synthesized from a bio- source using two different plant extract such as *Tabernaemontanadivaricata* and *Allium fistulosum.*The colour transformed from lighter brown to darker brown when the *Tabernaemontanadivaricata* and *Allium fistulosum* leaf extract were introduced droplet manner to the silver nitrate mixture, indicating Ag NPs formation. A variation in colour of the solution showed the AgNPs synthesis.

The color changing of $AgNO_3$ mixture from colorless to darker brown signifying the NP formation that were seen via naked eye and further verified by the UV. The absorption band intensity of *Allium fistulosum* and silver nanoparticle synthesized was identified as 225nm and 421nm respectively (Figure 1).The produced Ag NPs with plant extract of *Tabernaemontanadivaricata*. It was observed from that the plant extract of *Tabernaemontanadivaricata* and Ag NPs shows an extreme absorption band of 215 and 426 nm. The prominent hump specifies the creation of Ag NPs (Figure 2).

The Figure 3 illustrates the FTIR spectra of Ag NPs produced from two diverse plant extract. The nanoparticle synthesized Allium *fistulosum*using 3 bands in the arena of 3500 - 1500 cm^{-1} with a more influential band at 1609 cm^{-1}, and a lower height at 3360 and 2114 per cm related to C=O carbonyl group, O-H stretched vibration, aromatic C-H bond (Figure 3(a)).The FTIR spectra of nanoparticle synthesized by *Tabernaemontanadivaricata*extract revealed an absorption band at 3297 cm^{-1}, 1634 cm^{1},1347 cm^{-1},1324 cm^{-1}and1065 cm^{-1} which corresponds to existence of C-H stretch, C=O bonds, C-O bonds, C-O bonds and alkylamine groups (Figure 3(b)).

The morphological nature of AgNPsis examined by employing SEM. SEM shows solid block-like structures for *Allium fistulosum*mediated AgNPs while for *Tabernaemontanadivaricate* mediated AgNPs, it shows rod-like structure with some agglomeration where an average particle shape was 55 and 57 nm respectively (Figure 4)**.**The particle size, morphological, and crystalline were examined by employing TEM and Particle size analyser. A one drop solution of AgNPs were combined on to the carbon enclosed copper-based grid. *Tabernaemontanadivaricata*and *Allium fistulosum* plant extracts were used to synthesize AgNPs and TEM images of AgNPs with dimensions of 55 nm and 57 nm respectively were obtained. TEM pictures showed the produced AgNPs were comparatively even in diameter and size (Figure 5).

The antimicrobial action of Ag NP produced using plant extract was confirmed using well diffusion method, which is considered as one of the fastest and reliable method.The antibacterial efficiency of the Ag NP is analysed with the help of microbial species like *Enterococcus faecalis, Escherichia coli, Staphylococcus aureus,* followed by *Salmonella typhi.* The sample Ag NP synthesised using *Allium fistulosum* and *Tabernaemontanadivaricata* is represented as V andN Ag. The Table 1 represents the antibacterial efficiency of Ag NP against the bacterial culture. In the case of sample N(Ag), highest zone of inhibition is observed while treating with the *Salmonella typhi* then least is found with *Staphylococcus aureus* i.e.18 mm and 08 mm respectively. Similarly, in the case of sample V(Ag), highest zone of inhibition is observed while treating with the *Salmonella typhi* then least is found with *Staphylococcus aureus* i.e.18 mm and 10 mm respectively. The positive control used is ciprofloxacin and its zone of inhibition is observed within a ranging from 35-40 mm.

The antifungal efficiency was studied by employing well diffusion technique towards two diverse fungal straining like *Aspergillus niger* and *Candida albicans faecalis* (Figure 6 and 7).In the case of fungal strain, *Aspergillus niger,* the zone of inhibition followed the order V (Ag)>) > N(Ag)mediated AgNPs i.e. 11mmand 08 mm respectively. Similarly, in the case of fungal strain,*Candida albicans faecalis,* the zone of inhibition followed the order V (Ag)> N(Ag)mediated AgNPs i.e.10mmand 07 mm respectively.

Conclusion

Plant extracts such as *Allium fistulosum andTabernaemontanadivaricata* have been successfully used to synthesise Ag NPs in a lesser price, eco-friendly approach. UV, FTIR, TEM and SEM were used to characterise the synthesised Ag NPs. Biosynthesised AgNPs are non-toxic and have antibacterial and antifungalproperties. Ag NPs synthesised with *Allium fistulosum* demonstrated a good zone of inhibition against both bacterial and fungal strains among the two sources of synthesis. The findings are highly positive, demonstrating a significant increase in the activity of the undamaged fractions. The use of biological sources to synthesise nanoparticles adds a new dimension to all application areas.

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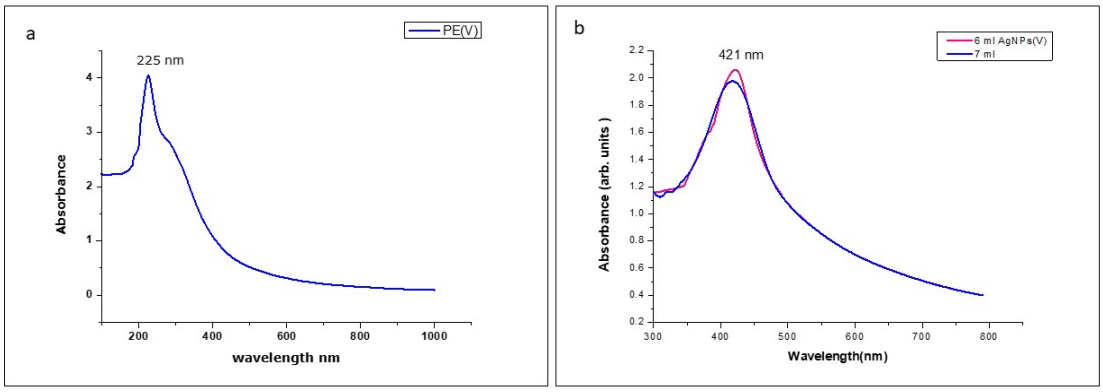

Figure 1: (a) UV spectrum of *Allium fistulosum* leaf extract. (b) UV spectra of silver nanoparticle formulated using *Allium fistulosum* leaf extract.

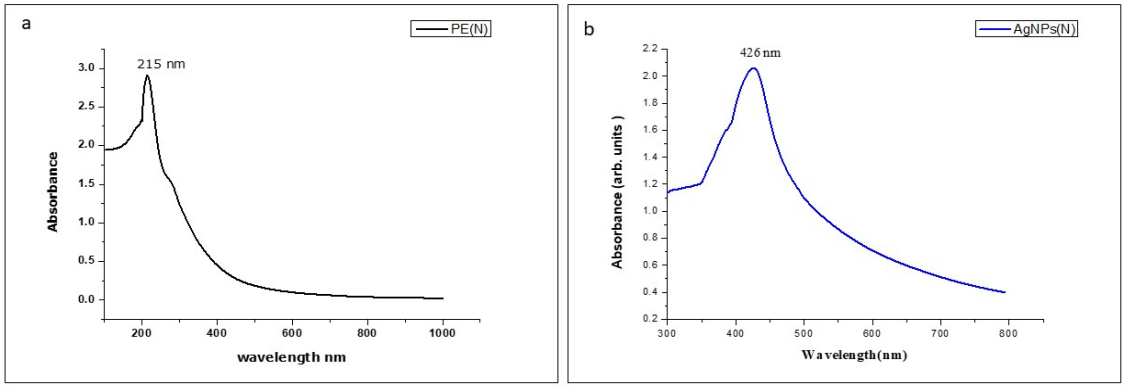

Figure 2: (a) UV spectrum of *Tabernaemontana divaricata* leaf extract. (b) UV spectrum of silver nanoparticle formulated using *Tabernaemontana divaricata* leaf extract.

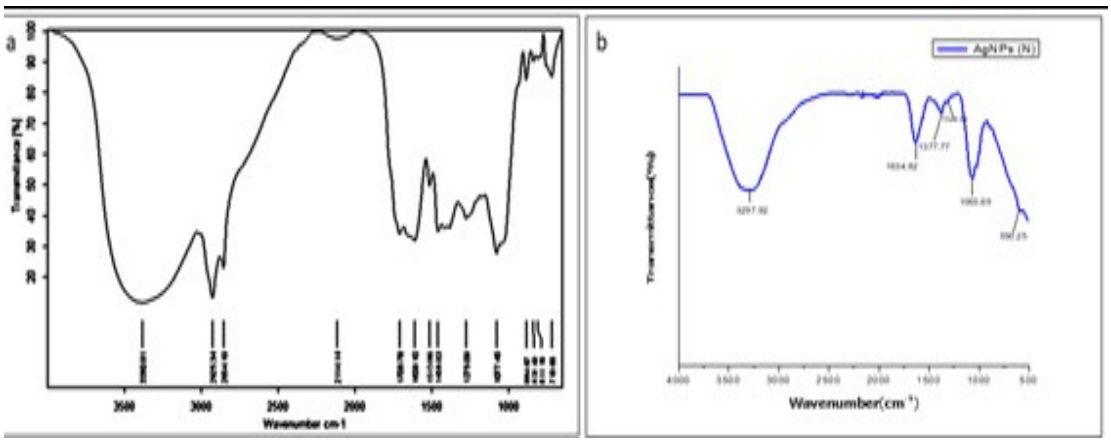

Figure 3: (a) FTIR spectrum of produced Ag NPs from *Allium fistulosum*extract. (b) FTIR spectrum of produced Ag NPs from *Tabernaemontanadivaricata*extract

(a) (b)

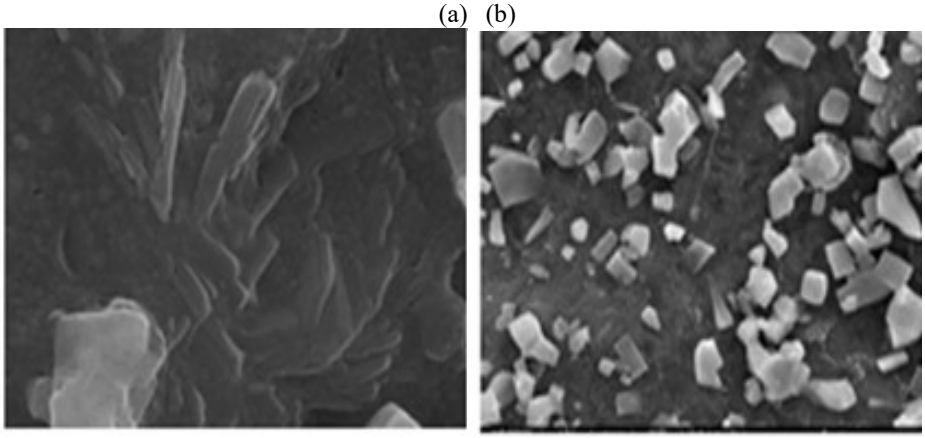

Fig. 4: (a) SEM picture of Ag NP produced through employing (a)*Tabernaemontanadivaricate* (b) *Allium fistulosum*

(a) (b)

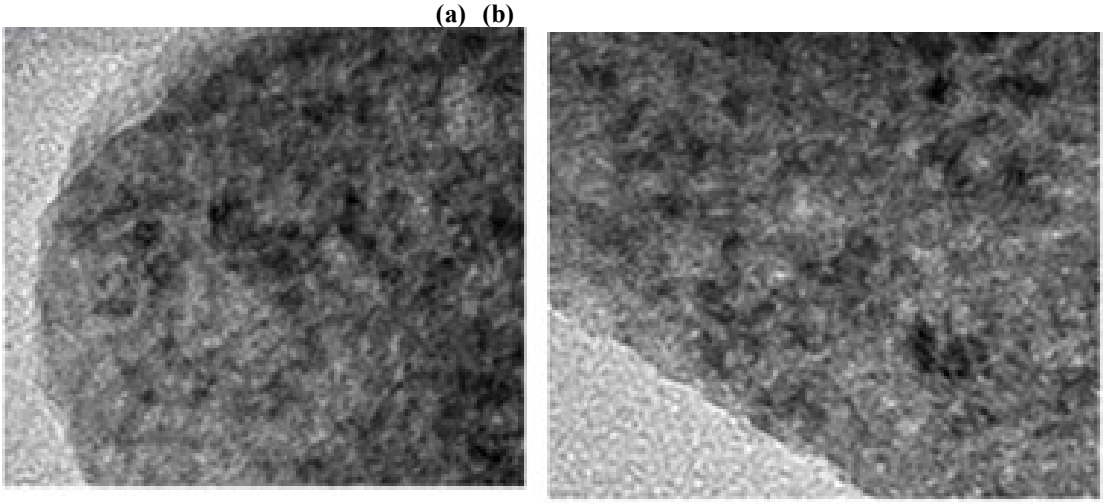

Fig. 5. TEM picture of Ag NPs synthesized from (a)*Tabernaemontanadivaricata* and (b) *Allium fistulosum*extract

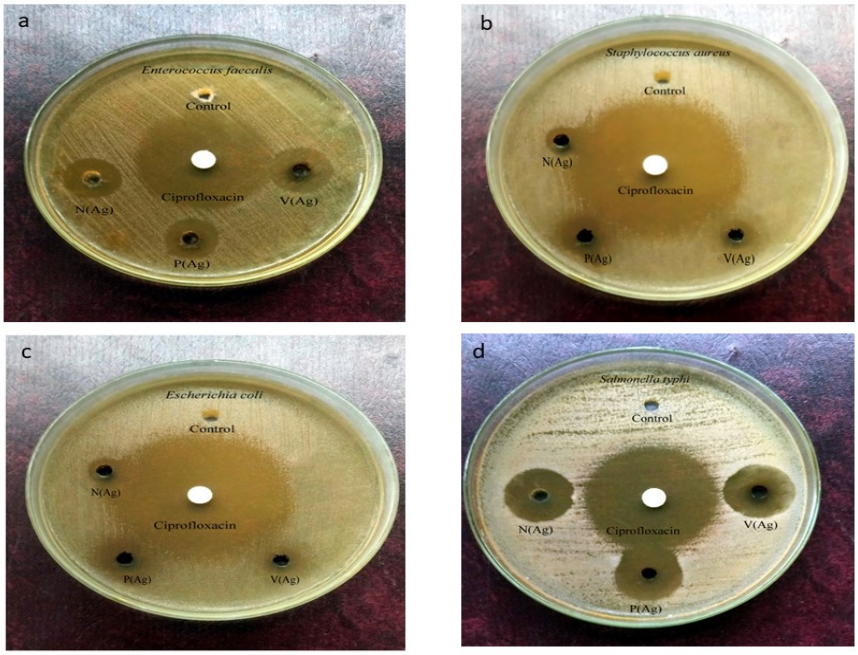

Figure 6 : The antibacterial effect of synthesized silver nanoparticle using (a), *Enterococcus faecalis* (b) *Staphylococcus aureus* (c) *Escherichia coli* (d) *Salmonella typhi*

Fig. 7. The antifungal effect of synthesized silver nanoparticle using (a), *Aspergillus niger,* (b) *Candida albicans faecalis*

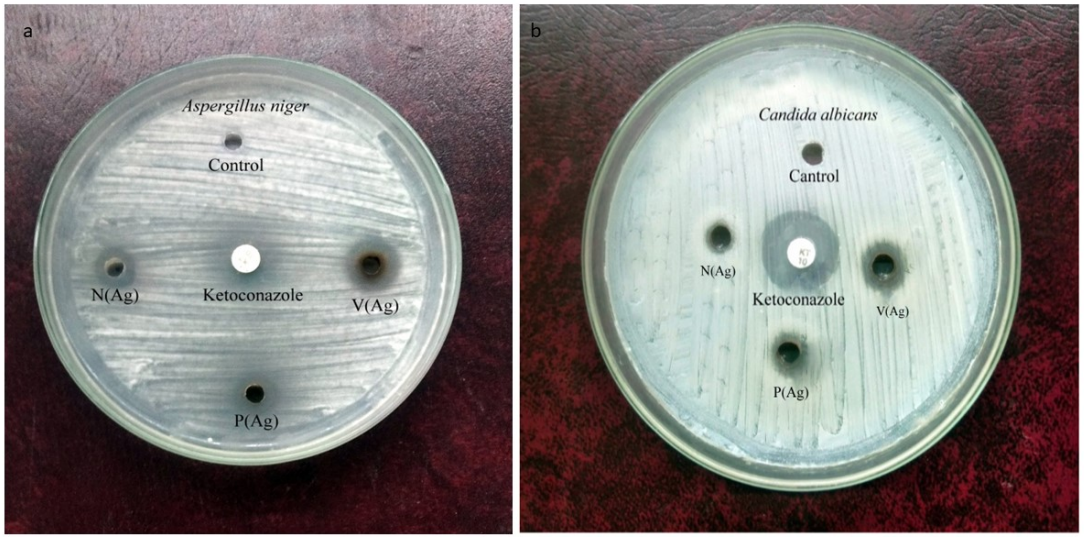

Fig. 7. The antifungal effect of synthesized silver nanoparticle using (a), *Aspergillus niger,* (b) *Candida albicans faecalis*

Table 1: Antibacterial efficiency of silver nanoparticles using well diffusion method

Microorganism	Control	*Allium fistulosum*	*Tabernaemontanadivaricate*	Ciprofloxacin
		Zone of inhibition in mm		
Enterococcus faecalis	-	17	15	35
Staphylococcus aureus	-	10	08	40
Escherichia coli	-	11	09	38
Salmonella typhi	-	18	18	35

ENDOPHYTIC FUNGI FROM MEDICINAL PLANTS *CITRUS MEDICA*:A TREASURE HUNT FOR BIOACTIVE METABOLITES

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Introduction

Plants are naturally associated with microorganisms both externally and internally in various ways. The microorganisms involved in these interactions are commonly referred to as endophyte. Endophytes constitute an important component of microbial diversity, and in the present investigation, *Citrus medica Linn*., known as bijapura in Ayurvedic literature, is widely used in traditional system of medicine . Ripe fruits used in sore throat, cough, asthma, nausea, vomiting, antiscorbutic, stomachic, tonic, stimulant, expellant of poison.Traditionally, the leaves, fruit of *C.medica* practiced as a medicine in ancient times in India. But to our knowledge, there is no report in endophytic fungus isolation from the particular medicinal plant. The mechanisms of action for these properties are not fully understood. Preliminary studies have found various constituents of *Citrus medica* exhibiting a variety of therapeutic effects in plants but the bioactive compounds using microbes was still unexplored.Due to enormous medicinal properties, it was selected for the isolation and screening of endophytic fungi for exploring biological potential of their secondary metabolites.

Methodology

Collection of plant and isolation of endophytic fungi

Sample Collection

Young and healthy parts are collected from disease free plants of *Citrus medica* from nursery, Coimbatore. The plant parts is cut and placed in a polythene bag and then taken to the lab.

Surface treatment

Collected plant parts are rinses in running tap water for 10 to 15 minutes followed by washing with double distilled water. first the leaf and stem sample is wash with 70% ethanol for 1 minute and then in 4% (NaClO) for 30 seconds.1 and 1.5 minutes and then wash with 70% ethanol for 10 seconds.Finally the samples are washed with autoclaved distilled water for 3 times and blotted on autoclaved blotting paper.

Isolation of fungal Endophytes:

The leaves and stem should be remove carefully and dissected into small pieces ($0.5x0.5cm^2$). The pieces are placed on petriplates containing oatmeal meal agar medium supplemented with streptomycin (200mg/L) and incubated for 21 days at 26±2°C in BOD cum humidity incubator. Tissues are observe for fungal growth at 2 days interval for 20 days. Actively growing fungal tips immerging from plant tissues are sub-culture on petri plates for identification and enumeration.

Microscopic examination of fungal endophytes

The microscopic examination of the fungal endophytes was done by preparing slides using lactophenol cotton blue dye, covering the sample with a cover slip and observing under the microscope at 10X and 40X magnifications.

Enzyme characterization

Amylase

The activity of Amylase is determined by inoculating the selected isolates in oatmeal agar. After 3-5 days incubation time, the fully formed cultures were flooded with 1% iodine in 2% potassium iodide. The clear halos are visualized around the colony.

Cellulase

For cellulolytic activity, the isolates are grown on yeast extract peptone agar medium amended with 0.5% Na-carboxy methyl cellulose and the culture is incubated. The plate were flooded with 0.1% Congo red and destained with 1M sodium chloride for 15 min. The clear halo is observed around the colony indicates the cellulose activity.

Lipase

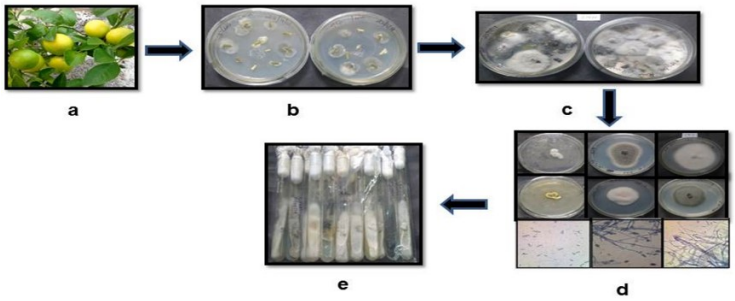
The lipolytic activity is observed by growing on the peptone agar media To the sterilized peptone agar culture media, previously sterile the Tween 20 is added in a final concentration of 1% (v/v) is added. This media was inoculated with the isolates and incubated. The precipitation of fatty acid crystals is observed around the colony indicates the lipase activity.

Protease

The proteolytic activity is determined by growing the isolates on agar media yeast extract, supplied with 0.4% gelatin and sterilized. To the sterilized culture media, separately sterilized 8g of gelatin in 100ml distilled water was added along it . After incubation, on the grown culture was flooded with saturated aqueous ammonium sulphate. The clear zone around the colony indicates a positive test for protease activity.

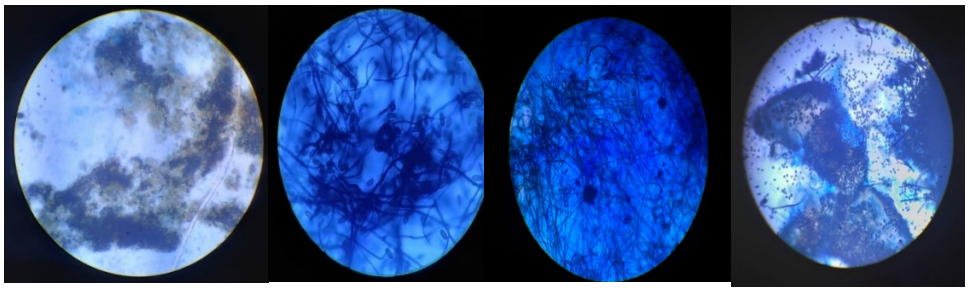
Results

A total of 10 morphotypes were isolated from the 20 segments. The results shown in Figures & tables.



a. *C. medica*plant,
b. PDA plates showing emerging endophytes from tissue samples ,
c. fungal endophytes emerges after 8 days in PDA plates ,
d. Pure culture of fungal endophytes,
e. Preservation of fungal isolates in slants.

Microscopic Examination Of Endophytc Fungi

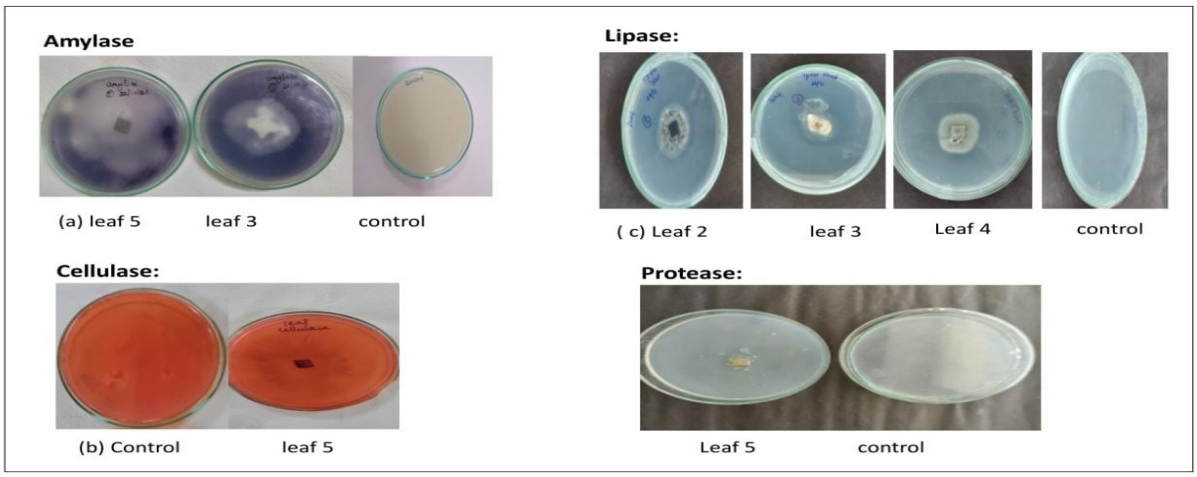


Leaf 1 **Leaf 3** **Leaf 5** **Stem 5**

Enzymatic activity of fungal endophytes

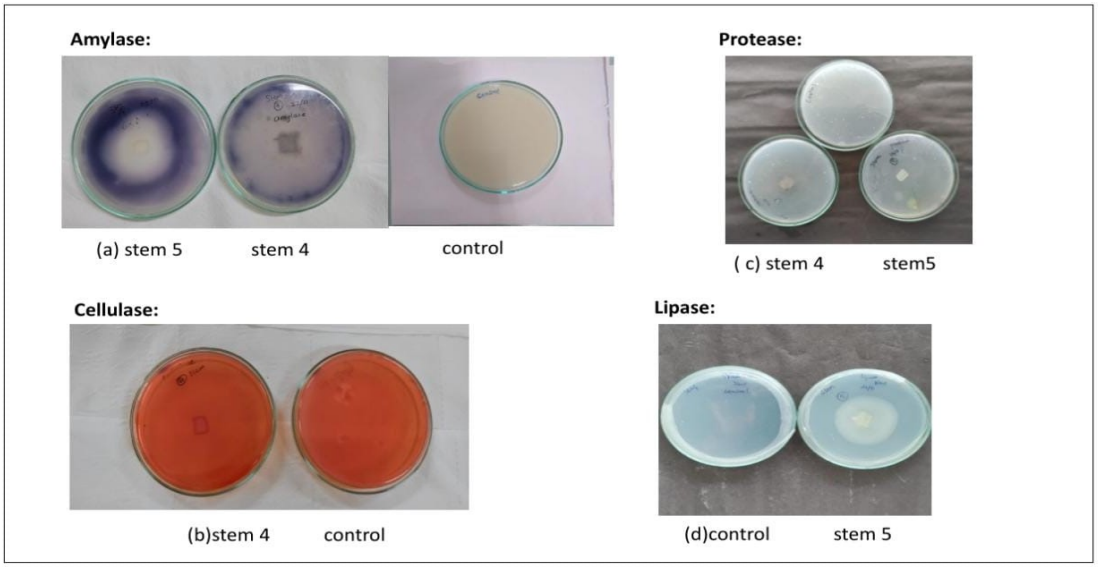
Endophytes survive inside the plant tissues and secret variety of extracellular enzymes in order to utilize the available nutrients. In this study, four extracellular enzyme productions were assayed by the isolated endophytic fungi of the plant. From the results, four fungal strains showed positive for amylase production (L3, L5 & S4, S5). In cellulase production two fungal strains showed positive (L5 & S4), for protease (L5 & S5, S4) and lipase (L 2, L3, L4 & S5).

Enzyme Characterization of Leaf Sample



S.No	Samples	Amylase	Cellulase	Lipase	Protease
1	Leaf 1	Negative	Negative	Negative	Negative
2	Leaf 2	Negative	Negative	**Positive**	Negative
3	Leaf 3	Negative	Negative	**Positive**	Negative
4	Leaf 4	Negative	Negative	**Positive**	Negative
5	Leaf 5	**Positive**	**Positive**	Negative	**Positive**

Enzyme Characterization of Stem Sample



S.No	Samples	Amylase	Cellulase	Lipase	Protease
1	Stem 1	Negative	Negative	Negative	Negative
2	Stem 2	Negative	Negative	Negative	Negative
3	Stem 3	Negative	Negative	Negative	Negative
4	stem 4	**Positive**	**Positive**	Negative	**Positive**
5	Stem 5	**Positive**	Negative	**Positive**	**Positive**

Conclusion

In present study 10 different fungal endophytic were isolated from from leaf and stem parts. The fungal isolates were observed under microscope and results were showed in the picture. The enzyme characterization such as amylase, cellulase, protease and lipase, which has grown on specific mediums. From the results, four fungal strains showed positive for amylase production (L3, L5 & S4, S5). In cellulase production two fungal strains showed positive (L5 & S4), for protease (L5 & S5, S4) and lipase (L 2, L3, L4 & S5). The L5, L3 & S5 fungal stains can be further characterization has to be carry out for production of better and more novel enzyme production for industrial application

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BIOTRANSFORMATION OF PARA PHENYLENE DIAMINE USING PURIFIED PEROXIDASE FROM THE LATEX OF *CALOTROPIS GIGANTEA* L.

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Abstract

Peroxidase (EC 1.11.1.7; donor: hydrogen peroxide, oxidoreductase) (POD) are a part of large group of enzymes associated with cell wall biosynthesis, response to injury, disease, resistance and wound repair. They catalyze the oxidation of various electron donor substrates such as phenols and aromatic amines in the presence of hydrogen peroxide. Peroxidase had attached considerable interest in recent years because of their activities towards a wide variety of chromogenic substance. The *Calotropis* peroxidase was purified from the latex of *Calotropis gigantea* using ion exchange chromatography and gel filtration chromatography. It was observed that after purification, the enzyme activity fold was increased as compared to crude enzyme extract. Optimum pH, optimum temperature and their stability, substrate and inhibitor were determined for *Calotropis* peroxidase. The substrate specificity of peroxidase was investigated using guaiacol, Para phenylene diamine (PPD) and o-Dianizidine. Although the PPD is genotoxic when exposed to human system *in vitro,* the peroxidase from *Calotropis gigantea* mediated metabolites of PPD is non – genotoxic as shown in DNA fragmentation assay. These results has gave clue that the peroxidase possess the ability to transform the genotoxic to non-genotoxic form of PPD.

Keywords: Peroxidase, Ion exchange chromatography, Gel filtration chromatography, Para phenylene diamine, genoto

Introduction

Calotropis is a genus of flowering plants in the Apocynaceae family and commonly known as milk weed or crown flower weed. Plant latex is a mixture of alkaloids, tannins, gum, sugars, starch, resins, protein and hydrolytic enzymes (Al-Yahya et al 1990). Leaves, roots, stem, flowers and latex of *Calotropis gigantea* are used in traditional medicinal system to cure several diseases and medicinal potential of the *C. gigantea* proved scientifically (Chitme et al 2005). The latex is used in Indian indigenous medicine in combination with *Euphorbia neriifolia* as a drastic purgative. The latex also used to induce abortion, infanticide (The Wealth of India, 2004). Peroxidases (EC 1.11.1.7), ubiquitous enzyme which belongs to the oxidoreductase class of enzyme, are widely distributed in nature and can be easily extracted from most plant cells, some animal tissues and fungus. Peroxidases they are divided into three superfamilies based on their structural and catalytic properties (Welinder, 1992). Plant peroxidases are receiving increasing attention due to their extensive bioactivation properties and potential applications in clinical, biochemical, biotechnological and related areas (Fatima et al 2007). The novel applications of peroxidase in agricultural, paper pulp, water treatment, pharmaceutical, and medical situations (McCarthy, 1985; Mougin et al 1994; Hernandez et al 1995; Hopfer, 1995; Rob et al 1996; Yazdi et al 2002; Veitch and Nigel 2004). In this study, peroxidase enzyme was isolated from the latex of *Calotropis gigantea*, purified, biochemically characterized and find its ability to biotransform carcinogenic compound into non-carcinogenic compounds.

Materials and Methods

Preparation of sample

The latex collected from plant was centrifuged at 10000 rpm for 10 minutes and the supernatant was taken. Though the centrifugation was repeated many times the wax content of the latex was unable to remove completely. Thus the solution was dialyzed against distilled water overnight and a frequent change of water was recommended to attain complete removal of wax. The dialysis was centrifuged at 10000 rpm for 10 minutes and supernatant was taken as source of enzyme.

Peroxidase assay and protein estimation

The peroxidase activity was determined using PPD as substrate slight modification in standard protocol according to Rompel method. 1ml reaction mixture containing (0.1U/ml), 1M sodium acetate buffer (pH-4), 8mM of PPD and 100mM of Hydrogen peroxide. The increase in an oxidation deduction was measured by absorbance at 492nm. One unit of the peroxidase activity is defined as the amount of enzyme that oxidizes substrates per min at 25°C. Quantitative protein

determination was achieved according to the (Lowry et al 1951) method, by measuring the optical density at 660 nm, with bovine serum albumin as standard.

Purification of *calotropis gigantea* peroxidase

Ion exchange chromatography

Further purification of partially purified peroxidase was carried out by ion exchange chromatography using diethyl amino ethyl (DEAE) cellulose column. The column was packed to the height of 7 cm in a glass column with an internal diameter of 4cm and equilibrated with 20mM sodium acetate buffer (pH 4) for 24 h. 1ml of the sample was loaded to the equilibrated column and loading break through was collected. The bounded protein with the cation resin was eluted by the gradient of 0.5M NaCl in equilibrated buffer (pH-4). The collected fractions were assayed and active fractions were pooled.

Gel filtration chromatography

Purified peroxidase enzyme (1ml) obtained from ion exchange chromatography was subjected to gel filtration chromatography using sephadex-G-50 column. The column was packed to the height of 70 cm in a glass column with an internal diameter of 1.5cm. Sample was poured on top of the column and eluted with sodium acetate buffer of pH 4. A total of 35 fractions of 1 ml each were collected at constant drop rate and both the enzyme activity as well as the protein content was determined for each separate fraction.

Characterization of peroxidase

Effect oF pH

The influence of pH on thermostable peroxidase activity was determined in the presence of buffers of wide pH range (pH 3.5–8.5) at a concentration of 100 mM. The following buffers were used: sodium acetate buffer (pH 3.5–5.5), phosphate buffer (pH6–7), and Tris–HCl buffer (pH 8–8.5), respectively. pH stability was determined by pre-incubating the purified enzyme (0.1U/ml) with respective 1M buffer at 25^{o}C.

Effect of temperature on enzyme activity and stability

The effect of temperature on enzyme activity was determined by pre-incubating the 100µl of enzyme with 20mM assay buffer at appropriate temperature (30^{o}C to 70^{o}C) for 10min. Pre-incubation was done for 1ml reaction mixture which contains 1mM Sodium acetate buffer (pH-4) and purified enzymes (0.1U/ml) for 10min. The thermal stability of the enzyme was checked by pre-incubating the 0.5 ml of enzyme with 20mM sodium acetate buffer (pH 4.5) at 70ºC for 1hr.0.1 ml of enzyme was collected at 10 minutes interval and stored at 4ºC until the assay was started. Substrates like p-Phenylenediamine, o-Dianisidine Hydrochloride, Tetramethylene Benzidine, Guiacol and Pyrogallol were taken with concentration of 4mM to 9mM to determine the enzyme activity. Enzyme kinetic parameters was calculated for PPD & o-Dianisidine Hydrochloride by Line-Weaver burk plot.

Kinetic characterization for ppd

Km values were calculated for the peroxidase reactions, using the Lineweaver-Burk graph. But transformation of the Michaelis-Menten equation Km and Vmax values for Para phenylenediamine/Hydrogen peroxide substrate pairs in order to able to and Vmax compare substrate specificity. In addition, this measurement was performed at constant concentration of enzyme with different concentrations of Para phenylendiamine.

Biotransformation of carcinogenic para phenylenediamine

The reaction was performed at 37^{o}C in 1ml of reaction mixture containing 20mM sodium acetate buffer (pH-4.0), 100µl of purified enzyme, 8mM PPD as substrate and 6mM H_2O_2 and allowed to settle the reaction product. The floating particles were pelleted out by centrifuging at 10,000rpm for 10mins. The pellets were allowed to air dry at room temperature.

Isolation of DNA from human blood

According to Miller *et al.,* (1988) method the DNA was isolated from human blood and dissolved in 1X TE buffer. 3µl of quantified DNA was mixed with 2µl of bromophenol blue and loaded in the 0.8% agarose gel.

DNA adduct formation with para phenylenediamine

25µl reaction mixture was prepared by adding different concentration of PPD (0.01mg, 0.02mg, 0.03mg, 0.04mg) and 50mM citrate phosphate buffer (pH 5.6) were added and incubated for 30min at room temperature. A control DNA was prepared with same composition except the PPD. The incubated mixture was loaded on 0.8% agarose gel and electrophoresis was carried out with 50V for 1hr to check for DNA parameters in comparison with control DNA (Balasubramanian *et al.,* 2013).

DNA adduct reaction with para phenylenediamine product

To the samples (containing 0.01mg, 0.03mg, 0.05mg of para phenylenediamine product), human blood DNA (2µg/25µl) and 50mM citrate phosphate buffer (pH 5.6) were added and incubated for 30min at room temperature. A control DNA was prepared with same.

Result and discussion

Purification of peroxidase enzyme

Table 1 summarizes the POD enzyme purification procedures from *Calotropis gigantea* extra cellular fluid. The extra cellular fluid was separated from the latex by centrifugation at 12000 rpm for 30 minutes. The dialysate was loaded on Diethyl amino ethyl (DEAE) column and fractions were collected. The washing buffer containing active fractions were considered as source for anionic peroxidase (Fig. 1a) and the eluted fractions were considered as source for cationic peroxidase. The bounded proteins were eluted using 0.5M NaCl (Fig. 1b). The active fractions pooled and dialyzed against distilled water. The active washing break through buffer was used as enzyme source and loaded on to CM Cellulose. The bound protein was eluted using 0.5 M NaCl (Fig. 2). The specific activity and purification fold was calculated for all the purifications steps. The purified peroxidase enzyme was further characterized.

Table 1. Purification profile of peroxidase enzyme from latex of *Calotropis gigantea*

raction	Protein (mg/ml)	Enzyme (U/ml)	Specific activity (U/mg)	Fold	Recovery (%)
Crude	5.71	5.19	0.908	1	100
DEAE	0.402	4.09	10.16	11.19	78.8
CM-Cellulose	0.995	3.85	3.85	4.26	74.2
Sephadex G-50	0.156	2.18	13.99	15.4	42

Fig. 1. Washing and Elution profile of DEAE

Peroxidase from latex of *Calotropis gigantea* was separated in DEAE column. a) unbound protein collected as washing breakthrough; b) bound protein eluted with 0.5M NaCl as elution breakthrough.

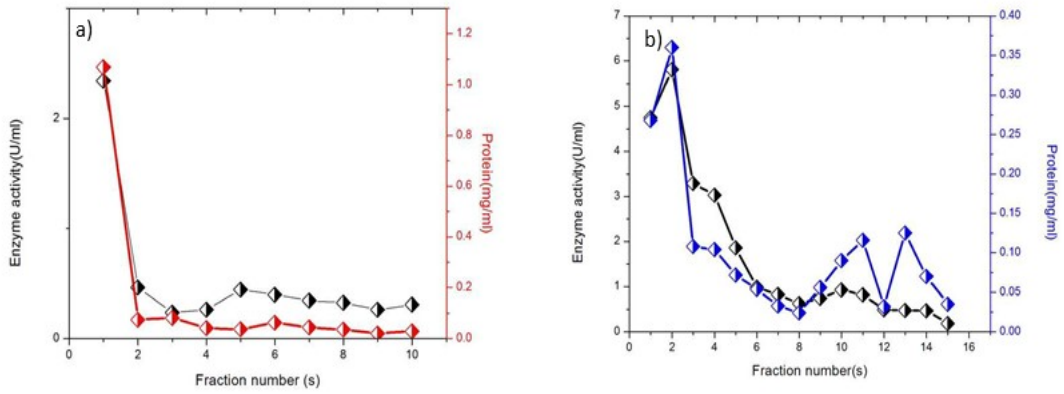


Fig. 2. Elution profile of CM Cellulose

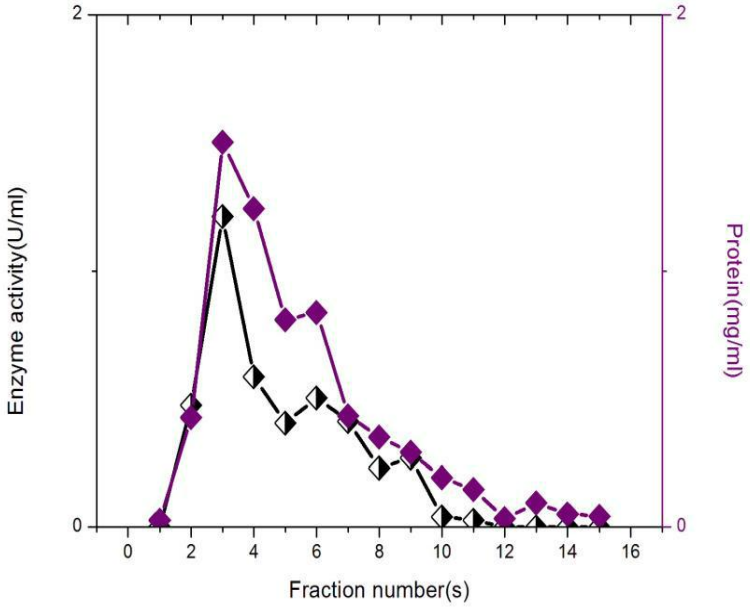
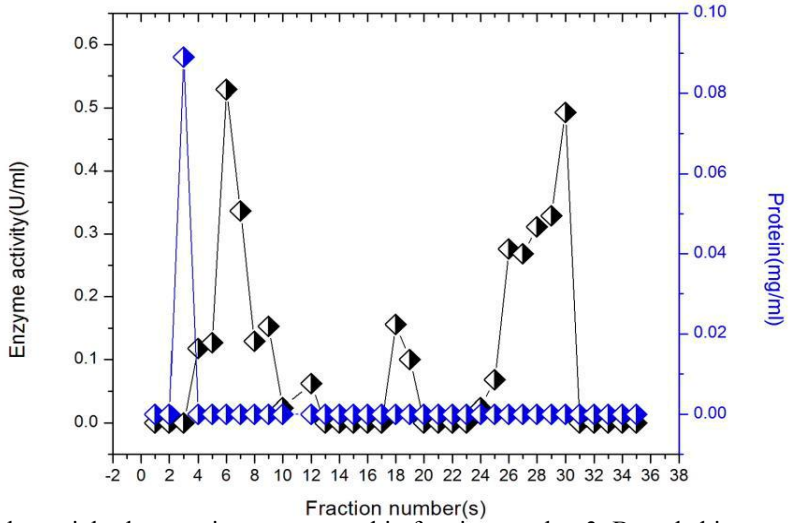


Fig. 3. Elution profile of Gel filtration column

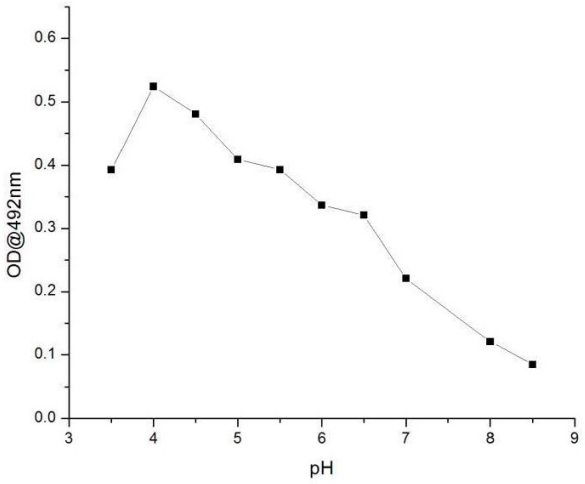


Based on the molecular weight the protein got separated in fraction number 3. Bounded isoenzyme were separated with two peaks at fraction number 6 and 30 in the elution profile.

Effect of pH on peroxidase

The optimum pH of the enzyme was determined by incubating the enzyme using different pH buffers ranging from 3.5-8.5. The results regarding the effect of pH on the enzyme activity are reported in Fig.4. The optimum pH was found to be 4.The peroxidase activity was low above and below the optimum pH. The pH at which an enzyme catalyses a reaction at the maximum rate is called the optimum pH. Change in pH above and below the optimum level affects the charges on the amino acids within the active site such that the enzyme enzyme-substrate complex formation is disturbed. Our findings are in close agreement with the reported results in which peroxidase isolated from, Turkish black radish and *Euphorbia cotinifolia* (Sisecioglu et al 2010; Kumar et al 2011) showed the same pH optima.

Fig. 4. Effect of pH on enzyme activit



Effect of temperature on peroxidase

The effect of temperature on activity of peroxidase was determined by incubating the enzyme at different temperatures ranging from 30-80ºC for 3 min and the results are represented in Fig. 5. The results indicated that the optimum temperature was 70ºC although there was not much decrease in activity up till 80ºC. These results reveal that peroxidases are stable in broad range of temperatures. Similar trend was obtained in case of peroxidase isolated from *L. leucocephala* (Pandey, 2011). An optimum temperature in the range of 40 to 55°C has been reported for turnip peroxidases (Duarte-Vazquez et al 2000).

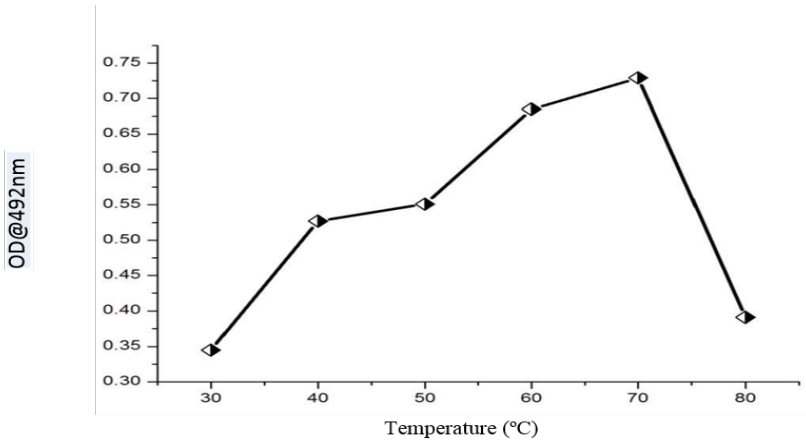


Fig. 5: Effect of temperature on enzyme activity

Thermal stability of the peroxidase enzyme

In general peroxidase is the thermo stable enzyme and the thermal stability of the enzyme was identified by incubating the POD enzyme for 1hr (Fig. 6). There is no change in enzyme activity up to 1hr incubation at 70ºC. It shows that the peroxidase enzyme from *Calatropis gigantea* was thermo stable. It was revealed that the soya bean seed coat peroxidase activity remained heat-stable at 75ºC (Ghaemmaghami et al 2010). In this studies, it was found that thermal stability of POD in temperatures up to 70ºC is comparable with the stability of peroxidase obtained from other sources. It was reported that the POD I and POD II were purified from the roots of cultivated *Raphanus sativus* L. were stable for 48hrs in temperatures up to 40ºC (Yazdi et al 1998). The present study revealed that the peroxidase obtained from *Calotropis* having more stability and thermo stability, so might be considered for use industrially by large scale production.

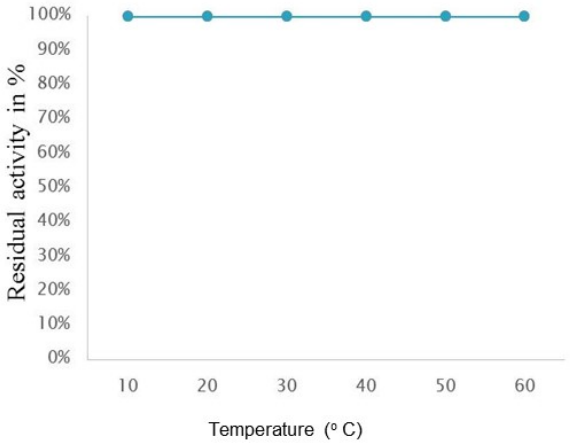
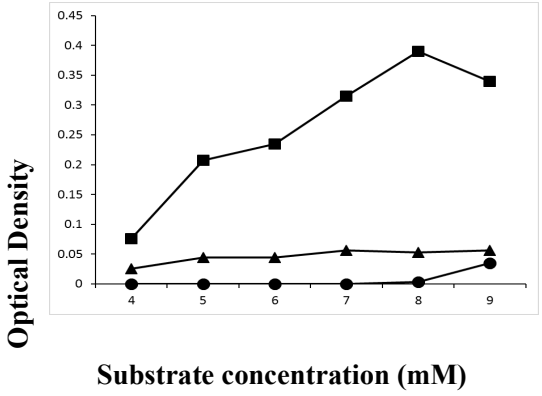


Fig 6: Thermal stability of the peroxidase enzyme at the time interval of 10 minutes.

Determination of substrate kinetics for peroxidase enzyme activity

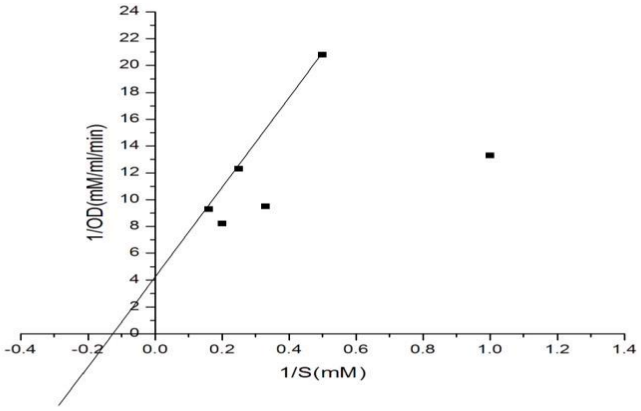
The substrate specificity of peroxidase was checked with Paraphenylene diamine, Pyragallol, and Tetramethyl benzidine. It was observed that the peroxidase shown measurable activity only with Paraphenylene diamine (Fig.7). The Michaelis - Menton Watanabe constant such as Km and Vmax were calculated 5mM as and 0.172mmole/ml/min respectively using Lineweaver-Burk (LB) plot for Paraphenylene diamine (Fig.8). The substrate specificity of peroxidase from *Sapindus mukorossi* was reported as guaiacol with *K*m value of 1.05mM (Jagtar et al 2012). The Peroxidase from different sources like horseradish (Putter et al 1983), cabbage (Kharatmol et al 2012), oil palm leaves (Deepa et al2002) and citrus peel (Nouren et al 2013) are mostly affinities towards guaiacol with *K*m values of 5.71mM, 0.7mM, 4.91mM and 0.66mM respectively. It was reported that peroxidase from the tender *Borassus flabellifer* was most specific towards guaiacol than o-Dianisidine and Benzidine as substrate *K*m value around 66µM (Aijthadevi et al 2012).

Fig. 7. Effect of substrate on enzyme activity. PPD (■), Pyrogallol (▲) and Tetramethyl benzidine (●). There was no measurable activity of peroxidase with Guaicol and pyrogall



The TCWP1 and TCWP2 isozymes has *K*m values of 1.63mM and 4.0mM respectively for the substrate o-Dianisidine. The *K*m values of this isozymes were lesser than the other peroxidase were found (Balasubramanian et. al., 2013). The reported *K*m value of soluble peroxidase were 7.4mM for kiwi fruit (Soda et al., 1991), 9.5mm for garlic peroxidase (Das et al 2011) and 2.14mM for *B. vulgaris* (Watanabe et al 2007). It was observed that substrate specificity of peroxidase were lower than the peroxidase from other sources.

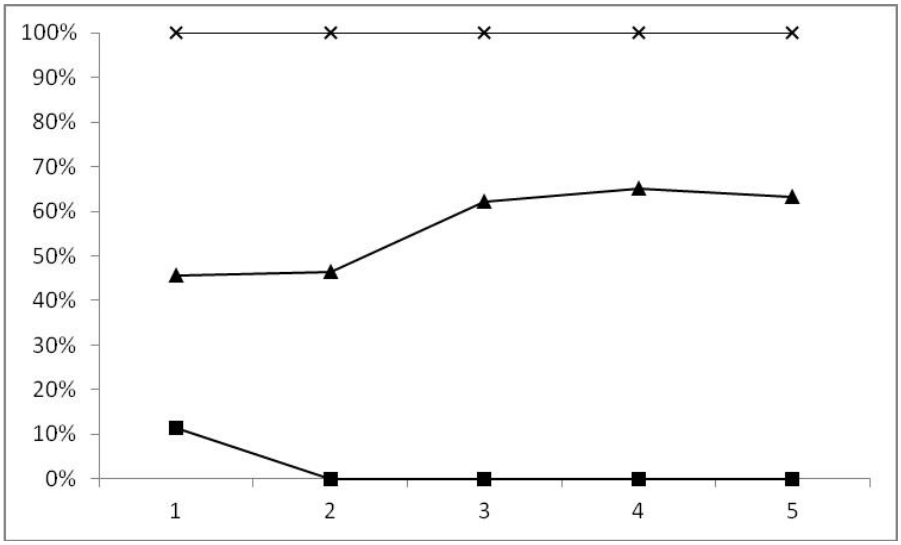
Fig. 8. LB plot for enzyme kinetics of PPD substrate



Inhibitior Kinetics of Peroxidase Enzyme

Typical inhibitors for peroxidase enzyme were sodium azide, phenyl hydrazine and hydroxylamine hydrochloride were used for this study (Mika et al 2003). Activity of the enzyme was completely inhibited at 2.5mM of by phenyl hydrazine and there was no change with hydroxylamine hydrochloride and sodium azide (Fig. 9).

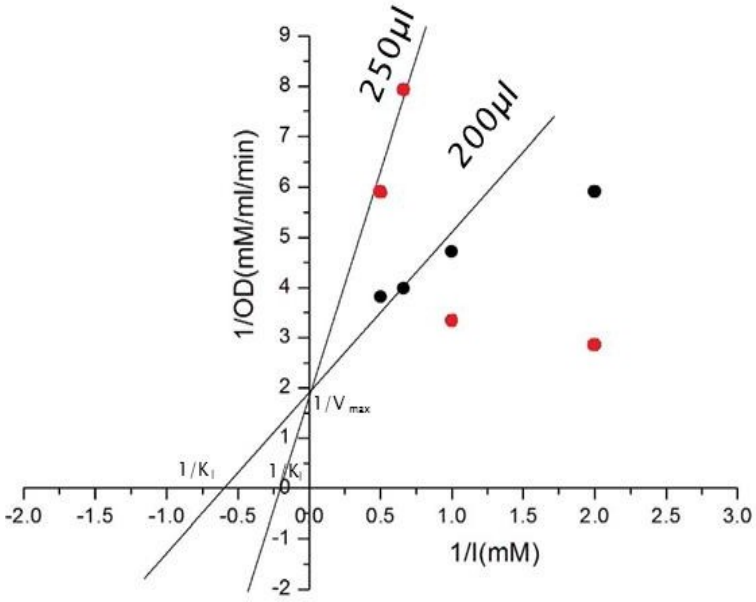
Fig. 9: Effect of inhibitor on enzyme activity



Inhibitors concentration (mM)

Different inhibitors like phenyl hydrazine, sodium azide and hydroxylamine hydrochloride were used to determine the effect of inhibitor. Among three inhibitors phenyl hydrazine (■) inhibited the activity of the peroxidase whereas even at increase the concentration of the sodium azide (▲) and hydroxylaminehydrochloride (×) there was no inhibition of enzyme activity.

Fig. 10. LB plot for enzyme kinetics of phenyl hydrazine



In Fig. 10 Phenyl hydrazine revealed the competitive type of inhibition with PPD at various volume of enzyme (0.2U/ml & 0.25U/ml).

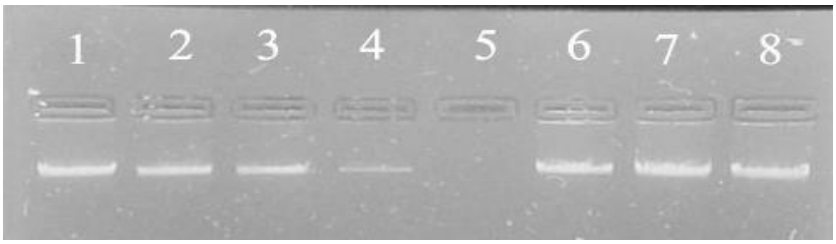
Some amino acids like cysteine, methionine, tryptophan and valine were act as inhibitor and decrease the activity of enzyme at the oxidization state of guaiacol (Kumar et al 2008). Sodium azide was reported as an inhibitor for activity peroxidase from sunflower and maize (Mika et al 2003) and *S. melongena.* From the result, we can conclude that phenyl hydrazine compound containing amine group may perfectly match in the substrate binding site rather than enzyme substrate complex.

Genotoxicity of PPD and its biotransformant on Human DNA

P-pheneylenediamine is an aromatic diamine used in dye industries. Most of the carcinogen induced DNA damage by forming a specific adduct with DNA. The genotoxicity of PPD on human DNA were observed. However at low concentration PPD with DNA, bands were similar as control shown in Fig. 11 (Lane 1, 2, 3). In contrast, when concentration of PPD increased that showed a gradual decrease in the fluorescent intensity (lane 4 & 5). This results indicated that up to 0.02mg of PPD not genotoxic effect on DNA, while incubation with 0.04mg of PPD adduct formation with DNA, prevent the ethidium bromide binding to DNA. Similar pharmacological effect was reported in dentric cells, where the cell death was occurred against the increasing concentration of PPD. However the o-Dianisidine possess the ability in the form to bind with DNA, it was reported that benzidine itself having DNA adduct forming ability in the form of nitrenium ion (Zheng et al., 2006).

The genotoxic effect of biotransformed PPD was observed in Fig. 11 (Lane 6 to 8). DNA was incubated with various concentration of biotransformed PPD (0.01mg, 0.03mg, 0.05mg) respectively, there was no changes observed when compared with control DNA. Carcinogen induced DNA damage involves the formation of specific adduct(s) with DNA. To form such DNA adducts, most carcinogens have to be converted into reactive metabolites.

Fig. 11: Genotoxicity of PPD and biotransformed PPD on human DNA.



Agarose gel electrophoresis of high molecular weight DNA from human blood (~2 µg per lane) exposed to reaction medium. 1) DNA; 2-5) increased concentration of PPD, system without PPD (0.01mg, 0.02mg, 0.03mg, 0.04mg) and 6-8) increasing concentration of PPD metabolites (0.01mg, 0.03mg, 0.05mg).

Conclusion

As a conclusion, the present study showed that enzyme from *Calotropis gigantea* produce copious levels of POD, which was purified and characterized to homogeneity. After purification the peroxidase activity of *Calotropis gigantea* extracts were increased as compared to crude enzyme extract up to 15.4 fold with their specific activity of 13.99 U/mg, respectively**.** The Michaelis - Menton Watanabe n constant such as Km and Vmax were calculated 5mM as and 0.172mmole/ml/min respectively using Lineweaver-Burk (LB) plot for Paraphenylene diamine. Although the PPD is genotoxic when exposed to human system *in vitro,* the peroxidase from *Calotropis gigantea* mediated metabolites of PPD is non – genotoxic in DNA fragmentation assay. These results confirm that the peroxidase possess the transformation ability to the genotoxic to non-genotoxic form of PPD. However suitability of peroxidase for biotechnological applications can be investigated through its kinetic characterization. It was showed that this enzyme is an interesting candidate for further studies such as chemical diagnostics.

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EXTRACELLULAR ENZYMATIC ACTIVITY OF ENDOPHYTIC FUNGAL STRAINS ISOLATED FROM MEDICINAL PLANT *Thevetia neriifolia*

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Abstract

Endophytic fungi exhibit a complex web of interactions with host plants and have been extensively studied over the last several years as prolific sources of new bioactive natural products. Fungal enzymes are one of them which are used in food, beverages, confectionaries, textiles and leather industries to simplify the processing of raw materials. They are often more stable than enzymes derived from other sources. Enzymes of the endophytes are degraders of the polysaccharides available in the host plants. The use of simpler solid media permits the rapid screening of fungi population for the presence or absence of specific enzymes. Seven fungal strains, isolated from medicinal plants.*Thevetia neriifolia* were screened for extracellular enzymes such as amylase, cellulase, lipase and protease on solid media. The array of enzymes produced differs between fungi and often depends on the host and their ecological factors.

Introduction

Fungal endophytes are reported from cryptogams to phanerogames. Endophytes have been observed from land plant to aquatic. The colonization of fungal endophytes in marine algae, bryophytes, fern and numerous gymnospermous plants have also been observed. Endophytes fungi are more diverse as well as host and tissue specific. A single plant leaf can harbor several dozens of endophytic fungi. Endophytic fungi have been reported from all parts of plant tissues such as leaves, rachis, bark, stems, buds, tubers, fruits and roots (Mishra et al., 2012, Gond et al., 2011). An endophytic fungus is recorded from brown alga Sargassum sp. with microbial activity (yang et al. 2006). Fungal endophytes play a major role in determination of plant biodiversity.

Endophytes are mainly transmitted in two ways: vertically or horizontally. Horizontal transmission takes place among individuals i.e. from one individual to another or among population. Vertical transmission takes place from parents to offspring through infecting the seeds or embryo of a host organism. Reproduction through asexual or sexual spores leads to horizontal transmission, where endophytes may spread between plants in a population or community.

Endophytes producing immunosuppressives, for example, subglutinols A and B are produced by Fusariumsubglutinans. Endophytes producing Podophyllotoxin (PDT), a well-known aryltetralinlignan with potent anticancer, antiviral, antioxidant, antibacterial, immunostimulation, and anti-rheumatic properties, are obtained from endophytic fungus Alternaria sp. isolated from Sinopodophyllum, and endophytic fungus ***Fusarium oxysporum*** **obtained from Sabina recurva.**

T.neriifolia belongs to the family Apocynaceae. It is also known as yellow oleander.It is a mortal plant after ingestion, all parts of these plants are toxic, and contain a variety of cardiac glycosides including nerifolin, thevetin A, thevetin B and oleandrin. Ingestion of oleander results in nausea, vomiting, abdominal pain, diarrhea, dysrhythmias and hyperkalemia. This plant served as a model plant in drug development. It is an evergreen tropical shrub or small tree. Its native range is tropical America. Its flowers are 3 inches(7.6 cm) long, have 5 overlapping petals that open in a spiraled pin wheeled. It is long funnel shaped sometimes fragrant yellow in color. It blooms from summer to fall. The fruit is deep red-black in colour. The broken foliage oozes toxic white latex sap. Its stem is green turning silver/grey. The plants spread by seed. It grows well in agricultural areas; urban areas etc. and also its can survive most of the soils. Its leaves are willow-like, lanceolate and glossy green in color. The leaves contain thevetin B & A, peruvoside, neriifolin, thevenerin and peruvosidic acid are some of the glycosides which were identified in the frozen leaves.

Materials and methods

Collection of plant and isolation of endophytic fungi

Sample Collection

Young and healthy parts were collected from disease free plants of *T. neriifolia*(leaf, root, bark) from Botanical garden, BHU campus, Varanasi. The plant parts was cut and placed in a polythene bag and then taken to the lab.

Surface treatment

Collected plant parts were rinsed in running tap water for 10 to 15 minutes followed by washing with double distilled water to remove the debris and soil particles adhered to it. To free the tissue samples from any epiphytic microbes, surface sterilization treatment was done adopting the methodology by Petrini and co-workers in 1992 with slight modifications. In this procedure at first the leaf sample was washed in 70% ethanol for 1 minute and then in 4% sodium

hypochlorite (NaClO) for 30 seconds, 1 and 1.5 minutes and then washed with 70% ethanol for 10 seconds. Finally the samples was washed with autoclaved distilled water for 3 times and blotted on autoclaved blotting paper. The stem samples was washed in 70% ethanol for 1 minute followed by 4% sodium hypochlorite (NaClO) for 1 and 2 minutes and then washed with 70% ethanol for 10 seconds to remove the epiphytic microbes. Finally the samples was washed with autoclaved distilled water for 3 times and blotted on autoclaved blotting paper. The same process was repeated for root sample, but sample was treated for 4% sodium hypochlorite for only 2 minutes. Remaining steps were similar.

Isolation of fungal Endophytes

Leaves were cut into small pieces of 5x1mm^2 with a sterile blade. The stem and root was removed carefully and dissected into small pieces (0.5x0.5cm^2). The pieces were placed on petriplates containing potato dextrose agar (PDA) medium supplemented with streptomycin (200mg/L) and incubated for 21 days at 26±2°C in BOD cum humidity incubator (Calton, NSW, New Delhi). The composition of PDA is mentioned in Table 2. Tissues were observed for fungal growth at 2 days interval for 20 days. Actively growing fungal tips immerging from plant tissues were sub-cultured on PDA petri plates for identification and enumeration.

Effectiveness of surface sterilization

Since the emergence of fungi decreased with increasing time of treatment with 4% sodium hypochlorite, therefore 4% sodium hypochlorite for 1.5 minute for leaf, 2 minute for stem were employed for surface treatment. All samples were imprints of surface sterilized leaf, stem and roots pieces were taken on the PDA to check the efficacy of surface sterilization. A control PDA plate without any tissue segment was also run parallel to the experiment to examine contamination.

Purification and preservation of endophytic fungi

The collective colonization frequency of the endophytic fungi in the root, stem/bark and leaf parts of *Thevetianeriifolia* was calculated using the following formula:

$$\% \text{ colonization frequency} = \frac{\text{Number of isolates emerged}}{\text{Number of tissues plotted}} \times 100$$

The isolates obtained from different plant parts were purified by transferring each isolate to a fresh PDA plate. A piece of agar with mycelial growth of the isolate was cut and transferred to a new PDA plate.The preservation of endophytic fungi was done by slant culture.

Enzymatic activity of fungal endophytes

Amylase

The activity of Amylase is determined by inoculating the selected isolates in oatmeal agar. After 3-5 days incubation time, the fully formed cultures were flooded with 1% iodine in 2% potassium iodide. The clear halos are visualized around the colony.

Cellulase

For cellulolytic activity, the isolates are grown on yeast extract peptone agar medium amended with 0.5% Na-carboxy methyl cellulose and the culture is incubated. The plate were flooded with 0.1% Congo red and destained with 1M sodium chloride for 15 min. The clear halo is observed around the colony indicates the cellulose activity.

Lipase

The lipolytic activity is observed by growing on the peptone agar media To the sterilized peptone agar culture media, previously sterile the Tween 20 is added in a final concentration of 1% (v/v) is added. This media was inoculated with the isolates and incubated. The precipitation of fatty acid crystals is observed around the colony indicates the lipase activity.

Protease

The proteolytic activity is determined by growing the isolates on agar media yeast extract, supplied with 0.4% gelatin and sterilized. To the sterilized culture media, separately sterilized 8g of gelatin in 100ml distilled water was added along it . After incubation, on the grown culture was flooded with saturated aqueous ammonium sulphate. The clear zone around the colony indicates a positive test for protease activity.

Results

Isolation, purification and preservation of endophytic fungi

Diversity and distribution of endophytic fungi from three different tissues (root, stem/bark, leaf) of *T.neriifolia* have been analysed and described from their emergence, purification and identification.

Emergence of endophytic fungi from healthy tissue

The incubated plates with healthy surface sterilized tissues were observed regularly at every alternate for 21 days. Emergences of endophytic fungi from some of the tissues were observed and hyphal tips of actively growing fungi were then transferred to fresh PDA plates for further processing. After screening *T.neriifolia*, four morphotypes were obtained from leaf samples, three from the bark sample, and one from root samples.

In *T.neriifolia,* a totalof 8 morphotypes were isolated from the 49 segments. In which TB2 showed the maximum colonization frequency of 14.28% and one morphotype produced least CF% (TR1). While six morphotypes showed CF% of about 7.14%. The CF% ranged from 14.28% to 4.76%.

Enzymatic activity of fungal endophytes

Endophytes survive inside the plant tissues and secret variety of extracellular enzymes in order to utilize the available nutrients. In this study, four extracellular enzyme productions were assayed by the isolated endophytic fungi of plants.The most of the endophytic fungi exhibited the production of protease and pectinase on growth medium (table1). Only two isolates gave a positive test for cellulose activity, which included TL4 and TL2.None of the fungal isolates showed amylase activity.

Table1. Enzymatic activity shown by the fungal isolates

S. No	Isolates	Enzyme activity screened			
		Amylase	**Cellulase**	**Lipase**	**Protease**
1	TL1	-ve	-ve	-ve	-ve
2	TL2	-ve	**+ve**	-ve	**+ve**
3	TL3	-ve	-ve	-ve	-ve
4	TL4	-ve	**+ve**	**+ve**	-ve
5	TB1	-ve	-ve	**+ve**	**+ve**
6	TB2	-ve	-ve	-ve	**+ve**
7	TB3	-ve	-ve	**+ve**	-ve

Conclusion

As a poorly investigated store of microorganisms 'hidden' within the host plants, fungal endophytes are a rich and reliable source of bioactive and chemically novel compounds with huge medicinal potential which may be of antibacterial, antifungal, antimalarial or antioxidant nature.

In the present study, we aimed at exploring the mycoflora of a medicinal plant, *T.neriifolia*, and evaluation of the biological activity by virtue of their secondary metabolites. We also aimed to evaluate the genetic diversity of the common endophytic genera isolated from the plants, but this particular objective we could not complete in time owing to some technical reasons and we will attempt in near future.

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INFRARED HEATING IMPACT ON PHYSICAL AND MECHANICAL PROPERTIESOF POTATO STARCH FILM

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Abstract

The objective of the study was to analyze the effect of infrared heating (IR) of potato starch and its film properties. Potato starch was heated with IR heater at different time durations (45 min, 65 min and 85 min) and polymer films were developed from the treated starch by the addition of glycerol as a plasticizing agent. The physical and mechanical properties of developed films were analyzed by their thickness, pasting properties of starch solution, tensile strength and tearing strength were calculated. The results show that the tensile strength of starch film increased from 2.47 MPa to 3.99 MPa as the heating time increased. The final viscosity and setback from tough values of native and modified starch were found in the range of 3812 to 4171cp and 701.7 to 1219cp respectively. The modified starch films thickness and tearing strength were increased from 0.11 to 0.142mm and 149.33 to 170.6g respectively. From the study, the results are showed that IR heat treatment has a significant effect on the physical and mechanical properties of starch film.

Keywords: Starch film, Infrared heating, Tensile strength, Tearing strength, Packaging

Introduction

Potato starch is found abundance in an environment, low cost, renewability, palatability, biodegradable and available as agricultural waste with good film-forming properties (Whitaker, 2020). The characterization of potato starch is large particle diameter, low gelatinization temperature, high paste viscosity and transparency(Gao et al., 2019). Worldwide, corn starch plays a vital role in commercial application due to increased moisture retention, retard crystal growth, and improved product quality. In industrial applications with high shear rates and forces, low acidity and different operating temperatures native starches cannot withstand the processing conditions. Therefore, modification of starch is needed to eliminate the shortcomings of native starches and obtain new functional and value added properties(Arce-Arce et al., 2014; Ismailoglu and Basman, 2015). In general, either by physical method or chemical modification, the characteristics of the native starch may have altered. But the cost is high in the usage of chemical and biological agents. In this context, to improve their properties, various physical treatments such as microwave, infrared (IR), ultrasound have been utilized to modify the structural and functional properties of starch(Chunli et al., 2020; Ismailoglu & Basman, 2015; Przetaczek-Rożnowska et al., 2019).

The utilization of infrared (IR) heat treatment in industries is gaining much attention in recent times due to its various advantages. The application includes a high drying rate, increases degree of starch gelatinization, and modify the structural and functional properties(Ismailoglu & Basman, 2015). The effect of IR treatment alters the colour, pasting properties and moisture content of starch when its exposed for a longer time at higher watts. The physicochemical properties of starch material such as solubility, surface and interfacial properties, gelling potential, linkage type and pattern, viscosity and mechanical properties are important in many material sciences and product development(Orezzoli et al., 2018). Morphological, rheological, thermal and textural properties of starch and polymer solutions vary with varieties, internal factors and operating conditions. Measurement of these properties supports handling, storage and mechanical transport phenomena such as friction or followability(Stasiak et al., 2013). The structural arrangements of the surface of starch granules are important to understand before being used as raw material for various purposes(Popescu et al., 2012). Thus, the present study investigates the effect of IR treatment on important pasting,physical and mechanical properties of potato starch.

Materials and methods

Raw materials

Potato starch and corn starch powders in white to off-white were purchased from M/s Angel Starch and Food Pvt. Ltd., Erode, India. Glycerol (99% purity) was obtained from the Sigma-Aldrich (Mumbai, India) is used for polymer solution preparation along with pure distilled water as a solvent.

Infrared heating system

The selected starch samples were heated by infrared light source of 250w. For the experiment, the starch samples 50gms were taken in the ceramic plates with different bed thicknesses of 5mm, 7mm and 9mm for infrared heating at 45min, 65min and 85min time intervals. The plates were placed under the IR lamp at about 200mm distance for heating.

Pasting properties

The pasting properties starch added film forming polymer solution were analyzed using a Rapid Visco Analyzer (Anton Paar, MCR 302 e, Gurgaon, India) with a rheoplus software. For the test, 3g of 7mm bed thickness IR treated at different time interval starch samples (dry basis) with 40% (w/w) of glycerol were mixed along with 25 ml of distilled water. The solution was correctly mixed to get a uniform dispersion and stabilized at 50^{o}C for 1 min. Then, the temperature was raised to 95^{o}C (5 min), and the suspension was cooled back to 50^{o}C for 1 min. The pasting parameters such as peak viscosity, pasting temperature, final viscosity and total setback value were calculated from the RVA curve(Arce-Arce et al., 2014).

Potato starch film

The potato starch film from the native and IR treated modified starch was developed along with the addition of glycerol as plasticizer. The glycerol was used as plasticizer for starch based films as because it provide more plastic like structure by increases their chain mobility structure with the starch network (Ballesteros-Mártinez et al., 2020; Bertuzzi et al., 2012). The visual observation of developed films from native and modified starch was shown in Fig (1) and it was noted that all films were transparent in nature.

Fig. 1. Potato starch film (a) Control, (b) 45min IR (c) 65min IR (d) 85min IR

Physical Properties

Thickness and density

A digital thickness gauge (Model: Insize 2871-10) with an accuracy of ±0.02mm was used to determine the thickness of potato starch film. The values were measured at five different positions for each film and the average was noted. The mean value of thickness was used for further calculation.

Film color

The surface color of the developed starch film was measured using the colour meter (Hunter lab, model. color flex EZ). The CIE Lab values indicates 'L*' from (0) black to (100) white; 'a*' values (-) green to (+) red; and 'b*' values (-) blue to (+) yellow were adopted to determine the film color. Initially, the instrument was calibrated using standard black and white plates. The test films were placed on the glass container covered with dark metal cup and their color values were measured(Ballesteros-Mártinez et al., 2020). The color difference (ΔE) was calculated by using equation (2)

$$\Delta E = \sqrt{(L^* - L)^2 - (a^* - a)^2 - (b^* - b)^2} \quad \ldots (1)$$

Mechanical properties

Tensile strength

The tensile strength of potato starch films was measured using a single column universal testing machine (Model: Tinius Olsen L-series-H5KL) equipped with a 5KN load cell. The film preparation for the study was carried out as reported by(Ibrahim et al., 2019). The film strip (200 mm×25mm) (LxW) were fixed between the two c-clamps with a grip separation height of 50mm. The peak force was calculated by running the instrument, and the tensile property of each film was calculated using the average thickness value of the sample. The tensile strength was calculated from the measured force and cross sectional area of the film given in equation (6)

$$Tensile\ strength\ (MPa) = \frac{Force}{Cross\ sectional\ area} \quad \ldots(2)$$

Tearing strength

The tearing strength of the film was measured using a tearing tester instrument (Model: Presto PTT-156). This method calculates the work done during tearing through a fixed length of the test specimen. The apparatus setup consists of a pendulum with a clamp aligned with a fixed clamp. The sample was cut into standard template size (100x70mm) and placed in between sample holders. Then, the test was performed by raising the pendulum level by applying a maximum potential (Dhamija & Chopra, 2007). The tearing strength indicated on a calibrated scale fitted on the pendulum by a pointer is calculated from the potential capacity factor value and scale reading used in equation (7)

$$Tearing\ strength\ (g) = 16 \times scale\ reading \ldots(3)$$

Results and discussion

Pasting properties potato starch

The pasting properties of the potato starch film forming solution (FFS) were studied by using rapid visco analyzer. This property in starch is important in the food as well as other industries in terms of viscosity. In heating process, the starch at continues rotating stress in rheometer causes starch granules to swell and results in bursting and the amylose in matrix leaches out and as a result in formation of paste(Bashir & Aggarwal, 2019; Chung & Liu, 2009). The pasting values of native and modified potato starch FFS were shown in Table.1 The peak viscosity represents the maximum viscosity in which there was reduction in viscosity from 5386cP to 4088Cp and the final viscosity (cold paste viscosity) refers to the viscosity obtained at the end of cooling phase where it provides the information on starch capacity to form paste after cooling.From the results, it was observed that the peak viscosity, holding strength, final viscosity and set back trough was decreased due to the infrared heating treatment. The reduction in peak viscosity of IR treated starch due to the partial gelatinization of starch due to the IR treatment condition. It was due to the degradation of amylose and amylopectin in starch by IR treatment that decrease the swelling capacity of starch that result in reduced peak viscosity. The decrease in the final and set back viscosity was due to the retrogradation (Chung & Liu, 2009; Li et al., 2013). All the results were found to be significantly different ($p<0.05$) as compared tocontrol film.Similar results were reported by Chung & Liu, 2009, the gamma radiated corn starch shoed decreased in the viscosity.

Table 1. Pasting properties of native and modified potato starch

Sample	Peak Viscosity (cP)	Pasting Temperature (^oC)	Holding Strength (cP)	Breakdown Viscosity (cP)	Final Viscosity (cP)	Setback from trough (cP)
potato starch (Native starch)	5386 ±3.05^a	75.1± 0.25^a	2101± 3.21^a	3289 ±3.21^a	4171 ±5.03^a	1219±4.16^a
Modified potato starch (45 min)	5152±4.58^b	75.6±0.04b^c	2065 ±4.04^b	3087± 5.51^b	3997±3.51^b	1155±3.79^b
Modified potato starch (65 min)	4088 ±3.06^d	75.9± 0.03^c	1742 ±3^d	2348± 4.51^d	3477 ±5.29^d	611.2±2.52^d
Modified potato starch (85 min)	4512 ±5^c	75.4± 0.02^{ab}	1856 ±3.51^c	2652 ±3.51^c	3812± 2.52^c	701.7±3.21^c

Values are mentioned as mean± standard deviation. Values in each column with different letters in superscript are significantly different ($p<0.05$) follows turkeys test.

Physical properties of starch film

Thickness is considered to be a basic property of the films, the thickness value of films prepared from native and IR treated modified starches are shown in Table 2. The thickness of film ranges from 0.104 to 0.142 mm and the highest value of 0.142 mm was found in film prepared with 85 min IR treated potato starch. The result found to be significantly different ($p<0.05$) in the film thickness of IR treated potato starch as compared to native starch. The thickness of the film affected by chemical composition of the material, molecular structure of film compounds and their packaging level during formation of film (Majzoobi et al., 2015).The color attributes (L, a, b and ΔE) of the biopolymer film developed from native and modified starch was shown in Table 2. The ΔE value is the color difference that was analyzed from the color parameters such as lightness (L), red-green (a), and yellowness-blue (b) value. The lightness value of control film was 83.26 and the higher reduction of 82.63 was found in films prepared from 45 min infrared treated potato starch. The color a value incresed from

4.54 to 4.59 and the b value of native starch film was -12.42 which was reduced to -13.04 (film from 85 min IR treated starch) but they are not statistically different ($p>0.05$). The color difference ΔE was 2.68 for native potato starch film and the higher color difference of 3.40 which was observed for films obtained from 85 min IR treated potato starch as compare to the standard white paper (L:82.04, a: 4.59 and b: -13.04) respectively .The higher the color difference value the more opaqueness of the film (Rodrigues et al., 2021). The color differences in the film may be due to the changes in the presence of some other compounds such as lipids, protein, mineral and pigments during heating treatment (Falade & Ayetigbo, 2015; Rodrigues et al., 2021).

Table 2. Physical properties of films from native and modified potato starch

Sample	Film thickness (mm)	Color value			ΔE
		L	a	b	
Native starch	0.104±0.015	83.26±0.59	4.54±0.08	-12.42±0.36	2.68±0.22
IR 45 min	0.11±0.019	82.63±0.08	4.53±0.05	-13.04±0.31	3.08±0.69
IR 65 min	0.126±0.23	82.31±0.29	4.57±0.01	-12.9±0.30	2.49±0.46
IR 85 min	0.142±0.02	82.04±0.19	4.59±0.01	-13.04±0.08	3.40±0.44

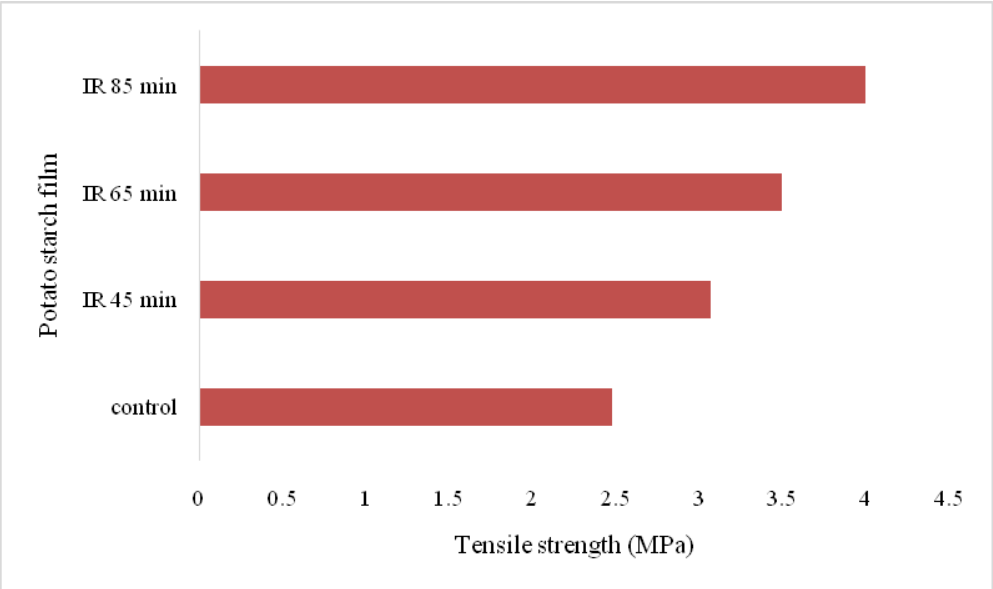
Values are mentioned as mean± standard deviation. Values in each column with different letters in superscript are significantly different ($p<0.05$) follows turkeys test.

Mechanical properties of starch film

Tensile strength

In mechanical properties, tensile strength plays an important role as it determines the usage of films in food applications. The tensile strength provides the information of film strength in resisting against the mechanical damage before the film breaks (Javadian et al., 2014; Othman et al., 2019).The tensile strength values were shown in Fig.2The value of tensile strength of potato films were ranges from 1.86 to 3.03 Mpa and they were found to be significantly different ($p<0.05$) as compared to control films. The increase in the tensile strength of film from 85 min IR treated starch was may be due to heating, where the starch powder breaks out and the amylose in the starch leaches out and form the complex of amylose-lipid with lamellar structure that improves film strength (Noranizan et al., 2010; Zhong et al., 2018).The obtained result was found to be closer to thefinding reported by other researchers (de Almeida et al., 2020; Sun et al., 2014)were the values were found to be 1.40MPa and 2.06MPa for waxy potato starch.

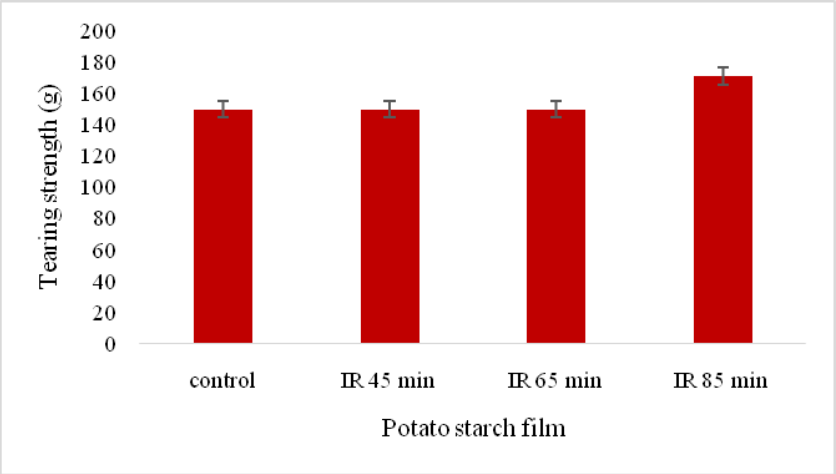
Fig 4. Tensile strength of films from native and modified corn starches. Different letters on the bars represents there was significant differences at ($p <0.05$)



Tearing strength

Tearing strength is considered to be one of the major tests for paper and for all films(Bebartta et al., 2020).The tear strength of the prepared films was shown in Fig. 5. It was observed that the tearing strength value was found to be increasing from 133.3 g to 272 g where the values were increasing as the infrared treatment time increases and the maximum strength was found for films from 85 min infrared treated starch film. The results were found to be significantly different as compared to the control native starch film. The tear strengthmeasures the resistance of film against tear propagation is of machine performance and also can be used as a primary packaging film in sealing and wrapping operations (Kittur et al., 1998).

Fig 5. Tearing strength of films from native and modified potato starch. Different letters on the bars represents there was significant differences at (p <0.05)



Conclusion

The physical, hydration and functional properties of film prepared with potato starch as native form and IR treated starch as modified form. The potato starch was treated with the infrared heat treatment for 45 min, 65 min and 85 min respectively. Various properties such as film thickness, color, tensile strength, tearing and tearing strength and film morphology wereanalyzed. The colour brightness of the film was ranges from 82.04 to 83.26 and it was observed the highest opaqueness was found for film from 85in treated potato starch powder. The tensile, and tearing strength also found to be higher at film from 85 min treated potato starch film. Thus, the infrared treatment has shown significant change on the prepared films. The heat treatment induces the complex formation between the amylose and lipids that present in the potato starch. The treatment doesn't affect the color of the film as all were transparent in nature. Thus, this study showed the effect of IR treatment on the potato starch based film.

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