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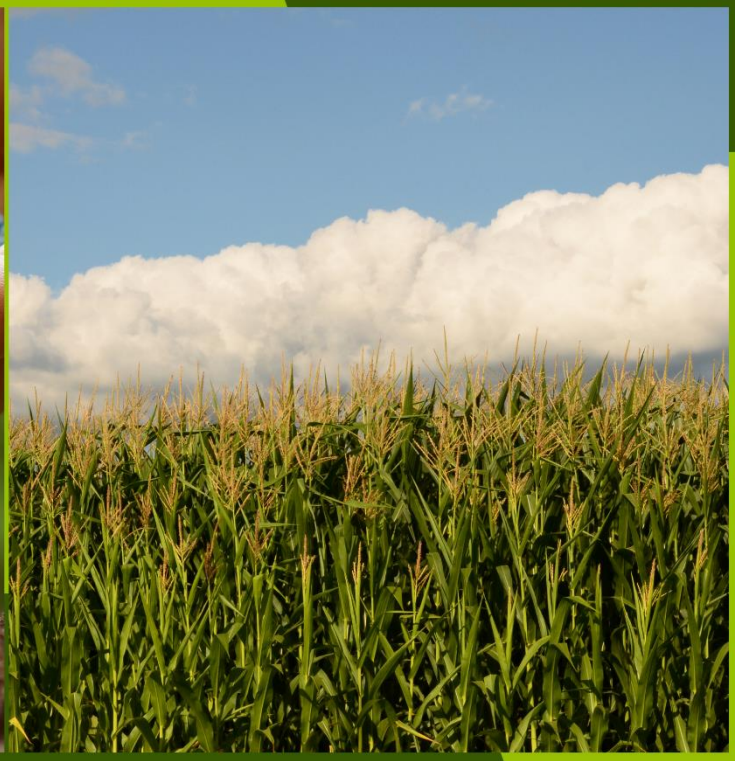
AGRICULTURE SCIENCE: RESEARCH AND REVIEW

VOLUME XIII



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PREFACE

We are delighted to publish our book entitled ***"Agricultural Science: Research and Reviews Volume XIII".*** *This book is the compilation of esteemed articles of acknowledged experts in the fields of basic and applied agricultural science.*

The Indian as well as world population is ever increasing. Hence, it is imperative to boost up agriculture production. This problem can be turned into opportunity by developing skilled manpower to utilize the available resources for food security. Agricultural research can meet this challenge. New technologies have to be evolved and taken from lab to land for sustained yield. The present book on agriculture is to serve as a source of information covering maximum aspects, which can help understand the topics with eagerness to study further research. We developed this digital book with the goal of helping people achieve that feeling of accomplishment.

The articles in the book have been contributed by eminent scientists, academicians. Our special thanks and appreciation goes to experts and research workers whose contributions have enriched this book. We thank our publisher Bhumi Publishing, India for taking pains in bringing out the book.

Finally, we will always remain a debtor to all our well-wishers for their blessings, without which this book would not have come into existence.

Editors

CONTENT

Agriculture Science: Research and Review Volume XIII
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PHYTOCHEMICAL ANALYSIS OF *MYRISTICA FRAGRANS* [L] AND QUANTITATIVE ESTIMATION OF TOTAL PHENOLIC CONTENT

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Abstract:

Myristica is a genus of trees in the family Myristicaceae. There are over 150 species distributed in Asia and the western pacific. All or nearly all species are dioecious. The most important commercial species is *Myristica fragrans*, the main source of the spices nutmeg and mace. *Myristica fragrans* is commonly named nutmeg or mace. It is an aromatic evergreen tree with spreading branches and a yellow fleshy fruit similar in appearance to an apricot. Nutmeg contains 25-30% fixed oils and 5-15% volatile oil (camphrene, eugenol) and other molecules such as myristic acid, myristicin and lignin compounds. Among these molecules, Eugenol is very effective in antibacterial activity against oral bacteria. Nutmeg belonging to the family myristicaceae, is a spice seed from the fruit of a tropical evergreen tree. Myriticaceae are found in humid low land forests, swamp forests, Sub montane forests, and cloud forests at elevation up to 2100m. Some of the anatomical characters presented by this family suggest that in the past they could live in xeric (dry) environments, but now their species are linked to tropical rain forests. The foliage is generally spicy- aromatic and the leaves are glossy, dark green, simple entire, two ranked, undersides often whitish or tomentose, with dark brown punctation or not usually with complex caduceus hairs colored golden yellow or red. The flowers are usually small, highly reduced, fragrant, with 3-5 sepals, inner perianth whitish – green, yellow, reddish pink to rusty – brown, arranged in axillary paniculate inflorescences or unbranched wart- like structures. The main objective of the study is to analyze the various components in myristica and to estimate it quantitatively so that it can be used for various pharmacological activities.

Keywords: *Myristica*, flavonoids, mace, phenolic content, nutmeg

Introduction:

The Myristicaceae are a family of flowering plants native to Africa, Asia, pacific islands, and the Americans and has been recognized by most taxonomists. *Myristica* species are natives of Moluccas, indigenous to India, Indonesia and Sri Lanka and now cultivated in many tropical countries of both hemispheres as well as in South Africa (Pal *et al.*, 2011). *Myristica* genus has nine species. *M. fragrans* is a spreading aromatic evergreen tree usually growing to about 5 to13 m high, occasionally 20 m. When ripe, the succulent yellow fruit coat splits into two halves revealing a purplish-brown, shiny seed (nutmeg) surrounded by a red aril (mace). Seeds (nutmegs) are broadly ovoid (2 to 3 cm long), firm, fleshy, whitish and transversed by red-brown veins. When fresh, the aril (mace) is bright scarlet becoming horny, brittle and with a yellowish-brown color when dried. Valero M, Salmerón M (2002) studied antibacterial activity of 11 essential oils from aromatic plants against the strain INRA L2104 of the foodborne pathogen Bacillus cereus grown in carrot broth at 16 degrees C was studied. Grover *et al. (*2002) studied recurrent diarrhea is prevalent in developing countries, particularly in tropical regions. Murcia *et al. (*2004) studied antioxidant properties of seven dessert spices (anise, cinnamon, ginger, licorice, mint, nutmeg, and vanilla) were compared with those of the common food antioxidants butylated hydroxyanisole (BHA). JY Chung *et al. (*2006) studied occurrence of dental caries is

mainly associated with oral pathogens, especially cariogenic Streptococcus mutans. Preliminary antibacterial screening revealed that the extract of *Myristica fragrans*, widely cultivated for the spice and flavor of foods, possessed strong inhibitory activity against S. mutans.

Jung *et al.* (2007) studied insecticidal constituents of hexane-soluble fraction from a methanolic extract of the seeds from *Myristica fragrans* (Myristicaceae) against adult females of *Blattella germanica* (L.). Somani and Singhai (2008) studied hypoglycaemic and antidiabetic activity of seeds of *Myristica fragrans* in normoglycaemic and alloxan- induced diabetic rats. Morita *et al.* (2003) studied the hepatoprotective activity of spices, 21 different spices were fed to rats with liver damage caused by lipopolysaccharide (LPS) plus d-galactosamine (D-GalN). Parle *et al. (*2004) studied the effect of *Myristica fragrans* (MF) seeds on learning and memory in mice. Chirathaworn *et al.* (2007) studied *Myristica fragrans Houtt*. (nutmeg) contains antibacterial, antiviral and anti-cancer activities. Sohn *et al.* (2008) studied cisplatin is one of the most effective antineoplastic drugs, but it has undesirable side effects such as hepatotoxic the protective effect of macelignan, isolated from *Myristica fragrans* HOUTT. (nutmeg), against cisplatin-induced hepatotoxicity and the possible mechanisms involved in these effects in mice. Gupta *et al.* (2013) studied antioxidant and antimicrobial activities of nutmeg (*Myristica fragrans Houtt)* seed extracts were evaluated.

Taxanomical Classification (Forrester, 2005):

Kingdom: Plantae – Plants	Subclass: Magnoliidae
Superdivision: Spermatophyta	Order: Magnoliales
Subkingdom: Tracheobionta	Genus: Myristica Gronov. – Nutmeg
Division: Magnoliophyta	Family: Myristicaceae – Nutmeg family
Class: Magnoliopsida	Species: *Myristica fragrans Houtt.* – Nutmeg

Fig.1: *Myristica fragrans*

Pooja *et al.* (2012) studied in anti-fungal consequence of hexane, methanolic, chloroform, in addition ethanolic extract attained from Mace. Lee *et al.* (2009) studied *M. fragrans* compounds have proven ability to stimulate osteoblastic differentiation. This study is based on the aqueous extraction of the leaf of *M. fragrans* which were then analyzed for the most important phytochemicals such as alkaloids, steroids, phenolics, tannins, glycosides, saponins and flavonoid present in it and to estimate the amount of phytochemical that is present in highest amount in the aqueous extract of *M. fragrans*.

Material and Methods:

Collection of plant materials

The leaves of *Myristica fragrans* were collected from the local area during the month of January 2020. The taxonomic identification of plant material was done with the help of Professors of Department of Botany, TKMCAS college. The plant samples were collected and properly washed in tap water and rinsed with distilled water. They were shade dried and pulverized during using a motor and pestle, to obtain a powdered form. The powdered form of this plant was stored in an air tight glass container and protected from sunlight until required for further analysis.

Chemicals

The chemicals required such as Mayer's Reagent, Wagner's Reagent, Dragendroff's Reagent, Lead Acetate, Ferric Chloride, Sodium Hydroxide, Glacial Acetic acid, Concentrated Sulphuric acid, Hydrochloric acid, Chloroform, Molisch's Reagent, Benedict's Reagent, Milon's Reagent, Sodium Nitroprusside, 10% Ammonia, Benzene, Folin's Ciocalteau's Reagent for the experiment was purchased from Himedia, Sigma Aldrich chemicals and Merck Life Science Pvt Ltd.

Preparation of aqueous extract of plant samples

The aqueous extract of each plant sample was prepared by soaking 10g of powdered sample in 200ml of distilled water for 24hrs. The extract was then filtered using filter paper.

Phytochemical screening procedure

Qualitative tests for alkaloids, flavanoids, carbohydrates, glycosides, saponins, terpenoids, proteins and Anthraquinone were performed according to the procedure described by Harborne *et al.* (1973). Mayer s test, Wagner's test for alkaloids, Benedict's test, Molisch test for carbohydrates, Ninhydrin test and Biuret test for protein.

Detection of alkaloids

A. Mayer' s test

To 1ml of the extract, added 1ml of Mayer's reagent added. A white creamy precipitate indicates a positive result.

B. Wagner' s test

To 1ml of extract, 1ml of Wagner's reagent was added a brownish or reddish precipitate indicates positive result.

C. Dragendroff's test

To 1ml of extract added 1ml of Dragendroff's reagent. Formation of a orange red precipitate showed the presence of alkaloids.

Detection of flavanoids

A. Lead acetate test

1ml of extract was treated with few drops of lead acetate solution yellow colour indicates the presence of flavanoids.

Detection of phenolic compounds and tannins

A. Ferric chloride test

To 1ml of extract 5% neutral ferric chloride was added, a dark blue colour indicates the presence of phenolic compound

B. Lead acetate test

To 1ml of extract prepared lead acetate is added. A white precipitate indicates a positive result.

C. Potassium dichromate test

To 1ml of extract potassium dichromate was added. Brownish green coloured precipitate indicates the presence of tannins.

Detection of saponins

A. Froth test

To 1ml of extract, 20ml distilled water was added and shaken for 15 minutes, foam developed in the tube indicates the presence of saponins.

B. Foam test

To 2ml of distilled water, added 0.5g of extract and shaken well. Presence of foam indicates the positive result for saponin

Detection of glycosides

5ml of extract was mixed with 2ml of glacial acetic acid and 1 drop of ferric chloride. To this mixture 1ml of concentrated sulphuric acid was slightly added along the sides of test tube. A brown ring formed indicates the presence of glycosides.

Detection of terpenoids and steroids

A. 5ml of extract was mixed with 2ml of chloroform and 3ml of sulphuric acid was added along the sides of the test tube. A reddish-brown colouration indicates the presence of terpenoids.

1ml of extract was dissolved in 10ml of chloroform and 10ml of concentrated sulphuric acid and kept for few minutes. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence, which indicates the presence of steroids.

Detection of carbohydrates

Small quantity of extract was dissolved in 5ml of distilled water and filtered. The filtrate was subjected to various tests to detect the presence of carbohydrates.

A. Fehling' s test

To 2ml of aqueous extract, added 1ml of Fehling's reagent (A&B). Boiled for a few minutes. Formation of a red precipitate indicates the presence of carbohydrates.

B. Benedict's test

To 0.5ml of extract, 5ml of Benedict's reagent was added and boiled for 5 minutes formation of a bluish green or orange precipitate according to the amount of reducing sugar present in the extract.

C. Molisch' s test

In the test tube containing 2ml of extract, 2drops of freshly prepared 20% alcoholic solution of α - naphthol was added and mixed. To this solution 2ml of concentrated sulphuric acid was added along the sides of test tubes so as to form a layer. Formation of violet ring indicates the presence of carbohydrate.

Detection of anthraquinones

To 1ml of the extract added 10ml of benzene and filtered. To the filtrate added 1% of HCl added 5ml of 10% ammonia and shaken well. Development of a pinkish coloured solution indicates the presence of anthraquinones.

Detection of phlobatannins

10ml of extract is boiled with few drops of 1% aqueous hydrochloric acid. A red precipitate developed indicates the presence of phlobatannins.

Detection of amino acids and proteins

A. Ninhydrin test

To 1ml of filtrate added a few drops of 0.2% Ninhydrin and heated for 5minutes. The blue colour gives the positive results for Ninhydrin test and indicates the presence of aminoacids.

B. Biuret test

To 2ml of extract added 2ml of 10% sodium hydroxide and 0.1% copper sulphate solution. Pinkish or purple violet colour indicates the presence of protein. Thus gives a positive result for protein test.

C. Millon's test

To 1ml of extract added equal volume of concentrated H_2SO_4 along the sides of test tube and added a few drops of Millon's reagent and then heated it. The yellow precipitate developed indicates the presence of amino acid.

Determination of total phenolic content

From the aqueous extract of *Myristica fragrans* total phenolic content can be estimated by Folin's Ciocalteau method using gallic acid as the standard. So, the materials required are 100mg of the plant extract, Folin's reagent and sodium carbonate (75gm in 100ml). Different volumes of standard solution (0.2- 1ml) were taken in test tubes marked (S1 – S5). All the tubes were made up to 1ml with distilled water 1ml of the distilled water was taken as blank 0.4ml Folin's reagent was added to all the test tubes and mixed well. All the tubes were kept for 5 minutes at room temperature. Then 4ml of sodium carbonate was added to all the test tubes. 0.5ml of sample was taken as test in a test tube marked 'T' and treated in the same manner. The blue colour developed was read at 750nm using a colorimeter. A standard graph was plotted using the values obtained by taking concentration of standard on X- axis and optical density along Y- axis. From the graph, the concentration of total phenolics from extract was estimated.

Results:

Phytochemical analysis

Table 1: Phytochemical analysis of aqueous extract of *Myristica fragrans*

Phytochemicals	Aqueous Extract
Alkaloids	++
Flavanoids	+
Phenols	+++
Saponins	+
Glycosides	+++
Phlobatanins	-
Carbohydrates	+
Anthraquinones	-
Terpenoids	++
Tannins	+
Steroids	++
Amino Acids	+
Proteins	-

+++ = present in higher amounts, ++ = moderately present, + = present.

The presence of phytoconstituents makes the plant useful for treating different ailments and has a potential of providing useful drugs for human use. The aqueous extraction of the leaves of *M. fragrans* which were then analyzed for the most important phytochemicals such as

alkaloids, steroids, phenolics, tannins, glycosides, saponins and flavonoid present in it and to estimate the amount of phytochemical that is present in highest amount in the aqueous extract of *M. fragrans*.

Estimation of total phenolics by folin ciocalteau method

By quantitatively estimating the aqueous extract with Folin's reagent, the total phenolic content was estimated. In acid medium, the phenolic compounds reacted with sodium tungstate and sodium molybdate in the Folin's reagent to form blue colour which was read at 750nm by reading the optical density from colorimeter and further the total phenolic content was estimated. As a result, from the graph, it was estimated that the aqueous extract of *M. fragrans* contained 20.1 mg/gm of phenolics.

Discussion:

Most of the traditional knowledge about medicinal plants was in the form of oral knowledge. There is no uniform or standard procedure for maintaining the inventory of these plants and the knowledge about their medicinal properties. Therefore, it is necessary that such procedures to be documented and studied for systematic regulation and widespread application. It is very important to undertake phytochemical investigations along with biological screening to understand therapeutic dynamics of medicinal plants and also to develop quality parameters. Qualitative preliminary phytochemical analysis was performed initially with different chemical reagents to detect the nature of phytoconstituents. Phytochemical analysis conducted on the leaf extract disclosed medicinal as well as physiological activities. Since most phytochemicals have antioxidant activity and protect our cells against oxidative damage and reduce the risk of developing diseases

The qualitative analysis of phytochemicals in the aqueous extract of leaves of *M. fragrans* indicated the presence of alkaloids, flavonoids, phenolic compounds, saponins, glycosides, terpenoids, steroids, tannins, carbohydrates (reducing sugars) and amino acid. The results are tabulated in table 1. Glycosides and phenolics were present in higher amounts, followed by alkaloids, terpenoids and steroids. Flavonoids, saponins, carbohydrates, tannins and amino acids were present in least amounts. But anthraquinones, phlobatannins and proteins were found to be absent in the aqueous leaf extract of *M. fragrans*.

Phenolics and glycosides were found to be present in higher amounts in the aqueous extract. Phenols are found in the natural world proved to have hypertensive effects and anti-oxidants properties. Since total phenolics was increased in the aqueous extract of leaves of *M. fragrans*, we have done the quantitative estimation by Folin's method. The result showed that the total phenolics present in 1-gram aqueous extract was 20.1mg/gm. This preliminary phytochemical screening may be used in the detection of bioactive principles and subsequently may lead to the drug discovery and development.

Conclusion:

The millenarian use of plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases. In the present study, we have found that most of the biologically active phytochemicals were present in the aqueous extract of *M. fragrans* leaf. The medicinal properties of myristica leaf extract may due to the presence of above-mentioned phytochemicals. Phytochemicals found present in leaf extracts of *M. fragrans* indicates their potential source of principles that may supply novel medicines. Further studies are therefore suggested to ascertain their pharmacological activities. Furthermore isolation, purification and characterization of the active principles of *Myristica fragrans* will provide a platform for the treatment of various diseases.

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DIGITAL TECHNOLOGY: THE GAME CHANGER IN AGRICULTURE

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Abstract:

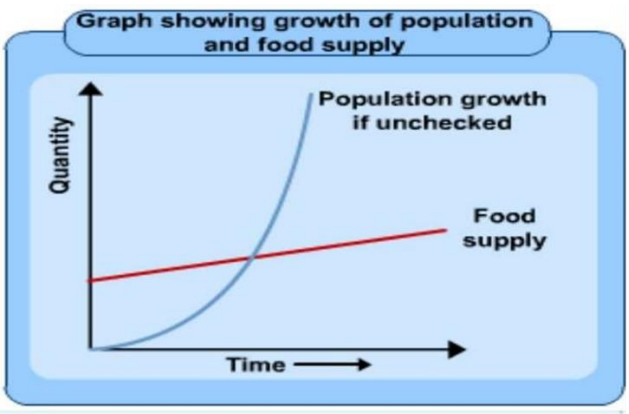
The projected food production to supply the growing demand is a challenging task as the world will reach 8.5 billion population by 2030. The world is witnessing yet another modification that employs application of modern information and communication technologies into agriculture i.e., digitalization of agriculture. Digital farming involves integration of advanced technologies into already persisting agricultural practices with a view to boost the food production, food quality and efficiency of farm activities. The precision tools reduce the heavy workload of the farm activities, inturn enhance the quality of work. The huge farm data collected and analyzed will help the farmer for precise decision making for higher agricultural production. Digital farming is a game changer in addressing the issues of population growth, climate change and labour issues in field operations from planting to harvest of the crops.

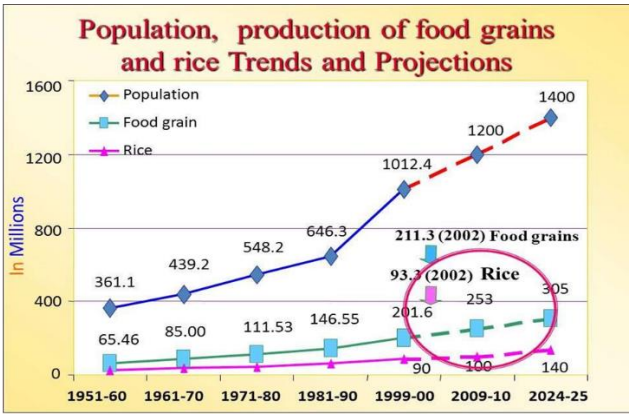
Keywords: Agriculture, Digital, Precision farming, Technology

Introduction:

In India, Agriculture is the core sector for food security, nutritional security, sustainable development and poverty alleviation. At present the world population is around 7.9 billion and approximately it would reach around 9.9 billion by 2050. Coming to India's context population is increasing day by day, it's around 1.44 billion as on now. The UN's food and agriculture organization has estimated that 60 per cent more food per year will be demanded by 2050. Keeping this in context, in just a 35 year period the earth is being tasked with producing more food than it has in the last 2000 years combined. Either we find very clever ways of sustainably producing more food on the same areaof land or we need to demand less. In reality we need to do both.

Notwithstanding the fact that there is no magic formula for producing enough to ensure global food security, it is clear that new technologies have a central role to play. One of the game changers of the past was the introduction of semi-dwarf varieties of cereals combined with improved irrigation and fertilizers resulted in increasing of the yields and resulted in green revolution. But the new revolution that the researchers are seeking for is agriculture's sustainable intensification, increasing yields on existing land without much impact on environment. Now a day's youths and people are migrating in search of new jobs, there is scarcity of labour in the rural areas because of urbanization. To combat all the above problems, a technology is the key source. New technologies will play an important role given the magnitude of the challenges for food security in the coming decades.





Growth of population, food supply and future trends and projections

By 2030, it is anticipated that there will be 8.5 billion people on Earth and close to 10 billion by 2050. At the same time, per capita arable land availability will be decreased. This will lead to a significant increase in demand for food and fiber as projections show that overall food production would have tobe raised up to 305 million tonnes by 2024-25, while production in the developing countries would need to be almost doubled. This clearly indicates that, the available resources should be managed efficiently to increase the food production along with maintaining sustainability.

Indian agriculture has been propelled by technology time and time again by strengthening market ties, overcoming production stagnation and improving farm management. These technologies help to solve major challenges confronting Indian agriculture which include declining total productivity, natural resource depletion and degradation, a rising global food demand (not just for quantity, but also for quality), stagnant agricultural incomes, dispersed land ownership, and unprecedented climate change are all factors. It has been proven that adopting technology modernizes farmers' production methods and results in predictable annual returns for farmers as well as decreased crop failure risk and higher yields.

The application of digital farming in agriculture has been instrumental in promoting data generation as well as the advanced analytics that allow farmers to make smart decisions about farming and to benefit from an economical use of inputs and labour. The aim in digital agriculture aims to enable the mechanization of sustainable agricultural processes by utilizing all knowledge and experience at our disposal.

Resources are anything that has utility and adds value to your life. Everything in nature that is useful to humans, including trees, tree, animals, land, metals and minerals, is a resource. Each of these resources has a different value depending on its usability and other elements. Metals like gold, silver, copper, or bronze, for instance, have economic worth, meaning that they may be traded for cash. Forests, rivers, mountains, and the sea are all resources, but they have no economic worth.

The two most crucial elements that can transform any material into a resource are time and technology. Technology and innovation allow people to turn a natural or artificial component into a resource. For example, sea minerals, fish, or other marine life can be used to make food and medication. Time also increases the worth of a resource in a similar manner. For instance, fossil fuels can be created from the remains of species that lived hundreds of years ago. A natural resource is anything and all of the things naturally present on earth.

As agronomists we are more concerned about the production resources. Production resources are nothing but the resources that are necessary for cultivation of crops. Examples for production resources are soil, water, seeds, livestock components, manures and fertilizers, weather, labours and machineries and economic resources.

Resource management

Resource management deals with managing the way in which people and natural landscapes interact. This focuses particularly on a scientific and technological knowledge of resources, ecology and the ability of such resources to maintain life. It acknowledges that people and their means of subsistence depend on the efficiency and soundness of our landscapes, and that their stewardship of the land plays a crucial role in preserving this productivity and health. Planning for land use, water management, protecting biodiversity and ensuring the long-term viability of sectors including forestry, mining, tourism, and agriculture are all included.

The various approaches involved in managing resources are

There are a number of challenges identified in the case studies related to natural resources and itsmanagement. These include,

- Rising levels of forest degradation and deforestation: The major causes of the deforestation include overexploitation, particularly illegal logging and forest conversion for other utilization, especially extensive oil palm and coal mining industries.
- Agricultural production and food security: Reduced agricultural production is due to climate change and has affected food security. Rapidly increasing population leads to increased demand for food. Marginal land such as peat land and upland, despite having low productivity, are used for food production. Using degraded land for food production has an impact on technological innovation that will increase land productivity and lessen environmental deterioration.
- Natural ecosystem degradation and biodiversity loss.
- Increasing disputes over the use, allocation and ownership of natural resources: Conflicts between governments and communities are developing as a result of the conflicting dual agendas of government and community for the maintenance and usage of protected zones. Over the land allotted for extensive, commercially oriented concessions and highland peasant cultivation, there is fierce competition and strife. However, there are significant difficulties with inequality in upland peasant societies for individuals taking advantage of several opportunities made possible by expansive agricultural and forestry concessions. As a result of these problems, local communities breach the law and indulge in careless resource collection, which has a negative impact on the natural resource base.
- Unsustainably high use of natural assets: The region's natural environment are frequently overexploited and used iniquitously, which has altered the quantity, quality and allocation of the ecological services.
- Rights and social welfare: Local laws that prohibit cutting down trees, hunting, farming land, and building homes restrict local residents' utilization of natural assets. In a number of instances, a local community's access to land and natural resources has been fully eliminated by leasing those rights to private enterprises within output forests or by designating the region as protected forests.

To overcome the above challenges mentioned, there is a need for technology in agriculture that can contribute towards resource management and have a positive impact on food production, security and sustainability of resources.

Digital agriculture

Digital agriculture is the evolution of Agriculture into a digitalized industry- making a farm's field operations more insight driven and efficient and it can improve on-farm decision making and execution, helping farmers to predict what is coming around the corner and to act upon it more effectively.

Benefits of digital farming

As innovators introduce new technologies, their commercial uses increase day by day. Some are as follows:

1. **Enhanced Production:** The farmer can improve production capabilities through comprehensive irrigation planning, adequate monitoring of crop health, increased knowledge about soil health and adaptation to environmental changes.
2. **Effective and Adaptive Techniques:** The use of digitized agricultural techniques provides farmers with regular reports on their crops and aids in the development of more effective farming methods. They can adjust to climate shifts and utilize resources efficiently.
3. **Greater safety of farmers:** It is safer and more convenient for farmers to use drones or robots or other digital farming technologies used to spray pesticides in terrains challenging to reach, infected areas, taller crops, and power lines. Also, it aids farmers avoid spraying their crops, which reduces contamination and chemical contamination of the soil.
4. **10x faster data for quick decision-making:** Digital farming technologies back farmers with accurate **data processing** that encourages them to make quick and mindful decisions without second-guessing, allowing farmers to save the time invested in crop scouting. The drone can be fixed with several sensors for other crops, allowing a more accurate and diverse crop management system.
5. **Less wastage of resources:** Enables optimum usage of all resources such as fertilizer, water, seeds, and pesticides.
6. A drone survey using geospatial technology has **a 99% accuracy** rate, which enables farmers accurately measure their acreage, divide their crops and map their soil.
7. **Helpful for Insurance Payments:** Farmers utilize the information obtained by drones to submit insurance claims for crop damage. Even if they are insured, they nevertheless analyses the risks and losses related to the land.
8. **Agri-drones are used by the agricultural insurance** industries to collect accurate and reliable data, which is evidence for insurance firms. For an accurate calculation of the financial compensation to be given to the farmers, they record the damages that have happened.

How digital farming technology help the farmers?

- Digital technologies may provide crop and input recommendations at the pre-harvest stage and help with insurance and financing applications.
- At the on-farm stage, support with disease and pest control, as well as weather advisories, are required.
- Real-time market information for both domestic and international markets is required at the post-harvest phase.

Components of digital agriculture

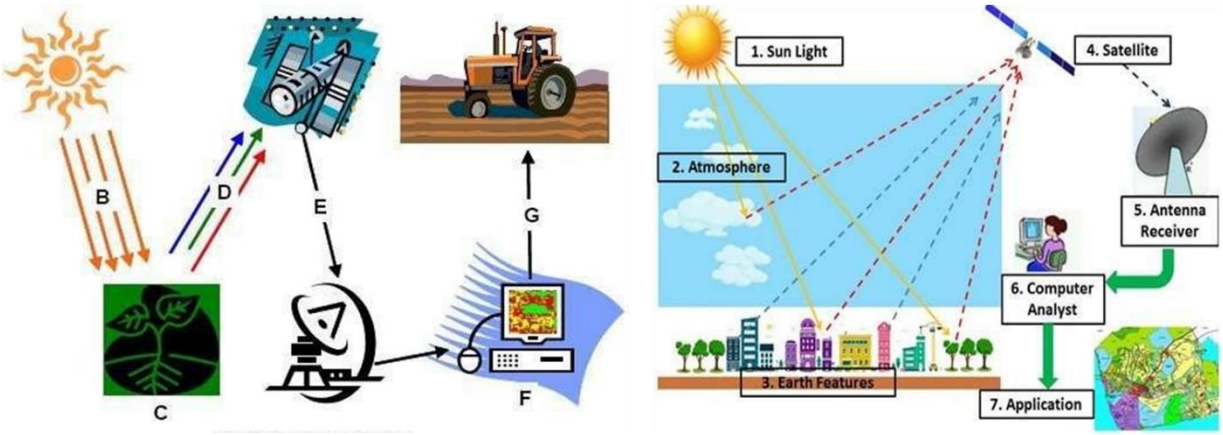
1. Remote sensing
2. GPS
3. GIS
4. Internet of things (IoT)
5. Artificial Intelligence (AI)
6. Robotics
7. Big Data Analytics
8. Information and communication technologies (ICT's)

1. Remote sensing:

As contrast to in situ or on-site observation, remote sensing is the method of gathering data about a phenomenon or object without actually coming into touch with it. The phrase is specifically used in reference to learning more about the Earth and other planets. Several areas of Earth science, such as hydrology, ecology, meteorology, oceanography, glaciology and geology, use remote sensing. It also has uses in the military, intelligence, commercial, economic, planning, and humanitarian sectors, among others.

Currently, the phrase "remote sensing" is used to describe the use of sensor technology based on satellites or aircraft to find and categories items on Earth. Based on transmitted signals, it encompasses the atmosphere, the surface, and the oceans (e.g. electromagnetic radiation). It can be divided into "active" and "passive" remote sensing, depending on how a signal is transmitted to an object by a satellite or aircraft and detected by a sensor (when the reflection of sunlight is detected by the sensor).

Process of remote sensing

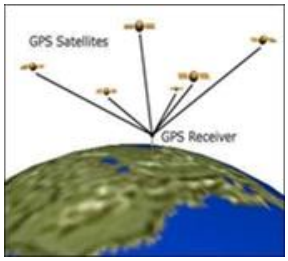


Process of remote sensing

Energy Source/Illumination **(A),** Radiation and Atmosphere **(B),** Interaction with the Target **(C),** Recording energy by sensor **(D),** Transmission, Reception, and Processing **(E),** Interpretation and Analysis **(F)** and Application **(G).**

Why Remote Sensing is required?

- Systematic data collection
- Information about 3D of real objects
- Repeatability
- Global coverage



- Assess to inaccessible areas
- Multipurpose information

2. Global positioning system (GPS):

The United States government owns and manages the satellite-based radio navigation system known as the Global Positioning System (GPS), formerly known as Navstar GPS. It is one of the global navigation satellite systems (GNSS) that gives a GPS receiver access to geo-location and time data from four or more GPS satellites from anywhere on or near the Earth. Mountains and structures can obstruct the comparatively weak GPS transmissions.

Although these technologies can improve the value of the GPS positioning data, the GPS does not require the user to submit any data and it operates without any telephonic or Internet reception. The GPS gives users in the military, civic and commercial sectors around the world essential location capabilities.

GPS Use in Agriculture:

- Tracking Livestock
- Tractor Guidance
- Fertilizing and Crop Protection
- Mapping, Scouting, and Sampling
- Harvesting
- Yield monitoring
- Soil sampling

3. Geographic information system (GIS):

A geographic information system (GIS) is a class of database that combines software tools for managing, analyzing and displaying data with geographic data (i.e., descriptions of phenomena for which location is relevant). A system like this may be thought of in a larger sense to include human users and support personnel, processes and workflows, a body of knowledge of pertinent concepts and techniques, and institutional organizations.

GIS data acquisition includes a variety of techniques for accumulating spatial data into a GIS database, which can be categorized into three categories: primary data capture, the direct measurement phenomena in the field (for example, remote sensing, the global positioning system); secondary data capture, the extraction of information from existing sources that are not in a GIS form, like paper maps, through digitization; and data transfer, the copying of current GIS data from external sources.

4. Internet of things (IoT):

IoT is inter-networking of physical devices. This system has ability to transfer data over a network without requiring human to human or human to computer interaction. IoT refers to a situation in which things, animals, or humans are given distinctive IDs that can transmit data over an Internet network without requiring human-human or interactions between people.

Five types of Internet of things:

1. Tagging Things: Using RFIDs, real-time item tracing and addressability.

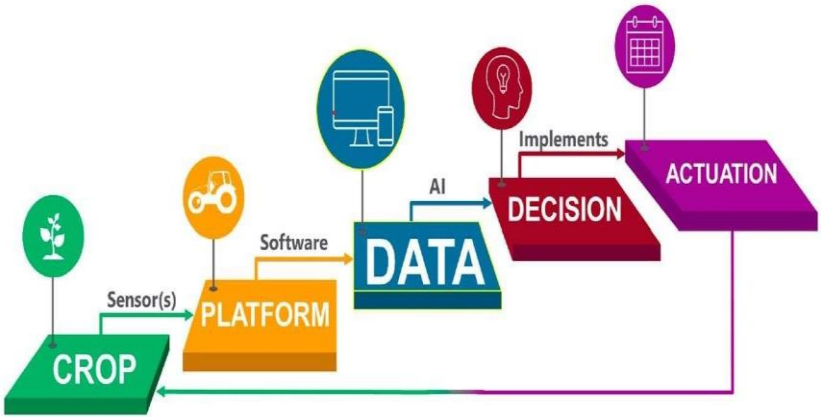
- Widely used in Transport and Logistics
- Easy to deploy: RFID tags and RFID readers

2. Sensing Things: Sensors are the main tools used to gather environmental data.

3. Shrinking Things: Because of miniaturization and nanotechnology, smaller objects can now communicate and link to smart gadgets.

4. Thinking Things: Using sensors, embedded intelligence in gadgets has created the network connection to the Internet.

Here the above figure represents the process of application of internet of things in field i.e., first sensors will collect primary data from the crop or field, then the collected data is sent to software for analysis. Based on the analyzed data with the use of artificial intelligence the system will take management decisions which is suitable for those conditions. Then those decisions are implemented back in the field by automation/actuation.



5. Artificial Intelligence (AI):

The simulation of human intelligence processes by machines, particularly computer systems, is known as artificial intelligence (AI). AI is when machines; Exhibit intelligence, perceive their environment and Make decision to maximize chance of success at a goal.

6. Drones and Robotics:

Robotics is an interdisciplinary branch of computer science and engineering. Robotics entails the creation, maintenance, use, and operation of robots. Robotics aims to create devices that can aid and support people.

Although manned planes and satellites won't likely completely disappear, unmanned aerial vehicles (UAVs) have a number of advantages over more conventional remote sensing techniques. The device can gather far higher-detailed information below the cloud cover than is typically available to developing nation analysts as satellite photography. They are simple to operate because the majority of drone mapping and data collection missions are now carried out autonomously, which effectively means that the UAV flies itself. Moreover, data processing software is become more affordable and user-friendly.

7. Big data analytics:

Big data means enormous amount of information collected from different sources and for the longer period like sensor data, social networking data and business data. The process used to combine the business and traditional analytics is called as big data analytics. Ex: e-Hadoop, HDFS.

8. Information and Communication Technologies (ICT):

Any device, tool, or application that permits the exchange or collection of data through interaction or transmission. A general phrase that covers everything from radio to satellite images to mobile phones to digital transfers of money.

Classification of ICT's:

1. Traditional ICTs –Radio, Television, Print media

2. New ICTs –Internet, Portals, Call centers, Mobile, Community radio, Video.

Benefits and drawbacks of digital farming:

Benefits:

1. Improved decision making
2. Community involvement
3. Reduced risk of crop failure

Drawbacks:

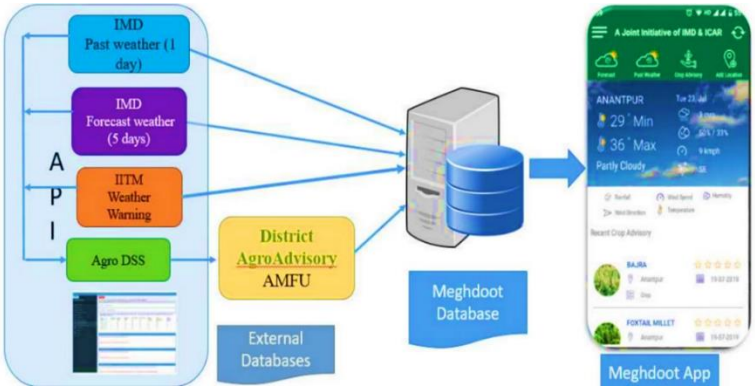
1. Inadequate Infrastructure
2. Small size and Fragmented plots
3. Technical problems with respect to devices

The globe is currently observing another change that incorporates the use of contemporary information and communication technology in agriculture, known as the "digital revolution of agriculture". Digital farming involves integration of advanced technologies into already persisting agricultural practices with a view to boost the food production, food quality and efficiency of farm activities. The precision tools reduce the heavy workload of the farm activities, in turn enhance the quality of work. The huge farm data collected and analyzed will help the farmer for precise decision making for higher agricultural production. Digital farming is a game changer in addressing the issues of population growth, climate change and labour issues in field operations from planting to harvest of the crops.

Use of digital technologies at different stages of crop production

1. Weather advisory:

Earlier farmers used to get weather related information from radio communication, newspapers etc. But now after digitalization of each and every sector, farmers can access weather forecasting information through various mobile applications, TV apps which provides weather prediction data which may specific to their location or particular to larger areas.



2. Selection of suitable varieties:

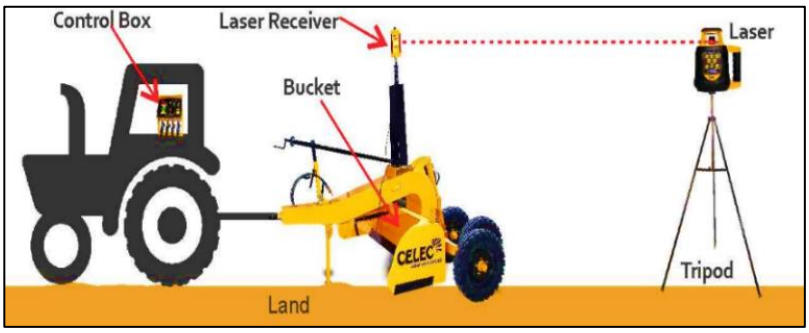
Before digitalization farmers used to select suitable crop varieties to their location based on advice by other farmers, extension workers etc. But now various online expert systems are developed which guide farmers to select appropriate varieties suitable to their region having high yield and high quality with more adaptability to that region.

3. Land preparation:

Laser land leveling is leveling the field within certain degree of desired slope using a guided laser beam throughout the field. The system includes a laser-transmitting unit that emits a laser beam that travels in a perfectly straight line. Beam received by a laser receiver that senses

the light and sends signals to a control box to activate an electro hydraulic valve which raises and lowers the blade of a scrapper & eliminates all undulations tending to hold water. Laser transmitters create a reference plane over the work area by rotating the laser beam 360 degrees. This is all accomplished automatically without the operator touching the hydraulic controls. There are two types of land leveling,

1. To provide a slope which fits a water supply.
2. To level the field to its best condition with minimal earth movement and then vary water supply.



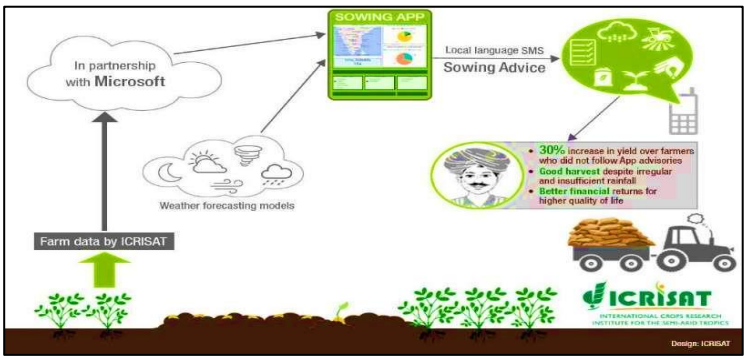
Laser land leveling working mechanism

Advantages of laser levelling:

- Saves fuel/electricity used in irrigation
- Saves irrigation water more than 35% due to uniform distribution
- Precise level, smoother soil surface & better top soil management
- Increase productivity up to 50% due to good germination and growth of crop
- Reduced weed in the field

4. Sowing:

In conventional agriculture farmers used to sow seeds by seed drill which may be animal or tractor mounted, hand sowing, broadcasting, line sowing etc. But after digitalization now automated seeding robots have been developed which is an interface between artificial intelligence and robotics. It sows seeds at appropriate depth with uniform spacing between two seeds.



Sowing advisory app (ICRISAT)

5. Water management:

Normally in conventional agriculture furrow irrigation, flood irrigation, sprinkler and drip irrigation systems are followed. But now smart technologies are used to develop automated drip irrigation system in which human interference is avoided and completely sensor controlled based on internet of things (IoT).

6. Nutrient management:

Normally in conventional agriculture nutrients are supplied by application of fertilizers either through broadcasting, foliar application or band placement. After digital technologies have developed nutrient management is done by developing GIS maps by using satellite/drone based remote sensing technique. Now a days nutrients are supplied by drone spraying of liquid fertilizers which is advantageous over human application.

7. Weed management: Normally farmers will do intercultivation, hand weeding, herbicides etc. But now after development of smart/digital technologies robotic weeding, spraying, sensor-controlled field sprayers have come up.

Weed management techniques:

1) Ground-based hyper spectral remote sensing techniques were developed for detection of crop Handheld hyper spectral radiometer is portable and effective device for rapid, early detection of herbicide injury of crops.
2) Sensor-controlled Field Spraying.
3) Automated weeders (Robotic weeders).

Original red, green and blue image

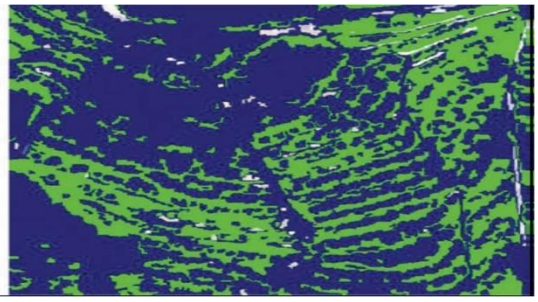
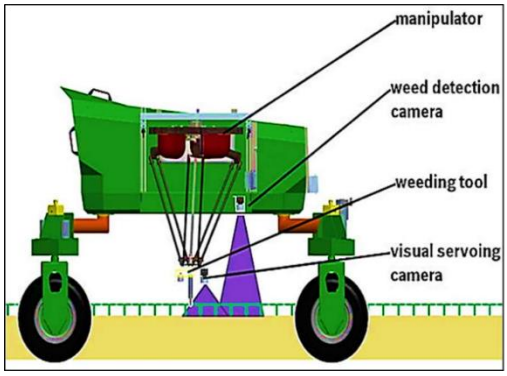
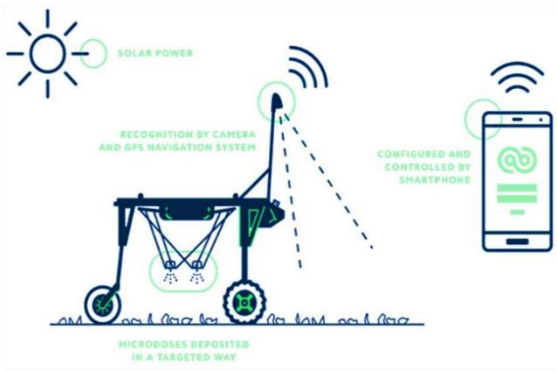


Image with weed and non-weed classifications





8. Pest and disease management:

In conventional method of pest and disease management manual spraying of pesticides / fungicides is done or biological control of pests/diseases is practiced. Now a days several mobile based apps are developed which helps farmer to identify the disease/pest and online recommendation of control measures is seen. In some cases, drones and remote sensing technologies are used for controlling pests/diseases.

Electronic solutions against Agricultural Pest (eSAP)

A plant protection ICT system is called Electronic Solutions against Agricultural Pests (e-SAP). It is an implementation science that successfully combines mobile communications, tablet-based technologies, and cloud solutions to bring different players in the agricultural ecosystem, such as farmers, agro - based universities, and policy experts, to interact on a unified

platform in real time and strengthen the agricultural sector of a country. The e-SAP specifically addresses crop health management issues and is built with digital field devices that can deliver information regardless of language or literacy constraints.

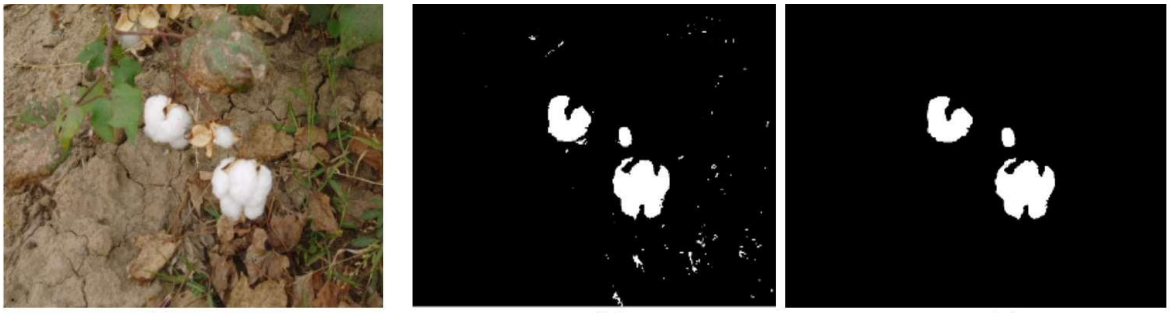
It is the first solution that makes it possible to immediately identify and quantify pest problems on-site. Additionally, it creates and compiles real-time data about insect outbreaks in a region or nation and makes it accessible to other stakeholders in the agriculture industry via its web solution. After pilot scale research in 2012 under the auspices of the University of Agricultural Sciences, Raichur, the deployment of e-SAP technology began in January 2013 and has since expanded to all the districts under the auspices of other Agricultural Universities in Karnataka.

Outcome of e-SAP usage

- **Large-scale implementation:** e-SAP has covered over 100,000 growers in 25 districts of Karnataka, encompassing all five (5) agrarian universities, and covering 26 crops.
- **Rural Jobs:** Options have been provided for more than 100 extension workers hired under different schemes.
- **Nominal application of insecticides:** The likelihood of selling ineffective (and occasionally fake) substances has significantly decreased. The amount of pesticides applied has also been in accordance with the prescription, which has decreased the use of pesticides without regard to need.
- **Systematic pest controlling:** e-SAP has facilitated farmers overawed a significant difficulty – dependable.

9. Harvesting:

Normally in traditional agriculture manual harvesting, combine harvesters are used but now after development of digital technologies robot harvesting has come up and are more used in protected agriculture i.e., in greenhouses/ poly houses.



(a) (b) (c)

Automatic cotton harvesting robot

Conclusion:

There are many new and exciting technological breakthroughs being made and this think piece just scratches the surface. If we are going to feed a growing population it is clear that we will need to use all of the tools in the toolbox, and provide a supportive environment to enable the development of new ones. Innovative technologies have enormous potential benefits, but these can only be attained if society is engaged from the start through two-way communication. The game changer is the digital technology in farming. The recent development of digital

agricultural technologies will hasten growth by ensuring higher crop yields and improving sustainability by lowering water use and pesticide use.

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BIO-MANAGEMENT OF *MELOIDOGYNE GRAMINICOLA* IN RICE NURSERY

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Abstract:

Rice (*Oryza sativa* L.) is the third most important cereal crop in the world, just behind wheat and maize, playing a strategic role in solving food security issues. Rice root-knot nematode, *Meloidogyne graminicola* are serious pests of rice, being, probably, the most economically important plant-parasitic nematode in rice. *M. graminicola* is an obligate sedentary endoparasite adapted to wide range of water regimes and soil's conditions and in India, this causes 16-32 per cent yield loss under irrigated and 11-73 percent under flooded conditions (Tian *et al.*, 2018). Various effective strategies should be applied in preventing the spread of *M. graminicola* from infected areas to uninfected areas by infected seedlings. Among these preventive or management methods, chemical control should be avoided due to their indiscriminate use which enhance the problem of resistance and risk to the environment.

Keywords: Rice, nursery, *Meloidogyne graminicola*, losses and bio-management

Introduction:

Rice (*Oryza sativa* L.) belongs to the Poaceae family and classified as semi-aquatic crop plants that thrive under variety of soil and water conditions. Approximately, 90 per cent of the world's rice is grown and consumed in Asia. In India, it was cultivated from ancient time and ranked first in area and second in production after China. Annually, the country produces approx. 175.58 million tonnes of rice (FAOSTAT, 2018). Rice is cultivated in almost all the states of country and among which West Bengal is the highest in rice production and Tamil Nadu has first place in productivity. As rice is grown under various conditions so it is affected by a number of abiotic and biotic factors. Among biotic factors, plant parasitic nematodes (PPNs) proved most damaging pathogens (Jain *et al.*, 2012) which causes economic losses of Rs. 23,272.32 million to ricein India.Due to these tiny worms on rice, annually estimated globally yield loss ranges from 10-25% (Bridge *et al.*, 2005). Among PPNs, four major nematode species of rice crop are taken into account i.e. *Meloidogyne graminicola*, *Aphelenchoides besseyi*, *Ditylenchus angustus* and *Heterodera oryzicola* that caused combined yield loss to be estimated 10.50 per cent (Jain *et al.*, 2007). However, rice root-knot nematode, *M. graminicola* has become the most destructive pest and serious problem in major rice producing countries of the world including in India (Jain *et al.*, 2012). In view of the enormity of the yield losses caused by *M. graminicola* in rice, it is necessary to minimize crop damage by adopting available environment friendly management methods. There are various methods of nematode management that may prove effective against rice root-knot nematodes. Due indiscriminate use of chemicals for the management of the rice root-knot disease enhances the problem of pathogen resistance and risk to the environment etc. Therefore, environmentally friendly alternatives are best and sustainable for nematode management. Therefore, in this chapter, we tried to define the various effective bio-management practices on rice root-knot nematode, *M. graminicola* under rice nursery conditions.

Symptoms:

Rice plants showed less vigor, stunting growth and yellowing in the nurseries and main field and production of partially filled kernels and late maturity of crop. The chlorophyll content ofleaves is also reduced (Swain and Prasad, 1988). The most characteristic symptoms produced by *M. graminicola* are the typical hook shaped galls which produced at terminal portion of root tip (Khan *et al.,* 2012).

Distribution and Dissemination:

Due to rice cropping intensification andincreasing scarcity of water, day-by-day*M. graminicola,* is becoming a constraint in riceproduction (Soriano and Reversat, 2003; Somasekhar and Prasad,2009) and is widely distributed in the countries of S.E. Asia, Burma, Bangladesh, Laos, Thailand, Vietnam, India, China, the Philippines, USA on both upland anddeep-water rice. In the Philippines, due economic reasonsand a decline in water supplies have prompted widespread use of the direct wet sowing method, in conjunction with chemical weed control and intermittent irrigation (Bhuiyan *et al.,* 1995). These operations favor the development of*M. graminicola* and have drastically increased the nematode's economic importance.

Bio-management practices:

In the year 2005, Anitha and Rajendran found that the combination of *P. fluorescens* at 2.5 kg/ha, neem cake (*Azadirachta indica*) at 1 t/ha and carbofuran at 1 kg a.i./ha was highly effective in enhancing plant growth parameters of rice and yield in both the nursery and main field. Prasad *et al.* (2005) evaluated the effects of castor (*Ricinus communis*), neem (*A. indica*) and *Simarouba glauca* (*Quassia simarouba*) seed cakes @ 2.5 or 5.0 g/kg soil as pre sowing soil treatments, and seed extracts of *S. glauca* @ 2.5 or 5% as root dip/soil drench on rice growth parameters and *M. graminicola* population. The lowest number of nematode eggmasses were found in soil application of neem cake @5 g/kg soil, seed extracts of *S. glauca* @ 2.5 or 5% as root dip/soil drench, and soil application of *S. glauca* cake @ 5 g/kg soil.

Poultry manure treatments with and without NPK fertilization had the lowest *M. graminicola* population dynamics and gall index (Amarasinghe *et al.,* 2007). A pot experiment was done by Dangal *et al.* (2008) to test the effect of some organic amendments such as buckwheat biomass, sesame biomass, neem leaves, chinaberry leaves and poultry manure against *M. graminicola* @ 1, 2 and 3 t/ha each in direct seeded rice. They observed that number of J2 and plant growth parameters were non-significant in different organic amendments including neem leaves (*A. indica*) when applied @ 1, 2 and 3 t/ha.

In the year 2008, Senthilkumar *et al.,* observed that soil application of carbofuran @ 1 kg a.i./ha was comparable with the effect of bio-pesticide, *P. fluorescens* @ 2.5 kg/ha for the management of rice root-knot nematode in rice nursery. Steffen *et al.* (2008) studied the 10 nematicidal and nematostatic activity of medicinal plant essential oils viz., *Lavandula angustifolia, Ocimum basilicum, Cymbopagon citratus, Eucalyptus globulus, Foeniculum vulgare, Rosmarimus officinalis, Matricaria chamomilla, Achyrocline satureioides, Origunum vulgare* and *Mentha pilerita* for the management of rice root-knot nematode in rice. *In vitro* testing of the nematicidal and nematostatic action of these medicinal plant oils on hatching and mortality of J2 was performed initially. Following that, the essential oils showed a higher nematode mortality percentage, and sixty-five days after inoculation, the effect of the oils on *M. graminicola* revealed smaller gall, reduction in numbers of egg and a 68 per cent reduction in *M. graminicola* reproduction.

Seenivasan *et al.* (2012) evaluated that talc based formulation of *P. fluorescens* and *P. lilacinum* significantly decreased the root invasion and soil population of rice root-knot nematode but *P. fluorescens* was highly effective when used as a seed treatment as well as soil application. The nematode trapping potential of *Arthrobotrys oligospora* was stuided under *in vitro* condition against rice root-knot nematode and five isolates of *A. oligospora* were isolated from different regions of India. All the isolates of *A. oligospora* parasitized and killed *M. graminicola.* The soil applications of *A. oligospora* was significantly increased plant growth parameters i.e. shoot length (56.4–68.8%), root length (44.0-54.5%), fresh weight of shoot and root (62.91-65.4%; 38.9-44.2%), respectively, as compared to the plants sown in nematode infested soil (Singh *et al.*, 2012).

In the year 2013, Mukesh and Sobita studied the nematicidal activity of plant extracts like *A. indica, Ziziphus mauritiana, Aegle marmelos, Parthenium argentatum, Eucalyptus globus, Jatropha curcas, Annona reticulate* and *Moringa oleifera* against *M. graminicola.* They observed that leaf extracts of neem, jatropha, eucalyptus, bael and congress grass significantly enhanced the plants growth parameters and reduced root's gall in different concentrations (50 and 100%. *In vitro* conditions 25, 50 and 100% concentrations of leaf extracts significantly decreased mortality of J2 after 24 and 48 h.

In the year 2013, Dongre and Sobita reported that extracts of neem, bael, congress grass, and eucalyptus were most efficient in decreasing the population of *M. graminicola* in rice and they also revealed that these extracts enhancing the plant growth. In a field study, Priya (2015) revealed that *Trichoderma viride* had the highest number of galls, gall index, yield, and nematode population dynamics in soil when compared to other bio-agents such as *P. fluorescens* and *Bacillus subtilis*. In the field experiment, Narasimhamurthy *et al.* (2017) found that the combined application of *P. fluorescens* at 20g/m² + *T. harzianum* at 20g/m² was observed to be the best treatment as it recorded maximum plant height (83.3 cm), root length (21.3 cm), maximum yield (45.6 q/ha) with minimum root-knot index (1.2) and nematode population (185.4/200g soil) with 72.8% reduction in nematode population 11 followed by carbofuran 3G at 0.3 a.i. /m2, *T. harzianum* at 20 g/m², *P. fluorescens* at 20 g/m² and *B. subtilis* at 20 g/m², respectively.

Shukla and Chand (2018) evaluated the bio-efficacy of different botanicals like *A. indica*, *C. longa*, *Zingiber officinale* and *Eucalyptus globules* against *M. graminicola* in pot conditions. The decomposed leaves of each plant @ 5, 10 and 15 g/kg soil was well mixed in the soil and susceptible rice were sown. Among the various botanicals *A. indica* @15 g/kg soil proved more effective, and significantly increased the plant and root length which was maximum as compared to other treatment. Devi *et al.* (2019) evaluated the efficacy of different organic amendments such as neem cake, caster cake, mustard cake @ 5g and 10g per kg of soil against *M. graminicola* in rice nursery. As compared to untreated check, all the cakes and carbofuran (Furadan 3G) reduced nematode galling. However, castor cake @ 10 g/kg of soil was found best in improving plant growth and reducing nematode galls and reproduction.

Kumar (2019) studied the effect of integration of different pre sowing nursery treatments on the plant growth parameters and nematode multiplication parameters under screen house conditions on rice infested with *M. graminicola.* Maximum and significantly higher plant growth parameters were recorded in nursery applied with neem cake @ 50 g/ pot + *P. fluorescens* @ 50 g/pot compared to other treatments. Minimum and significantly lowest nematode reproduction multiplication parameters were observed in neem cake @ 50 g/ pot + *P. fluorescens* @ 50 g/pot. Khan *et al.* (2021) compared the efficacy of five indigenous bio-control on rice cv. PS-5 against

M. graminicola in pot culture. *P. fluorescens, P. chlamydosporia* and *A. niger*, were found to be highly effective in reducing nematode infestations. In without any bio-control agent treatments, terminal galls developed on the roots of nematode-infested plots, and plant growth and yield were reduced by 19-31%. The root dip with one soil application at 15 days after planting with *A. niger* or *P. chlamydosporia* was shown to be highly efficient against this nematode. Kumar *et al. (*2022) evaluated the efficacy of different organic amendments (neem cake and FYM) and bioagents (*Pseudomonas fluorescens, Purpureocillium lilacinum* and *Trichoderma viride*) in nursey pots under screen house conditions. The nursery treatment neem cake @ 50 g/pot + *P. fluorescens* @ 50 g/pot was highly effective in reducing nematode reproduction and multiplication parameters and also improve the plant growth parameters.

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GENE FLOW AND ITS IMPLICATIONS IN PLANT BREEDING

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Abstract:

Gene flow is a natural phenomenon that serves as a mechanism to maintain biological diversity that helps ensure the long-term survival of populations and species in variable environments. In animals, this transfer often results from the interbreeding between populations of closely related individuals. In plants, such an exchange of genetic information typically occurs through pollen dispersal. This section explains the types, mechanisms, models, impacts, and other aspects of gene flow.

Introduction:

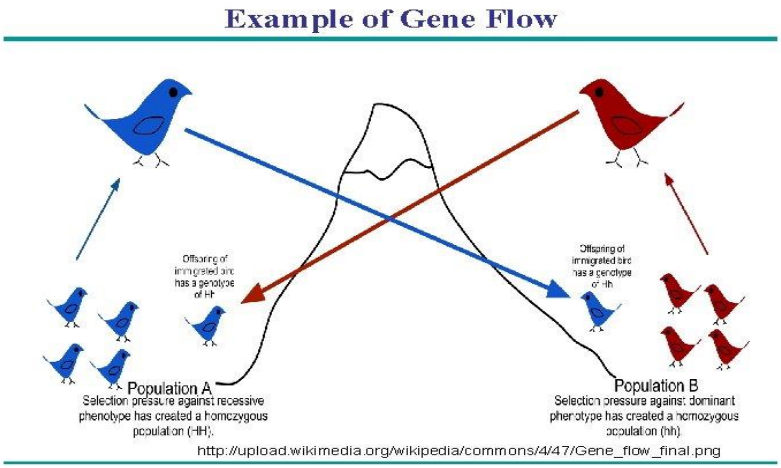
In population genetics, gene flow is the movement of genes from one population to another, conferring new traits – biophysical characteristics of the organism – to individuals of the recipient population. This occurs by cross-pollination (also called hybridization), the pollination of members of one population or genetic pool with those of another. If the rate of gene flow is sufficiently high, then the two populations will have equivalent allele frequencies and, therefore, can be considered a single effective population. It has been shown that it takes only "one migrant per generation" to prevent populations from diverging because of drift. Populations can diverge owing to the selection, even when they are exchanging alleles, if the selection pressure is sufficiently strong. Gene flow is an important mechanism for transferring genetic diversity between populations.

Migrants alter the distribution of genetic diversity among populations by modifying allele frequencies (the proportion of members carrying a particular variant of a gene). High rates of gene flow can reduce the genetic differentiation between the two groups, thereby increasing homogeneity. For this reason, gene flow has been thought to constrain speciation and prevent range expansion by combining the gene pools of groups, thus preventing the development of differences in genetic variation that would have led to differentiation and adaption. In some cases, dispersal resulting in gene flow may result in the addition of novel genetic variants under positive selection to the gene pool of a species or population. (Adaptive introgression).

One of the birds from population A immigrates to population B, which has fewer of the dominant alleles, and through mating incorporates its alleles into the other population.

Types of gene flow

1. Horizontal gene flow 2. Vertical gene flow



1. Horizontal gene flow: when genes are transferred by processes other than reproduction between unrelated species. The acquisition of genes is passed over, *i.e.*, 'horizontally', from one organism to another by means other than inheritance.

- It occurs through transformation, transduction, bacterial conjugation.
- In this only fewer gene are transferred.
- Introduces new traits to adult organisms.

Example: A new study by Fay-Wei Li and colleagues, published recently in *PNAS*, demonstrates a remarkable case of HGT between distantly related plants. Most ferns possess an unusual photoreceptor, neochrome, which consists of red-sensing phytochrome fused to blue-sensing cryptochrome. How ferns came to have this photoreceptor had remained an unsolved mystery until, using data from a large transcriptome sequencing initiative, Li *et al*. found neochrome in another group of plants, the hornworts. More striking, however, was the discovery that fern neochrome was acquired from hornworts more than 200 million years after the vascular plants, which include ferns, and hornworts diverged from their common ancestor.

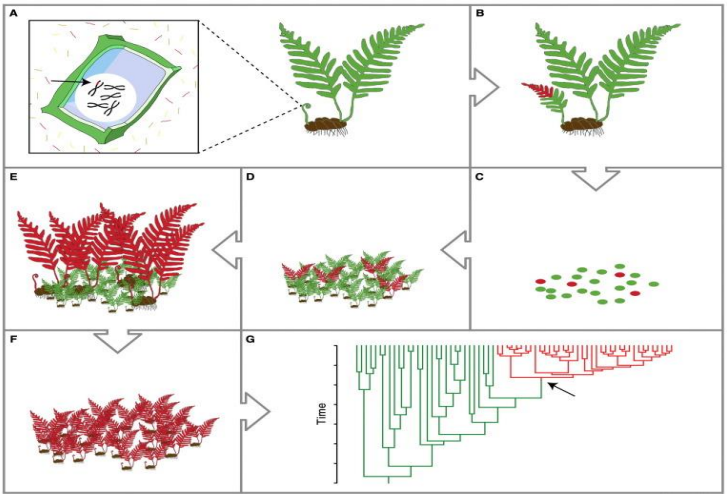


Fig. 1: Horizontal gene transfer of a selectively advantageous gene drives an adaptive radiation

First, exogenous DNA must have made its way into a cell and from there, into its genome (Figure 1A). As a protein-coding gene, the full open reading frame must have been incorporated into the genome in such a way as to be successfully transcribed and translated. The translated protein had to be able to interact with existing cellular networks to produce a phenotypic outcome. Next, the mutant cell must have continued to grow and divide, eventually producing reproductive organs and thereby passing the newly acquired gene to the next

generation (Figure 1B–D). The presence of the new gene would have conferred a competitive advantage and, over many generations, it became fixed in the population (Figure 1E–F). Acquired in this way, neochrome allowed ferns to diversify rapidly (Figure 1G) into the range of newly created niches in angiosperm-dominated forests.

2. Vertical gene flow: The outcrossing of genes is said to be 'vertical' as the genetic information is passed 'down' from parents to offspring. Transfer of genetic materials between different populations of a species through reproductive processes (David Quist., 2010).

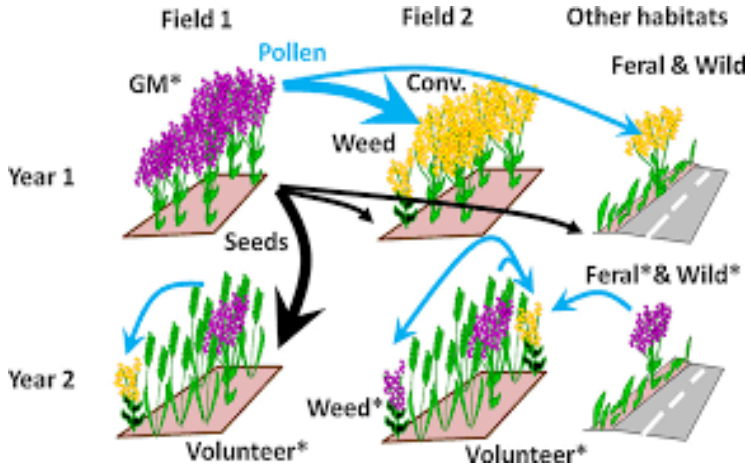
- Transmission of GM from a parent organism to offspring.
- Occurs either through asexual (or) sexual reproduction.
- Whole genome is transferred.
- Responsible for the inheritance of parental traits to the offspring.
- Example: In the case of a cross between a transgenic crop with herbicide resistance gene &a non – transgenic crop.

Processes through which gene flow occurs in plants

- Pollen mediated gene flow
- Seed mediated gene flow
- Vegetative propagules mediated gene flow

Pollen mediated gene flow: The movement of genes through pollination between individuals of different population. Transfer of genetic material (or) information from one plant to another compatible plants. Factors responsible for gene flow: outcrossing rate of recipients, the pollinating agents like wind, water, animals, insects.

Seed mediated gene flow: Seed movement can occur through space &time. Transport and processing of transgenic crop seeds can lead to unwanted gene flow. Common types of seed dispersal events will be spillage either pre (or) post-harvest through seed shattering & related mechanisms the results in the direct transfer of seeds to the environment (or) mechanical mixtures.



This example talks about both pollen mediated geneflow and seed mediated gene flow.

Vegetative propugules mediated gene flow: Gene flow as a result of asexual propagation would result in the long term survival & establishment of a plant in an abandoned cultivation area (or) the spread of a plant to areas outside of cultivation through expanding new growth.

Type of gene flow	Occurrence	Influenced by affinity between donors and recipients	Factors that constrain gene flow
Pollen-mediated	Common	Yes	Outcrossing rate of recipients, pollen loads of donors, pollen competition between donors and recipients, the pollinating media (e.g. wind, animals), and climate conditions
Seed-mediated	Common	No	Seed dispersal media (e.g. wind, water, animals, and humans) and sometimes climate conditions
Vegetative-propagule-mediated (usually for perennial)	Not common	No	Vegetative-organ dispersal media (wind, water, animals, and humans)

Barriers to gene flow

Where the gene flow is blocked by the physical barriers. That doesnot allow population of the same species to exchange genetic material. Physical barriers to gene flow are usually, but not always, natural. They are two types .

1. Natural Barriers 2. Artifical Barriers

In natural barriers : They may include impassable mountain ranges, oceans, vast deserts.

In Artifical barriers : This is also called as man made barriers. In that one example is the great wall of china, which has hindered the gene flow of native plant populations.





Ulmus pumila ***Prunus armeniaca*** ***Ziziphus jujuba***

One of the native plants,*Ulmus pumila* demonstrated a lower prevalence of genetic differentation than the plants *Vitex negundo*, *Ziziphus jujuba*, *Heteropappus hispidus*, *Prunus armeniaca* whose habitat is located on the opposite side of the great wall of china where *ulmus*

pumila grows; this is because *ulmus pumila* has wind pollination as its primary means of propagation & later plants carryout pollination through insects. samples of the same species which grows on either side have bben shown to have developed genetic differences, because there is no gene flow to provide recombination of the gene pools.

Models of gene flow

They are three models of gene flow :

- Contient island model
- Full island model
- Stepping stone model

Contient island model : This model tells about that process of gene flow during one way migration.

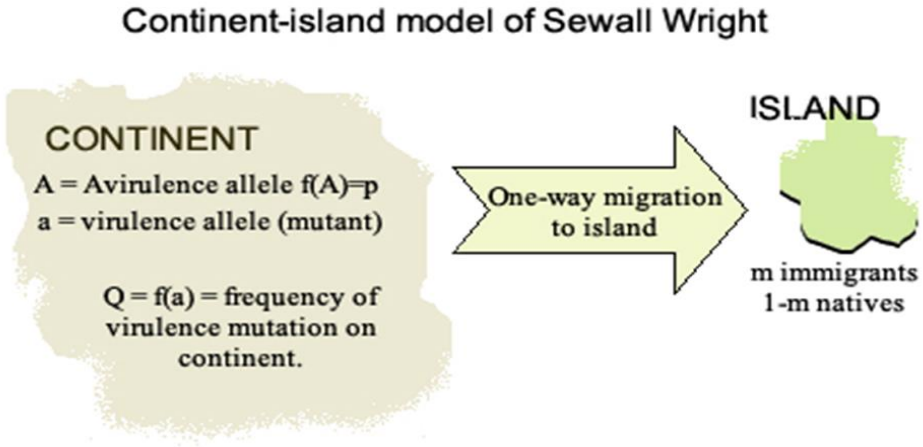


Fig. 1: The continent-island model assumes that gene flow occurs in only one direction, from a donor population (continent) to a recipient population (island)

Let:

m = the proportion of the island population that consists of migrants

1-m = the proportion of the island population that consists of natives

Q = the frequency of the virulence allele **a** in the "donor" (continent) population

$\mathbf{q_o}$ = the frequency of the virulence allele **a** in the "recipient" (island) population

After one cycle of gene flow,

we find that:

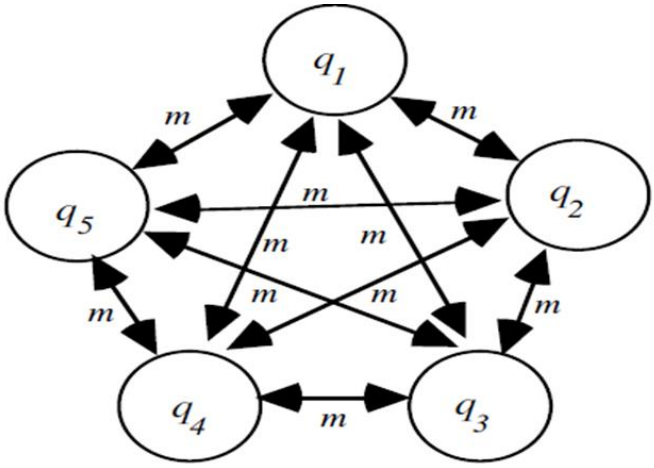
$q_1 = (1-m)q_o + mQ$, $q = -m(q_o - Q)$, where $q = q_1 - q_o$

Assumptions :

- No mutation
- No selection
- Each population persists indefinitely
- No geographic substructure apart from the division into island

Full island model: An ancestral population that was broken up into an array of partially isolated demes,each of effective size N. In the absence of selection ,the genetic differentation among the sub population results in an equilibrium between genetic drift & gene flow. Individuals migrate from one sub popluation to another with different migration rate values.

$$F_{ST} \approx \frac{1}{4Nm+1}$$



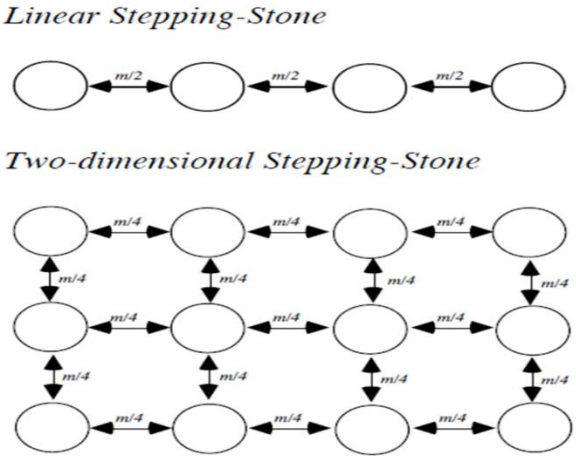
Stepping stone model : This model was proposed Kimura and Weiss (1964). In this again two types: a. one dimensional

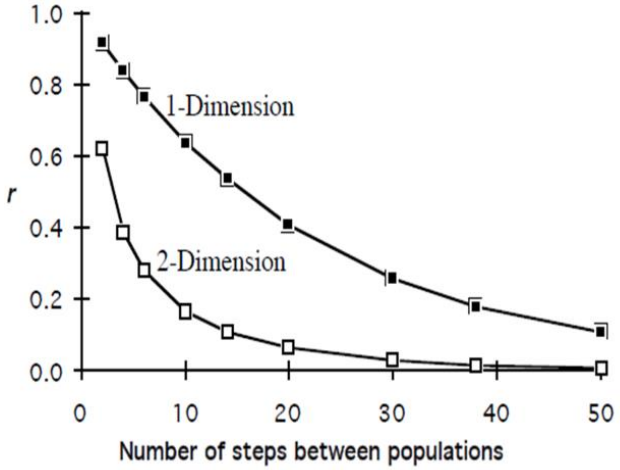
b. two dimensional

One dimensional: In this each generation an individual can migrate at most one step in either direction between colonies.

Two dimensional : The entire population consists of a rectangular array of colonies, each of which occupies a point denoted by a pair of integers (k_1,k_2).

We will also assume that in each generation ,an individual colony exchange migrants with four surroundings colonies, but the effective population number in each colony remains the same.(Ne). The rate of migration may be different in x & y directions : let M_{1A} be the rate of migration along the x-axis (or) horizontal direction, such that $M_{1A}/2$ is the proporation of individuals exchanged between a pair of adjacent colonies in this direction. Similarly let M_{1B} be the rate of migration per generation along the y- axis. The proportion of individuals which migrates to four neighbouring colonies per generation is $m_1=m_A+m_B$.





The correlation in allelic frequencies (r) between the pairs of populations fall off more steeply with distance as more dimensions are added to the stepping stone model.

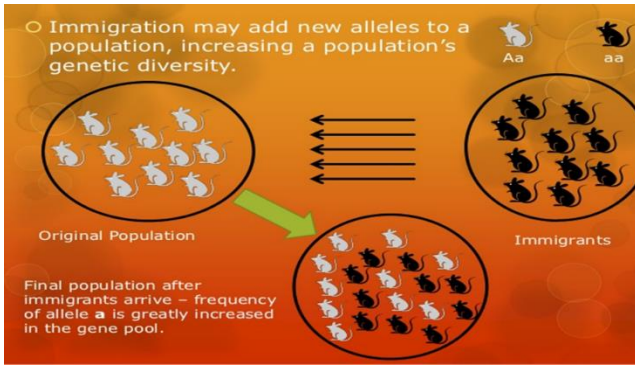
Effects / implications of gene flow

Gene flow as an evolutionary force : When one population entering into another population the gene pools of the two populations are different. Thus, new combinations of alleles get together and new phenotypes can arise. This changes allele frequency just by the gene flow will lead to evolution.

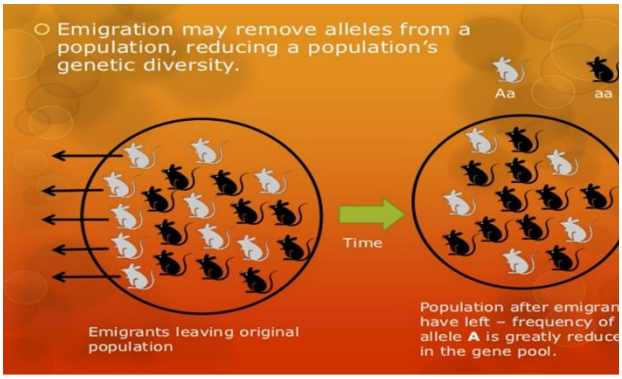
Gene flow result in changes in allele frequencies : Migration into or out of a population may be responsible for a marked change in allele frequencies (the proportion of members carrying a particular variant of a gene).

Immigration :

Immigration may add new alleles to a population, increasing a populations genetic diversity.



Immigration



Emigration

Emigration :

Gene flow leads to species extinction :

A. *Cercocarpus traskiae*, is endemic and is native to a single gully on an island off the coast of California.

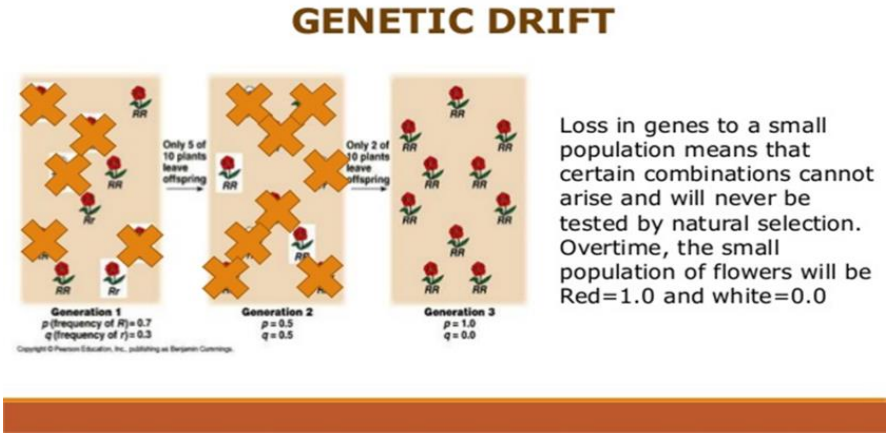
B. It hybridizes with the more common and widespread species *Cercocarpus betuloides.*

Mountain-mahogany

- Since the discovery of the island endemic, the adult population size has plunged from more than 40 to 11.
- DNA and isozyme analysis revealed that almost half of the total reproductive population, five adults, are of hybrid origin.
- Clearly, if future hybridization occurs, it will rapidly send the species to extinction (Wolf *et al.*, 2001).

Effect of gene flow on genetic drift

Genetic drift is change in allele frequencies in a population from generation to generation that occurs due to chance events.



▶ The effects of genetic drift can be overcome by gene flow.

▶ If enough individuals are exchanged between two populations that are experiencing independent genetic drift, then the drifting populations become genetically linked and population subdivision will not occur.

Sewall Wright population genetic parameter N_em :

N_e is the effective population size (a measure of genetic drift)

m is the percentage of the recipient population made up of immigrants (a measure of gene flow).

If $N_em = 0$ then :

- No migrants are exchanged between populations.
- The result is that different alleles can be fixed in different populations through genetic drift.
- Populations diverge and population subdivision occurs.

If $N_em = 1$ then :

- The effects of drift are exactly counterbalanced by the effects of gene flow.
- The populations do not diverge or converge.

If $N_em > 1$ then :

- On average one or more individuals are exchanged between populations each generation.
- Then populations will not diverge by genetic drift
- They will gradually become similar

Transgenic crops-why gene flow matters

▶ The rapid advance of transgenic biotechnology, more and more transgenic crop varieties are being released into the environment.

▶ Those have provided new opportunities for global food security and new developments in life sciences.

▶ However, the release and use of transgenic products have also caused tremendous concerns about biosafety. The potential ecological risks associated with transgene escape through gene flow are foremost among these concerns.

▶ Transgene flow from engineered crops to other cultivars or to their wild and weedy relatives is one of the major concerns in relation to the ecological risks associated with the commercial release of transgenic plants.

- Crops may interact with related wild plants forming crop-weed complexes (sugar beet and sea beet).
- These weed populations can act as reservoirs of foreign genes, potentially including genes introduced by genetic engineering.
- These weed populations can also act as bridges, allowing gene flow between crops and wild species that are usually unable to interbreed.
- Transgenes may disseminate within the weedy or wild populations through sexual reproduction and/or vegetative propagation.
- If the they are responsible for resistance to biotic and abiotic stresses (such as disease and insect resistance, drought and salt tolerance, and herbicide resistance) that can significantly enhance the ecological fitness of weedy and wild populations.
- The escape of these transgenes will probably cause ecological problems, e.g. producing aggressive weeds. Such weeds might get out of human control, and result in unpredictable damage to local ecosystems.

Implications in plant breeding

- Increases the effective size of the population
- Lower rates of gene flow prevents speciation
- Higher isolation standards to prevent the gene flow during seed certification
- It can overcome Genetic drift
- Evolution
- Mating system

Conclusion:

The most obvious implication of gene flow by pollen in plant breeding is that interpopulation gene exchange should increase the effective size of the populations and reduce the threat of genetic drift-based hazards such as the depletion of genetic variation and inbreeding depression. Gene flow is an important force in both plant evolutionary biology and plant conservation biology. Research on gene flow has lagged because gene flow rates have been assumed to be insignificant. However, more recently gene flow rates have been recognized to occur frequently at the levels that can influence the gene/allele frequencies of populations.

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CUSTOMIZED FERTILIZER - A NEXT GENERATION FERTILIZERS

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Introduction:

The world's current population of 7.5 billion people is expected to grow by one billion in the following 12 years, to 9.6 billion by 2050. By 2020, India's population is around 1.3 billion, rising to 1.67 billion by 2050. Food grain production in India is 285.17 million tonnes (Mt) in 2018-19, 296Mt in 2019-20. According to the 2nd Advance Estimates for 2021-22, overall foodgrain output in the country would reach a new high of 316.06 Mt, up 5.32Mt from the previous year's production and it is expected to 335 Mt in 2025 according to the reports. As a result, the country's focus has been on increasing food production to meet the growing demand (Majumdar and Prakash 2018). To feed India's rising population by 2050, land productivity must improve fourfold, with a threefold increase in water productivity and a six fold increase in labour productivity, all while focusing on energy conservation and low-emission technologies. Soil is the 'The final frontier' declared by 'Science'. Since 1970, the net cultivated area has stayed almost unchanged at roughly 140–142 million hectares (M ha) and there is little chance of increasing the area under cultivation beyond the current 155.22 M ha in 2015, much of the growth in food grain output will have to be achieved through increasing productivity per unit area.

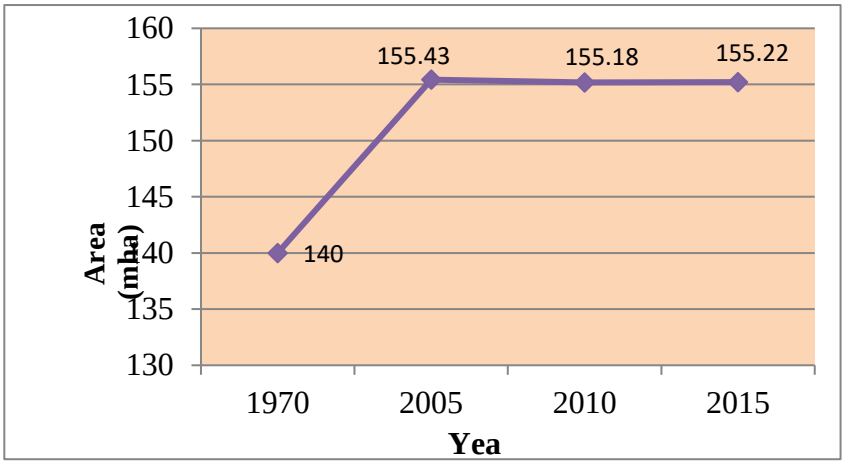


Fig. 1: Area under crop production (1970-2015)

The productivity of key crops must increase by 3.0–7.5% annually to meet production demands. A sufficient amount of additional plant nutrients must be provided externally for each additional tonne of foodgrain produced. Fertilizer is an essential component of Modern Agriculture. To meet the growing food grain need of a population, the only option available is increasing productivity through proper planning and optimum utilization of resources such as fertilizers, seeds, water etc. In India, among the nutrients, NPK remains the major ones for increased and sustained productivity (Hegde *et al.*, 2007). It is estimated that 45 Mt of N + P_2O_5

+ K_2O will be required yearly by 2025 to meet the foodgrain demand. Nonetheless, on average fertiliser productivity has been steadily dropping.

However, the development of high yielding systems will like exacerbate the problem of secondary and micronutrient deficiencies, not only because larger amounts are removed, but also because of the inadequate and imbalance use of N, P and K to achieve higher yield. To attain high future targets, balance fertilization, site specific nutrient management and customized fertilizers will play a very important role. Customized Fertilizer is defined as multi-nutrient carrier of macro and micro nutrients known for its sound scientific nutrition principles. This Fertilizer is tailored to meet the region, soil, crop specific needs. It includes both organic and inorganic sources and is designed through specialised smart Fertilizer technology, manufactured through systematic granulation process (Rakshith, *et al.,* 2012). These are manufactured by three methods namely Bulk Blending, Complex and steam granulation. The development of site and crop specific readymade customized fertilizers based on scientific principles may prove to be more effective to meet the plant requirement and enhance the nutrient use efficiency. This not only helps correct nutrient imbalance in the soil caused by prolonged inadequate and indiscriminate use of fertilizers. This approach is also likely to boost crop yield & arrest soil fertility decline in a long run in an eco-friendly manner.

Major concerns in Indian Agriculture

- Depleting soil organic matter
- Imbalance in Fertilizer use
- Emergind multi nutrient deficiencies
- Declining nutrient use efficiency
- Declining crop response ratio
- Negative soil nutrient balance

Nutrient inadequacy and imbalance are mostly to blame for agricultural production stagnation and trends of declining sustainability and profitability in crop production. Intensive efforts must be done to restore soil health so that agricultural production may expand at the necessary rate to meet the population's food, fibre, and fuel needs on a long-term basis. To break the cycle of agricultural output stagnation, balanced fertilisation beyond NPK is required. Balanced fertilisation must be promoted to maximise nutrient use and increase crop output for food and nutritional security. Multi-micronutrient blends make it simpler to apply a variety of plant nutrients to suit specific crop requirements at different growth stages, especially when using SSNM approaches. In order to provide individualised solutions and increase the overall effectiveness of all plant nutrients, new products must be created. In order to maximise yields, crops need be provided with nutrients at the right rate throughout the growing cycle (Majumdar and Prakash 2018).

Customized fertilizer:

Customized fertilizers are multi-nutrient carriers that make it easier to apply the whole spectrum of plant nutrients in the proper proportions to meet the unique needs of a crop at different stages of growth. Customized fertilizer formulas can be created based on a region's soil fertility state, climate and cropping pattern. Customized fertilizers are distinct, ready-to-use granulated fertilizers that are created using reputable scientific plant nutrition principles, knowledge about the soil, comprehensive laboratory studies and field testing. With effect from 2008, the Government of India categorised Customized Fertilizers as a separate category under

the Fertilizer Control Order (Rakshit *et al.*, 2021). NFCL is the first firm in India to gain formal approval for the manufacture and sale of Customized Fertilizers from the Department of Agriculture and Cooperation, Ministry of Agriculture and Government of India.

Customized fertilizers are designed to contain macro and micronutrient forms, manufactured through a systematic process and tailored to the crop's nutritional needs, as determined by a scientific crop model developed by an accredited fertilizer manufacturing or marketing company, and validated by a scientific crop model developed by an accredited fertilizer manufacturing or marketing company. Customized fertilizer is a soil-crop-climate-based fertilizer that increases nutrient uptake and reduces nutrient loss. Not only do you get core nutrients with customized fertilizer, but you also get secondary and micronutrients. It's made from both organic and inorganic sources, and it's granulated in a systemic process to meet the crop's nutritional needs, which are unique to its location, soil, and stage, and it's backed up by a scientific crop model established by an accredited fertilizer manufacturer. The cost of fertilizer application is reduced with customized fertilizer.

Site Specific Nutrient Management (SSNM) and Precision Agriculture both use customized fertilizer to promote optimal fertilizer usage efficiency (FUE) of the applied nutrients in a cost-effective manner. To calculate the best grades of personalized fertilizer, prospective makers or marketers are expected to employ computerized tools such as the Decision Support System for Agro Technology Transfer (DSSAT) crop model, among others. It has been shown to be a useful tool for determining soil variability within a field. Once essential soil or nutrient qualities have been determined, maps can be created and prescriptive procedures can be taken to treat field issues as needed. Precision maps based on soil or crop attributes can be created, and the field can be divided into management zones where varying fertilizer rates can be applied according to the prescription map. A management zone can be defined as a group of people who work together to achieve a common goal (Doerge, 1999). A field can be separated into several characteristic zones for variable rate fertilizer or water application since soil type or organic matter is a major component in creating yield variability.

Challenges in fertilizer industry:

- Produce more fertiliser at a cheaper cost to enhance the condition of soils owned by numerous small and marginal farmers.
- To provide a balanced delivery of various nutrients to crop plants, fertilizers supplying P, K and micronutrients are made available in sufficient quantities at the appropriate times and at reasonable prices.
- The current high price of P fertilizers discourages the balanced application of N, P, and K fertilizers.
- Creating unique macro- and micronutrient blends to provide the soil with various nutrients based on its requirements.
- Create as quickly as feasible high-efficiency fertilizer products.

Need for customized fertilizer:

Fertilizer is a crucial element for agricultural productivity and output. Fifty-five percent of the additional food output is due to fertilizer alone. Fertilizer is the main cart puller because there is no way to increase the cultivable land; the only option is to raise production per unit area. Prior to mixing, a custom-mixed fertilizer is made in accordance with the precise requirements offered by the customer. Certain lands demand significantly more balanced

fertilizer mixtures in granulated form for soil application, water soluble form for drip irrigation, micro sprinkler and foliar spray systems. A different way to describe customized fertilizer is as a multi-nutrient carrier that contains macro and micronutrients from inorganic or organic sources, is made through a systemic granulation process, and satisfies the crop's nutritional requirements.

- Improper fertilizer use
- Increasing secondary and micronutrient deficiencies over time
- Crop output is stagnant
- The emergence of genotypes susceptible to several fertilizers
- Demand for more FUE
- Boost the soil's health
- Need to lower input costs depending on fertilizer
- Pollution of the environment

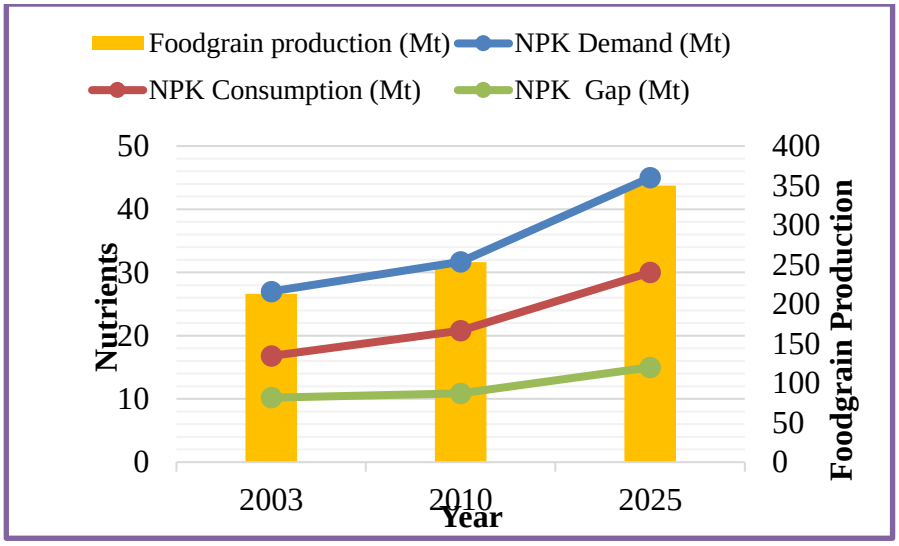


Fig. 2: Grain production *V/s* Fertilizers

Fertilizers are a crucial component that boosts crop output by 55%. The need for food grain in the existing environment might be met by increasing the production of food grain, which could only be accomplished through optimal fertilizer application and balanced NPK nutrition.

Converting energy to food security

Despite the fact that producing and utilising fertilisers uses a lot of energy, there are several advantages to using energy to increase food security. A further 218 kg of grain, or enough to meet one person's caloric needs for an entire year, is created for every 1 million Btu of energy used in the fertiliser business.

Objective of customized fertilizer:

In order to maximize fertilizer usage efficiency for the supplied nutrient while staying cost-effective, the customized fertilizer aims to provide site-specific nutrient management. The customized fertilizer may contain combinations of primary nutrients, secondary nutrients and micronutrients. Important components like urea, diammonium phosphate (DAP) and potash are supplemented with micronutrients like sulphur, zinc and boron at a ratio that is suitable for certain crops and soil types.

The customized fertilizer includes the combination of nutrients on the basis of:

1. Site
2. Soil testing

3. The requirement for crops
4. The crop's stage

Evaluation of customized fertilizer in India:

2005	Concept paper in IJF and presented in FAI seminar
2006	FAI working group
2007	FAI and DAC proposal on CF
2007	CF formulations and field validation trial by Tata, Nagarjuna and Deepak Fertilizer companies
2007	Series of CF workshops conducted
2008	CF guideline issued by GOI on 11 March, 2006
2008	DAC approved 12 CF grades
2009	TATA initiates setting up HI tech CF plant
2010	GOI support for availability of raw material
2010	TATA's CF plant starts production on 22 Nov , 2010

(*Source*: Vidyashree and Arthanari, 2020)

Formulations of customized fertilizer in India:

Prior to mixing, a fertilizer is manufactured in accordance with the consumer's specifications, which are often based on the findings of soil testing. Fertilizers are designed for the particular crop, soil, water, and nutrient needs. To obtain the required ratios of N, P, K, and S in customized fertilizers, urea, DAP, MOP, ZnS, bentonite sulphur, and boron granules are blended and crushed. In order to create a homogeneous product with the same nutritional composition in every grain, the mixture is subjected to steam injection, drying, sifting, and chilling. The need for more research to boost fertilizer effectiveness is highlighted by the sharp increase in fertilizer prices. Continue assisting Indian farmers to produce more with less fertilizer, i.e.

Fertilizers	**Efficiency (%)**
Nitrogen	40 to 50
Phosphorus	15 to 20
Potassium	50 to 70
Sulphur	8 to 12
Micronutrients	< 5

Customized fertilizers formulation in India:

The Fertilizer Control Order (FCO) certified around 36 customised fertiliser formulas. Over 1 lakh tonnes of customised fertiliser are now being marketed by four Indian companies.

The important customized fertilizers producing companies in the Indian market are:

- Tata chemical Ltd.
- Deepak Fertilizers
- Nagarjuna fertilizers
- Coromandal industries Ltd. Etc.

Sl. No.	**Crops**	**Formulations (N:P:K: Zn/ N:P:K:S:Mg:Zn:B:Fe/ N:P:K:S:Zn:B)**	**Geography/area**
1.	Wheat	10:18:25:3:0.5:0	Muzaffarnagar, Barielly, Bijnore, Hathras, Pilibhit, Mathura, Meerut
2.	Sugarcane	7:20:18:6:0.5:0	Moradabad, KR Nagar, Farukhabad
3.	Rice	8:15:15:0.5:0.15:0	Ghaziabad, Rampur, Shahjahanpur, Mainpuri and US
4.	Groundnut	15:15:15:9:0.5:0.2	Andhra Pradesh
5.	Maize	20:0:15:0:0:0.2	Andhra Pradesh
6.	Potato	8:16:24:6:0.5:0.15	Agra, Aligarh, Budan, Bulandshahar
7.	Paddy	15:32:8:0.5 18:33:7:0.5 18:27:14:0.5	Andhra Pradesh
9.	Grape, Sugarcane	10:20:10:5:2:0.5:0.3:0.2	Aurangabad, Nasik, Pune Ahmednagar
10.	Paddy (basal)	16:22:14;4:1:0	East & West Godawari, Krishna, Guntur
11.	Maize (basal)	14:20:15:4;0.6:0	Karimnagar, Warangal, Nizamabad
12.	Groundnut (basal)	17:17:17:4:0.5:0.2	Anantapur, Chitoor, Kadapa, Kurnool, Mahaboobnagar

(*Source*: Rakshit *et al.,* 2012)

Characteristics/quality of customized fertilizers:

1. Granular in texture
2. Minimum 90% between 1-4 mm IS sieve, and 5% shall not be allowed below 1 mm
3. Grade 100% water soluble
4. At least 30 units of each nutrient should be present in a grade's minimum nutritional content.

Methods of customized fertilizer manufacturing:

There are basically three method of customized fertilizer production are

1. Bulk Blending
2. Compound Granulation/Steam Granulation

3. Complex/Chemical Granulation.

1. Bulk blending:

The cheapest and easiest method is to simply combine solid fertilizers in the right amounts to obtain the appropriate nutrition ratio. All that is required is the utilization of a warehouse, weighing, and mixing apparatus. It has the benefit of having a smaller decentralized production capacity, which makes it well suited to give the customer the precise NPK ratio he requires. The choice is most prevalent in the US, but it is becoming more frequent in Europe. All fertilizers and basic materials used in bulk mixes should meet the same form and size requirements as per the physical standards. For instance, if granular urea is permitted in bulk mixtures, it must have the same size and shape as DAP. However, because to the separation.

Second, the price of raw materials has increased significantly, driving up the price of fertilizer as a whole, as a result of the rigorous criteria on raw material size and form. Such fertilizers are not available in India, and it would be expensive to import them in large quantities with the necessary physical standards. As a result, the bulk mixes method seems like a remote option for India's fertilizer customization.

2. Steam granulation:

This manufacturing technique uses only solid raw materials. Only the agglomeration process in the dryer, which uses water, steam, and heat, results in granulation. These fertilizers are referred to as compound granulation, steam granulation, or physical granulation. Additionally, many people still refer to them as "mixtures," which is erroneous. Compound NPK granulation is not a sort of combination, although bulk mixes are. This method of producing fertilizer sits in between bulk blending and the chemical procedure. While it does not provide as much adaptability as bulk mixes, it does offer a lot more versatility when it comes to producing various fertilizer grades than chemical granulation. It requires little investment.

3. Chemical granulation:

Chemical granulation is also known as slurry granulation or complex granulation. The majority of the time, ammonia and acid combine chemically to create an ammonium sulphate or ammonium nitrate salt, which is then granulated with discrete K2O in some facilities in either solid or liquid form. Accrual + agglomeration is the process used to create granules.

The limitations of this technology are:

It requires a sizable plant and building the infrastructure needed to store and handle acid and ammonia is quite expensive. To make it easier to unload liquids like phosphoric acid and, in certain cases, ammonia, it should preferably be close to the coast. Being a large-scale manufacturer, it is unable to produce a variety of grades. Only two grades can be produced by this kind of plant. The method option is cumbersome when a lot of custom NPK grades need to be produced.

How to arrive at customized fertilizer?

- Georeferencing the region of interest
- Choosing sampling locations based on the best statistical method
- Actually sampling the sites
- Examining a sample of places
- Examining samples of soil, plants, and water for nutrients and a few soil traits
- Setting up management zones
- Targeting yield in key management zones

- calculating crop nutrient removal
- Estimating the amount of nutrients needed
- Combining nutrients depending on information generated

Customized real-time VRF blending of individual nutrients:

Radite *et al*. (2000) developed and evaluated a blended variable rate applicator in paddy fields. The system may apply precise variable rates of two different granular fertilizers using a broadcast type granular applicator with 12 nozzles fed by six metering units. A variable rate applicator that can simultaneously apply two different liquid fertilizers at different rates while using a control system on a side dressing applicator was attempted (Yang, 2001). To fertilize grain sorghum, two uniform and one variable rate fertilizer treatments were employed (Sorghum bicolor). The two fertilizers' average application rate errors in 1997 and 1998, 32N-0P-0K and 11N-16.2P-0K, were 2.5 percent and 5.2%, respectively. Potassium (0 kg ha^{-1}) and phosphorus (53 to 158 kg ha^{-1}) were used by Sedlak *et al*. (2001).

The greatest growth and production-attributing characteristics, including the largest net returns (60,062/ha) and grain yield of rice (7.0 t/ha) with harvest index (HI) (46.39%) Under 150% dosage of CF, cost ratio (2.31) is recorded compared to other treatments, i.e. 50% and 75% dose of CF and recommended dose of fertiliser (RDF), which failed to significantly increase yield and nutrient absorption in plants when compared to the optimal amount of tailored fertiliser (Dwivedi *et al*. 2014a).

In Anju *et al*. (2018) study, tailored fertiliser (CF) formulations were made for elephant foot yam (EFY), which was cultivated as an intercrop in coconut gardens in Kerala's two agro-ecological units (AEUs), AEU 3 and AEU 9. To find the best formulation and the ideal rate of application, these formulations were evaluated over the course of two seasons at various sites within AEUs 3 and 9. The best product was found to have a N: P_2O_5: K_2O: Mg: Zn: B grade of 7:12:24:2.5:1.25:0.4 at 625 kg ha^{-1}. These were tried once more for larger yam at the ICAR-CPCRI under intercropping in coconut, as well as for several genotypes of cassava and sweet potato (var. Sree Arun) on station.

The impact of tailored fertilisers (CF) on wheat cultivated on clayey soils at IGKV, Raipur during the winter seasons of 2010–11 and 2011–12 was assessed by Dwivedi *et al*. (2014b). The highest grain yield of wheat (4.4 t/ha) was generated by applying 150% of the state-recommended dose of CF, which was 28.27% higher (120:60:40 kg NPK ha-1). The application of a 150% dose of CF resulted in an increase in NPKS and Zn absorption as well. Application of 150% CF produced the highest net returns (Rs. 37,676/ha) and benefit:cost ratio (2.7), which were then followed by applications of 125% CF and 100% CF, respectively.

During the rabi season of 2007–2008, Goel et al. (2008) conducted a Customized Fertilizer Grade (CFG) trial for pomegranate on inceptisol (typical haplustepts) to validate the customised fertiliser grade (made by Deepak Fertilisers and Petrochemicals Corporation Ltd., Pune). Three treatments (T1 - Farmers Practice, T2 - University Practice, and T3 - CFG) of the pomegranate crop were cultivated in four replications. Customized fertiliser is a multi-nutrient carrier made using a systematic procedure to incorporate macro and/or micronutrient forms, satiating the nutritional requirements of the crop that are unique to its site, soil, and stage. The T3 (21.6 t ha-1) and T2 (20.6 t ha-1) treatments both produced higher marketable yields than the T1 (19.6 t ha-1) treatment. With a marginal cost-benefit ratio (MCBR) of 16, the application of CFG - N 20: P 10: K 10: S 5: Mg 2: Zn 0.5: B 0.3: Fe 0.2 at 1000 kg ha-1 (T3) as a base

application to pomegranate trees resulted in a significantly higher number of large size "A" grade fruits (32%), compared to the other treatments (T1 and T2). As a result, it was discovered that using a specific fertiliser grade will increase pomegranate output and improve quality.

Kalaiselvi (2016) carried out a field experiment to investigate the impact of a TNAU-tailored fertiliser mixture on crop production, maize nutrient uptake and soil fertility. The grain yield was 32% greater than the farmers' practise. The dry matter production, yield characteristics and grain and straw yield of maize were all maximum in CF3- RDF with TNAU micronutrient mixture @30 kg ha-1 as EFYM (T6). The above-mentioned treatment had the highest levels of nutrient uptake for both macro and micronutrients, which was followed by the application of RDF (NPK) with TNAU micronutrient mixture at 15 kg ha-1 as an EFYM (T4). Even after the crop has been harvested, the application of CF3- RDF with TNAU micronutrient mixture at 30 kg ha-1 as EFYM (T6) has shown that the availability of macro and micronutrients has risen rather than decreased. The application of CF3- RDF with TNAU micronutrient mixture @ 30 kg ha-1 as EFYM (T6) for maize can maximise the yield with better net profit, according to the data.

N: P: K mixture (CF I) and N: P: K: Zn mixture (CF II) have been developed based on the general recommendation (BR) of straight fertilisers being followed in Tamil Nadu. Field experiments were carried out by Kaleeswari (2013) during kharif 2011 at Tamilnadu Rice Research Institute, Coimbatore to study the effect of customised fertilisers on yield and soil properties of lowland rice ecosystem. Three levels of CF I and CF II, 50%, 75%, and 100% of RDF, were contrasted with 100% RDF from conventional fertilisers. The outcomes showed that the application of 100% RDF in the form of CF II improved the number of productive tillers (21), the length of the panicle (27.70 cm), and the number of filled grains per panicle (203 nos.). The maximum grain production, 6878 kg ha-1, was obtained from the application of 100% RDF in the form of CF II, which was followed by the application of 100% RDF in the form of CF I + 25 kg Zn SO4 ha-1 (6622 kg ha-1). The use of 100% RDF in the form of CF II resulted in a yield increase of 22.2% above the application of straight fertilisers.

In a field experiment conducted by Kamble and Kathmale (2015), it was discovered that the highest plant height (57.77 cm), stem diameter (6.03 cm), and bulb diameter (15.13 cm) at the time of harvest, fertiliser use efficiency, bulb yield (22.34 t ha^{-1}), and benefit:cost ratio (2.56) of onions were recorded in 100% recommended dose of NPK through the use of customised fertilisers (CF). In comparison to the other treatments, the available nitrogen (213 kg ha^{-1}) and phosphorus (14.42 kg ha^{-1}) concentrations were significantly higher for the 125% recommended dose of NPK through CF in two equal split doses and the 100% recommended dose of NPK through CF in three equal split doses for available K (804 kg ha^{-1}). Onion appears to benefit from the application of the full recommended dose of fertiliser (100:50:50 $N:P_2O_5:K_20$ kg ha^{-1}) in two or three splits during CF by increasing soil fertility, yield, and onion yield-contributing characteristics, as well as generating higher net financial returns.

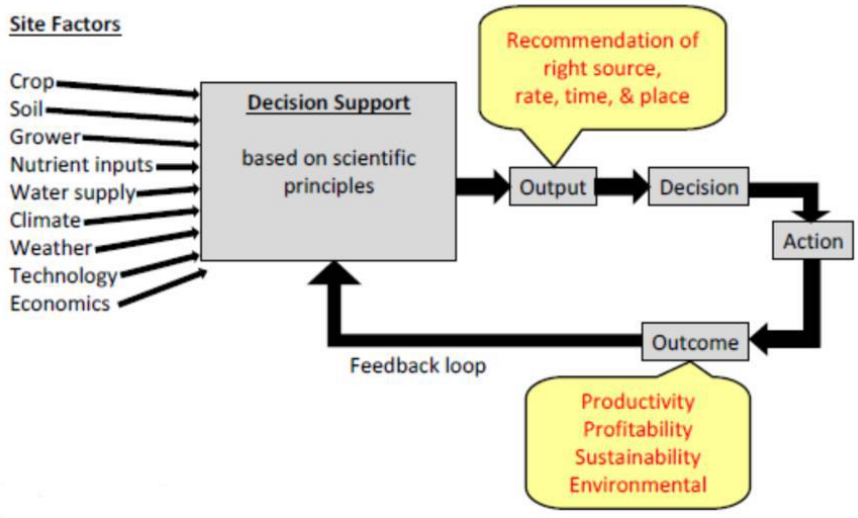
Meshram *et al*. (2016) investigated the impact of customised fertiliser on rice growth, yield, energetic study and nutrient uptake and found that, among the various doses of customised fertiliser (CF) and other nutrient management practises, the best result was found under 150% dose of CF (T6), but it recorded the highest growth and yield attributing characters, namely grain yield of rice (6.9 t/ha) with HI (46.18%) and Net energy output (183.2 MJ Under this therapy, NPK and Zn uptake were also increased. In comparison to the optimal level of tailored fertiliser,

other alternative dosages of CF and RDF—50% (T2) and 75% (T3)—failed to significantly increase yield and nutrient uptake in plants.

During the Rabi season of 2010–11, Mohammad Irfan conducted a field experiment to investigate the impact of moisture regimes and specialised fertilisers on the performance of potatoes (*Solanum tuberosum* L.). The 6 cm irrigation at 1.0 IW/CPE ratio and tailored fertiliser produced the highest WUE efficiency (F4). The treatment combination I_2 F_4 (6 cm irrigation at 1.0 IW/CPE ratio + 8: 18: 26: 1: 0.1: 6), which was used, computed the maximum net return and benefit-cost ratio, respectively, of Rs. 79309.00 ha^{-1} and 1.78. (N:P:K:Zn:B:S 150 : 67.5 : 97.5 : 3.75 : 0.37 : 22.5 kg ha^{-1}).

The effect of customized fertilizers (20:17:11:3:0.4% of N:P_2O_5:K_2O:S:Zn) on the productivity of finger millet was studied by Mudalagiriyappa *et al*. (2015) and reported that In comparison to the absolute control, 50, 75, 100 and 125% customised doses, the application of 150% customised fertiliser resulted in increased plant height (102.5 cm), number of tillers/hill (7.11), total dry matter accumulation (100.41 g/hill), ears/hill (6.21), and test weight (3.65 g). When compared to other treatments, the grain and straw yields from the application of 150% customised fertiliser dose were significantly higher (3279 and 4510 kg/ha, respectively), although it was comparable to the applications of 100 and 125% customised fertiliser. A customised 125% fertiliser dose application resulted in higher net returns and a higher B:C ratio.

According to Shyla et al. (2016), who studied the effects of different levels of customised fertilisers on V1 mulberry grown under irrigation, mulberry raised with 150 percent of the nutrients through customised fertilisers had significantly higher leaf moisture content (81.75%), chlorophyll 'a' (1.78 mg/g), chlorophyll 'b' (0.81 mg/g), carbohydrates (25.26%), soluble protein (10.04%), soluble sugar (12.97%), and reducing sugar (2 The leaf moisture content (66.61%), chlorophyll 'a' (1.53 mg/g), chlorophyll 'b' (0.54 mg/g), carbs (20.33%), soluble protein (8.28%), soluble sugar (11.36%), and reducing sugar (1.75%) were all at their lowest levels in control plots on the 75th day following pruning.



SSNM and customized fertilizers:

Current fertilizer recommendations for food crops are frequently "blanket" recommendations with set rates and timings for large regions of land. The substantial field-to-field variability of soil nutrient availability inhibits fertilizer effectiveness when broad-based blanket fertilizer recommendations are used. In order to maintain healthy soil, SSNM makes sure

that nutrients are administered in a field in accordance with the demands of the crop. The fertilizer industry supports SSNM by producing customized fertilizers. Over time, these are being improved.

Variable rate fertilization

Farmers preference of customized fertilizers due to:

1. Increased crop quality and output
2. Maximum efficiency of nutrient usage
3. Depending on the place and crop, specifically
4. Accessible in a balanced, ready-to-use form
5. Boost soil fertility
6. Environmentally conscious
7. Applicable to a variety of field situations

Customized Fertilizers and Opportunity

Customized fertilisers can help meet the issues of food and nutritional security in a big way.

Agronomic Opportunity

It is widely recognized that a lack of micronutrients is one of the main reasons for the stagnation of agricultural yields. On the other hand, farmers tend to avoid employing micronutrients because of their low usage efficiency and improper dosage and administration methods. Because customized fertilizers are the most effective micronutrient carriers, they not only boost efficiency but also provide a great deal of convenience and uniformity in micronutrient application.

Marketing Opportunity

The Government of India's lax regulations on personalized fertilizer recommendations present a chance for the fertilizer industry and talented entrepreneurs. For the benefit of farmers, strong production and marketing standards may be established in this sector of the business since it has the financial and technological resources. There is no room for government monitoring, thus these regulations must be strictly adhered to. However, creating regulatory norms and making sure they are followed may be challenging for both the government and the customized fertilizers industry. They must stop inconsiderate players from breaking the regulations and profiting from the shortage of fertilizers by doing so. On the other side, the customized fertilizer industry runs the danger of making significant investments in cutting-edge production.

Customized fertilizers for 4R nutrient stewardship:

Customized Fertilizers (CFs) are unique, granulated fertilizers that are ready to use and intended to maximize the usage of nutrients for good crop quality, farm productivity, and profitability. The Government of India established Customized Fertilizers as a separate category under the Fertilizer Control Order with effect from 2008. The Department of Agriculture and Cooperation, the Ministry of Agriculture, and the Government of India officially approved NFCL as the first company in the country to manufacture and distribute customized fertilizers.

The procedure for authorizing customized fertilizer formulae that are made for sale has been outlined in detail by the Indian government. The process of creating customized fertilizer (CF) formulations using crop models and decision support systems, trial manufacturing of CF formulations, and multi-location testing includes selecting a targeted geographic location or cluster, gathering socioeconomic and agro-ecological data about the location cluster, choosing

the crop or cropping system, collecting soil samples for lab analysis, identifying limiting nutrients, and determining availability status. Even though the CF development process is drawn out, the final outcomes are quite positive. A group of soil scientists, agronomists, crop specialists, and field managers will create customized fertilizers.

The East and West Godavari Districts of Andhra Pradesh have built a model showcasing a knowledge transfer method for customized fertilizers for paddy crops. Indicators of 4R stewardship and the promotion of Fertilizer Best Management Practices include the appropriate source (geography and crop-specific customized fertilizers), the right quantity (doses) prescribed at the right times (i.e. during three crucial crop stages), and the appropriate location (basal, top dress) (FBMPs). The cluster idea entails providing goods and services related to fertilizers to the farming community as a whole. To entice farmers to adopt and use the plant nutrition solutions offered to them, NFCL developed and implemented the cluster idea. This procedure is divided into three steps:

Stage 1: Awareness

Prior to the planting season, the market development team adopted a number of villages. A survey was conducted in these cluster villages, and using internally developed criteria, a group of progressive farmers was found. Specialists created training sessions and generated appropriate communications to emphasise the need of balanced fertilisation for increasing production and profitability as well as the usage of customised fertilisers (including methods and quantities needed at different stages of crop growth). Technical videos on FBMPs on crop production methods and product awareness have been produced by NFCL and are being screened across the cluster villages using audiovisual vans.

Because of this cluster-based communication strategy, many farmers in Andhra Pradesh have started using customized fertilizers, which has led to higher agricultural income and productivity. In the next years, NFCL aims to connect with farmers in additional Indian states.

Stage 2. Implementation

A group of farmers will get fertilizers and be instructed on how to use them appropriately during the cropping season. Development officers and their teams will work with them to adopt these values during the course of the season. Several observations will be made during the course of the crop's growth. Just prior to harvest, other farmers will be invited to observe the results of customized fertilizers and will have the chance to engage and ask any questions. Dealers and government officials will be present.

Stage 3. Evaluation

Throughout the third phase, a thorough assessment procedure will be carried out, and farmer comments will be recorded. Farmers will be encouraged to compare their practices, crop quality, pest/disease incidence, fertilizer costs, incomes, and other factors while discussing the product and yields gained. Discussion participants will be requested to be local farmers. The media, government representatives, and agricultural specialists will all be involved at this time. Farmers that are successful will be compensated for their work. Effective fertilizer use by farmers requires not just arduous internal scientific work but also the delivery of pertinent services. To properly convey knowledge and information to farmers and communicate it to their counterparts, each programme has to be evaluated and improved. Information must be disseminated from farmer to farmer, as farmer opinion is a potent channel. With this in mind, NFCL places a premium on offering value-added fertilizer products and services in tandem.

Merits of Customized Fertilizers:

- It provides the plant with all the available nutrients in an appropriate quantity and ratio.
- Customized fertiliser is a soil-crop-climate-based fertiliser that is less affected by soil, plant and climatic conditions, resulting in more nutrient absorption and decreased nutrient loss.
- Customized fertiliser provides secondary and micronutrients in addition to fundamental nutrients.
- Customized fertiliser lowers the cost of application, which lowers the overall cost of agriculture.
- Site specific nutrient management and precision agriculture, which support the highest fertilizer usage efficiency of the supplied nutrients in a cost-effective way, heavily rely on customized fertiliser.
- Site and crop-specific fertilisers can help increase soil health.

Conclusion:

Innovative ready-to-use or made-to-order solutions for nutrient management at particular sites and crops under certain agroclimatic conditions are known as customized fertilizers. Customized fertilizers increase yield, crop quality, and fertilizer usage efficiency of the provided nutrient in a cost-effective manner by delivering the optimum amount of plant nutrients at the right time for different growth stages of the crop. Customized fertilizers also sustain post-harvest soil fertility because they utilize appropriate quantities of macro and micro nutrients that are particular to the place.

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PLASMA IN AGRICULTURE: FARM TO FORK

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Abstract:

The population on earth is estimated to reach 10 billion by 2050 which raises a challenge of high food production to meet global demand (FAO, 2017) and other challenges are emerging pathogens in food production process, intensive use of chemicals for higher food production leading human health and environmental pollution, food wastage due to lack of proper handling processes in which microbes and pathogens are major constraints. In this scenario we review Cold plasma is one of the innovative technologies recently drawn interest in food and agricultural sector. Plasma is a fourth state of matter which contains free electrons, positive and negative ions. Production of reactive oxygen and nitrogen species (RONS) in cold plasma enhances the structural and biochemical changes of the treatment material which leads to increase in seed germination, phyto hormones, growth and which finally results increase in yield and also promote the post-harvest technologies like processing, increase in shelf life, storage.

Keywords: Cold Plasma, Promotion, Growth, Quality, Agriculture, Reactive oxygen.

Introduction:

By 2050, the population on earth is estimated to reach 10 billion which points to the need for innovative approaches for food production and processing to meet the global demand for food and nutritional intake (FAO, 2017). The greatest challenge is to produce safe food with high quality considering the new risks encountered during the food production process due to emerging pathogens. Furthermore, intensive use of chemical fertilizers and pesticides used for increased yield brings hazards to human health and environment. Therefore, newer strategies and technologies having least or no environmental risks are currently being focused on by researchers.

United Nations Food and Agriculture Organization predicted that the global food shortages will be three times more likely as a result of climate change owing to industrialization and urbanization (FAO, 2017). As the horizontal expansion of cultivable land is possible only to limited extent, the only way to address the food shortage is by increasing the crop yield through an economically viable process.

Food wastage is also a major problem due to lack of farming practices and post-harvest processing. Even with higher growth and production, the lack of handling practices led to huge food losses. Microbes and pathogens are the major constraints that cause food spoilage at altered times from farm to fork (Alexander *et al.*, 2017).

Obtaining high yields in agricultural production is essential due to the world's population growth and increased food demand. At the same time, adverse effects of agriculture on environment need to be kept to a minimum. A new rapidly developing field called 'Plasma

agriculture' shows promise as an efficient green technology for enhancing productivity while maintaining good food quality and safety in the many steps of the food cycle.

What is plasma?

The plasma state is often referred to as the fourth state of matter. Much of the visible matter in the universe is in the plasma state. This is true because stars, as well as all visible interstellar matter, are in the plasma state (Bogaerts *et al.,* 2002).

Plasma is charged gas with strong electrostatic interactions and consists of neutral and excited atoms, free radicals, negative and positive ions, UV photons with net zero electric charge. Plasma contains free electrons, positive and negative ions. It was discovered by W. Crookes in 1879 as the fourth state of matter. The term 'plasma' is a Greek word meaning 'moldable substances' first coined by the chemist Irving Langmuir in 1920 (Rajvanshi, 2008).

Plasma is classified into thermal plasma and non-thermal plasma based on thermodynamic temperature of their constituents like electrons, neutrons and ions. Thermal plasma is also called as 'hot or high temperature plasma' because the temperature of all the constituents (electrons, ions, neutral species) are the same. This is true for stars as well as for fusion plasmas. High temperature is required to form these plasmas, typically ranging from 4000 to 20,000 K.

Non-thermal plasma is known as low temperature plasma (LTP) or cold plasma or cold atmospheric plasma as it can be produced at atmospheric pressure. It implies that the temperature of all the constituents is different. More precisely, the electrons are characterized by much higher temperature than the heavy particles like ions, atoms and molecules (Bogaerts *et al.,* 2002). LTP sources can produce a chemically rich environment at close to room temperature both at reduced and at ambient pressures, a unique condition that enables the delivery of highly reactive plasma species in a non-destructive and beneficial way to even extremely heat sensitive surfaces.

Production of cold plasma

Different types of cold atmospheric plasma (CAP) have been developed for agricultural uses. Thermal, electric or light energy can be used to produce plasma. Usually, the discharge needed to produce CAP is induced electrically. Some of the methods to produce CAP are dielectric barrier discharge (DBD), atmospheric pressure plasma jet (APPJ), plasma needle and plasma pencil.

Dielectric barrier discharge: Preliminary experiments on DBD was conducted by Siemens in 1857. DBD consists of two flat metal electrodes that are covered with dielectric material. A carrier gas (H_2, O_2, Ar, He) moves between two electrodes and is ionized to create plasma. Between electrodes, one is a high voltage electrode and other is a grounded electrode.

Atmospheric pressure plasma jet: Plasma jet devices are made up of two concentric electrodes. The outer electrode is grounded and the inner electrode is connected to external energy source such as radio frequency (RF) source and creates RF energy. Radio frequency interaction with the working gas in the target chamber causes ionization and exits through nozzle giving a 'jet-like' appearance.

Plasma needle and Plasma pencil are the modified methods of plasma jet.

Treatment of cold plasma

Cold plasma can be applied by two methods.

i) Direct treatment ii) Indirect treatment.

Direct treatment: This treatment is also known as material/ seed treatment. In this type, seeds or materials are exposed to the plasma directly placed in between electrodes or under plasma regime like in plasma jet.

Indirect treatment: In this type, materials are treated with plasma activated water (PAW). PAW is produced by application of cold plasma to the water surface or underneath the water surface by using plasma sources. Water is activated for a period of time with metastable species of cold plasma which results in activation of relatively long-lived species such as reactive nitrogen species (RNS) like nitrates, nitrites and reactive oxygen species (ROS) like hydrogen peroxide, atomic oxygen, singlet oxygen etc. The resulted plasma water is then used for treatment of certain fresh produce by immersion or spraying.

Properties of plasma activated water

The formation of reactive neutral species attributes some important change in properties of water such as pH, electrical conductivity (EC), H_2O_2 concentration, nitrites and nitrates concentration.

pH

It is the measure of hydrogen ion concentration of a solution. Upon activation of liquid, temperature of the liquid rises slightly which can lead to decrease in its viscosity and increase in its ion mobility. Also, higher dissociation of molecules will induce increase in its overall ion concentration (Barron *et al.,* 2011). The reactions taking place between the chemical species formed in plasma and water results in the acidification of liquid (Judee *et al.,* 2018). Nitrate radicals formed in the plasma treated water lowers the pH of treated liquid to a range of 5-6 (Peethambaran *et al.,* 2015).

Electrical conductivity

It provides the information on both nature and concentration of ions present in the electrolytic solution. In a research study, a fall in conductivity was observed during the first 5 minutes (min) of activation, from 647.33 ± 15.07 μ S/cm to 614 ± 10.60 μ S/cm. After 5 min, EC increased linearly with the activation time, on a range covering values from 614 ± 10.60 μ S/cm to 731.33 ± 19.37 μ S/cm for 5 and 30 min respectively. It was concluded that variations in EC result from charged species consumption (quick concentration decay of bicarbonate ions *i.e.,* decrease in EC) and production (formation of nitrate ions leading to increase in EC) mechanisms occurring in the liquid phase (Judee *et al.,* 2018).

Hydrogen peroxide

Aqueous and gaseous hydrogen peroxide can result from the recombination of two hydroxyl radicals in liquid or in plasma phase. In plasma phase, hydrogen peroxide can be also generated from interaction between excited water molecules and hydroxyl radicals. Hydrogen peroxide causes dormancy release by down-regulating the blockage of abscisic acid (ABA) and induces important effects on development, fruit growth and quality (Maheux *et al*.,2015).

$$OH + H_2O \longrightarrow H_2O_2 + H$$

Nitrite and Nitrate concentration

In plasma, NO is rapidly converted to nitrogen dioxide by reactions with oxygen. In liquid phase, nitrogen dioxide is dissolved in water, leading to the formation of nitrites, nitrates and hydronium ions.

$N + O_2 \longrightarrow NO + O$

$NO + O \longrightarrow NO$ Gaseous phase

$2NO_2(g) + H_2O \longrightarrow NO_2^-(aq) + NO_3^- + 2H^+(aq)$ Liquid Phase

In open air, surrounded oxygen concentration is stable during plasma treatment, leading to a linear increase of nitrites and nitrates (Judee *et al.,* 2018).

Ammonia is directly created in plasma phase due to interaction between excited nitrogen and hydrogen (Maheux *et al.,* 2015). Once ammonia is dissolved into water, it can be converted into ammonium ions by acid-base reaction.

$N2 + 3H_2 \longrightarrow 2NH_3(g)$

$NH_3(aq) + H^+ \longrightarrow NH_4(aq)$

Dihydrogen and excited molecular nitrogen both present stable concentrations in the gaseous phase during plasma activation which induces linear increase in ammonium ions and ammonia in PAW (Judee *et al.,* 2018).

Applications of cold plasma in agriculture

Applications of cold plasma in agriculture deal with both preharvest and postharvest systems. Pre harvest applications include seed treatment, seedling treatment and management upto harvest while, post-harvest applications include disinfection during storage and enhancing produce quality.

The beneficial effects of plasma arise from the cocktail of reactive neutral species, charged species (electrons, ions), electric fields, and ultraviolet radiation produced in the discharge. Production of reactive oxygen and nitrogen species during cold plasma production enhances the structural and biochemical properties of the treatment material which lead to increase in seed germination, phyto hormones and growth which finally results in increase in yield. The action of plasma species contributes to the seed germination, seed disinfection, plant growth, insect control, retention of quality of agricultural products, and soil remediation, which altogether can contribute toward an increased food production and realization of sustainability. (Table 1)

Table 1: Effect of cold plasma on different aspects of growth and production

Increases	**Decreases**
Germination potentialPlant growth Water uptake Nutrient uptake Stress tolerance Quality of produce Quantity of produce Shelf life of produce	Duration of vegetative growth Water requirement External inputs Wastage of produce Pollution of environment

Improving seed germination

Many researchers reported enhancement of germination in plasma treated seeds due to increase in water absorption, smoothening of seed coat, sterilization of the seed surface and decrease in contact angle (Fig 1). A significant benefit of plasma technology is associated with

its synergistic effects on seed germination and seedling vigour without any synthetic chemical residues. In general, an apparatus for plasma treatment simply consists of electrodes for plasma generation, a treatment chamber that accommodates the electrodes, and electric power to supply current to the electrodes. When a high electric discharge is applied to air or an aqueous solution in a chamber, ROS, e.g., superoxides, singlet oxygens, atomic oxygen, ozone, hydrogen peroxide, and hydroxyl radicals), RNS, e.g., nitric oxide, nitrogen dioxide, nitrate, nitrite, and peroxynitrite), and ultraviolet (UV) photons are mainly generated from the plasma discharge. ROS, RNS, and UV have been independently used to scarify seeds (a technique to soften the seed coat while keeping the seed viable), inactivate seed-borne pathogens, and enhance antioxidant defense systems in crop plants (Jisha *et al.*, 2013).

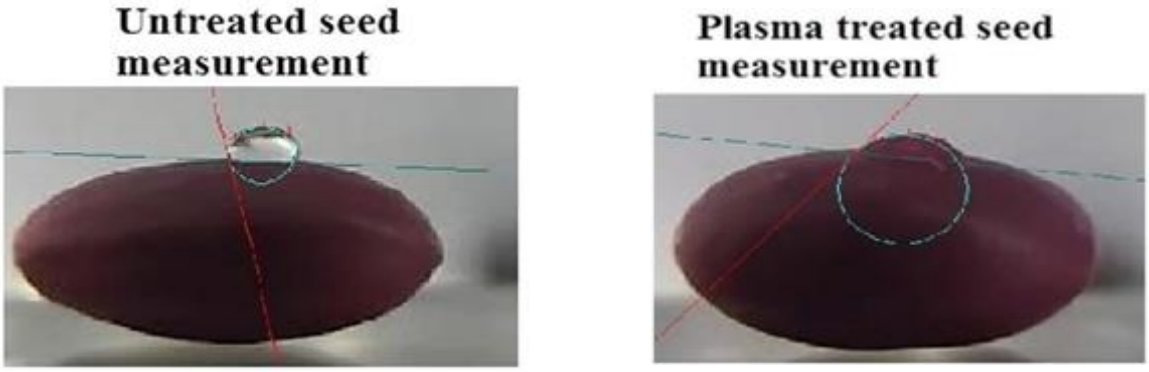

Fig. 1: Contact angle for the control and plasma treated seed

Khamsen *et al.* (2016) obtained the highest germination parameters for seeds stimulated with plasma. The analysis of the contact surface angle showed decerement to its mean values. Analysis of the scanned electron microscopy scans revealed the increase in seed pattern intensity which could be attributed to removing of the surface parts of cuticle possibly covered with wax upon short time (2-5 min) plasma treatment. Such a phenomenon can act similarly to mechanical scarification of seeds.

Breaking seed dormancy

A physical way to break dormancy using cold plasma is known as recent stimulation (Dhayal *et al.*, 2006, Zivkovic *et al.*, 2004). Several agents that can play a role in dormancy breaking are generated by plasma treatment. If the plasma is directly applied to the material, the etching and erosion of the surface are probably the most dominant processes, which lead to promotion of water permeability and water uptake which in turn enhance the amylase and protease activities in metabolism of sugars and proteins that are essential to the germination process (Sera *et al.*, 2009).

Enhanced nutrient availability and uptake

Plasma treatment through water or soil supply the essential nutrients to plants. Plasma could double the nitrogen content in the leaves, increase leaf area and dry weight. It could also reduce the bacterial density in drainage water which was irradiated by plasma. Plasma irradiation produced the aqueous nitrous nitrogen and nitrate nitrogen which were absorbed by the plant and resulted in higher plant growth with respect to dry weight.

Improving plant growth and development

Field experiments were carried out for wheat seeds treated with 80W cold plasma. Compared with the control, plant height (20.3%), root length (9.0%) and fresh weight (21.8%)

were improved significantly at seedling stage. At booting stage, plant height, root length, fresh weight, stem diameter, leaf area and leaf thickness of the treated plants, were increased by 21.8, 11.0, 7.0, 9.0, 13.0 and 25.5% respectively. At the same time, the chlorophyll content (9.8%), nitrogen (10.0%) and moisture content (10.0%) were higher than those of the control, indicating that cold plasma treatment could promote the growth of wheat (Jiang *et al.,* 2014). The low temperature plasma pre-treatment produced changes in endogenous hormones (auxins, cytokinins and their catabolites and conjugates), which could be correlated with increased growth of the pea seedlings (Stolarik *et al.,* 2015).

Tolerance to biotic and abiotic stress

Plasma treatment could alleviate the adverse effects of drought stress on growth of wheat plants. Proline and soluble sugar levels under drought stress were improved after the DBD plasma treatment, whereas the malondialdehyde content decreased. Content of ROS under drought stress were reduced after the DBD plasma treatment, whereas the activities of superoxide dismutase, catalase, and peroxidase were promoted. Plasma treatment with DBD promoted ABA generation in wheat seedlings, and it also regulated functional gene *LEA1* and stimulated regulation genes *SnRK2* and *P5CS* to resist drought stress.

Reduction in water consumption

Plasma treatment has contributed to the reduced water consumption and higher fecundity of *Arabidopsis thaliana.* Cold plasma was used to treat deionized water for irrigation of *Arabidopsis thaliana* plants for 5 weeks 20-30% reduction in water consumption was observed in treated plants in comparison with control (Fig 2). Plasma treatment decreased overall water consumption and water requirement for irrigation simultaneously enhancing plant growth and yield (Peethambaran *et al.,* 2015).

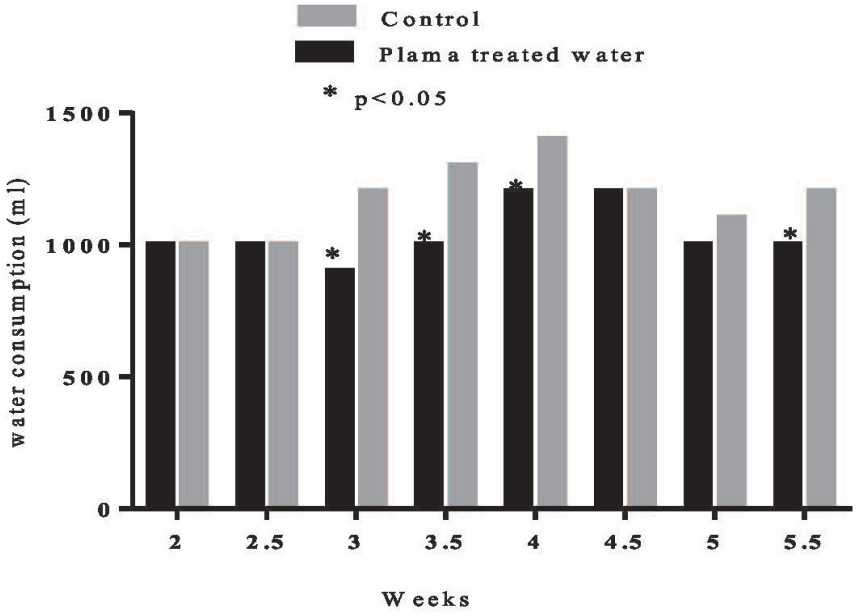

Fig. 2: Influence of plasma water on water consumption of Arabidopsis plant

Enhancement in yield and yield attributes

Plasma treatment of *Arabidopsis thaliana* seeds recorded growth enhancement from germination to harvest with 11% shorter harvest period, 56% increase in total seed weight, 12% increase in individual seed weight and a 39% increase in seed number compared to control (Koga *et al.,* 2015).

In berseem, increase in yield was observed along with the length of plant and reduced leaf stem ratio for each cut (Aragi, 2016).

Disinfection of food products

Cold plasma treatment has been proposed as a promising alternative for seed and grain decontamination (Table 2). It offers high reactivity at moderate temperature which is required to treat temperature sensitive substrates like plant materials, yet does not produce any toxic residues if properly engineered. Moreover, plasma-generated reactive species act only on the surface of the substrate, where typically most of the microbial contamination is found and therefore ensures efficient decontamination without affecting the bulk properties (Butscher *et al..*, 2020). Plasma treatment could reduce the pathogens present on the food surfaces and made them good to consume. Cold plasma treatment eliminated the *E. coli* and *Aspergillus* on almond surface after 20 min treatment.

Soil properties

Plasma activated water does not affect the soil physico chemical properties. In a recent study conducted by Simeckova *et al. (*2020) it was showed that PAW improves the soil water retention by 30% in comparison with control. A minimal change was observed in natural evaporation with PAW. The soil pH remained in the neutral range of values even after the highest used number of PAW applications, therefore the soil was still in the best conditions for growing.

Soil remediation

The treatment of soil with atmospheric pressure DBD plasma for 25 min was found to result in 93% degradation of acid scarlet and 74% removal of chemical oxygen demand. Ozone has the dominant role in the degradation process. Decolourization of polluted soil increases with high voltage and discharge frequency. The degradation efficiency of the process was found to depend on the moisture content of the soil, inducer gas and applied voltage.

Upon plasma treatment with TiO_2 catalyst for 10 min, it was observed that 88.8% of para nitrophenol was removed. The active species (O_3 and H_2O_2) enhanced para nitrophenol degradation and mineralization. The degradation was higher in moist soil compared to dry soil. The main intermediates were hydroquinone, benzoquinone, catechol, phenol, benzol, trioxole, acetic acid, formic acid, NO_2^-, NO_3^- and oxalic acid (Wang *et al.*, 2011).

Plasma research in India

The Institute for Plasma Research (IPR) is an autonomous physics research institute located in Gandhinagar, Gujarat, India. The institute is involved in research on aspects of plasma science including basic plasma physics, magnetically confined hot plasmas and plasma technologies for industrial applications. It is a large and leading plasma physics organization in India. The institute is mainly funded by Department of Atomic Energy.

The Facilitation Centre for Industrial Plasma Technologies (FCIPT) is a division of Institute for Plasma Research working in the field of industrial plasma technologies. This centre was set up in the year 1997 to promote, foster, develop, demonstrate and transfer industrially relevant plasma-based technologies to industries, thus enabling technology commercialization. This centre acts as an interface between the institute and industries. FCIPT has developed technologies related to waste remediation and recovery of energy from waste, surface hardening

and heat treatment technologies such as plasma nitriding and plasma nitrocarburizing, plasma-assisted metallization technologies and space related plasma technologies.

FCIPT has developed APPJ source which can be used to degrade pesticides. Results showed that dicholorvos concentration goes down by 3 times at 9 min treatment. Recently, FCIPT in collaboration with Anand Agricultural University developed glow discharge plasma for degradation of pesticides in fruits and vegetables. Another research conducted at FCIPT has shown that microbial cells can be killed when fruits and vegetables are exposed to plasma activated water (Nema, 2018).

Future prospects

The efficient application of cold atmospheric or low-pressure plasma requires experimental evidence and mechanistic studies. Although enhanced plant vitality and development due to plasma treatments have been well documented, evidence for the applied usage of plasma in agricultural fields and facilities are still in infancy stage. Moreover, available experimental data are biased towards laboratory conditions. Thus, field and facility application studies are required. The underlying mechanisms of the plasma effects are also relatively unexplored compared to phenotypic effects discussed here. More information about the modes of plasma action on plant production and sustainability is necessary to optimize and upgrade the plasma systems and applications.

Conclusion:

It can be concluded from several researches that plasma has efficiently enhanced the germination rate, crop growth and yield, protection of crops from stress, decontamination of food products and enhancing shelf life of food products. Many researchers have reported that RONS which are produced in plasma were responsible for the improvement of all the above applications. Both the sensory and nutrient qualities are well preserved after proper plasma treatment. With its decontamination effects, cold plasma treatment is beneficial to the extension of shelf life of fruit and vegetables. Therefore, it is an alternative technology to reduce food wastage. It is environmentally friendly and does not leave any contaminated aqueous waste that could potentially affect the environment as well as human health.

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EXPLORING SUSTAINABLE APPROACHES FOR MANAGING ROOT-KNOT NEMATODES IN AGRICULTURE

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Introduction:

Plant-parasitic nematodes (PPN) are a major biotic factor limiting agricultural yield and, ultimately, crop production. Besides from causing direct crop output losses, plant-parasitic nematodes also play a significant role in disease complexes including other diseases. They drain nutrients, obstruct water transport, make plants more susceptible to secondary infections, and serve as viral vectors. Although it is difficult to measure their impact, estimations imply that PPN diminish worldwide yields by 10-25% (Nicol *et al.*, 2011). A critical examination of crop losses has been conducted using data provided by AICRP on Nematodes over the years. Plant-parasitic nematodes cause 21.3% crop losses of Rs. 102,039.79 million (1.58 billion USD) per year; losses in 19 horticultural crops were estimated at Rs. 50,224.98 million, while losses in 11 field crops were calculated at Rs. 51,814.81 million (Kumar *et al.*, 2020).

There are more than 4,000 species of PPN known, most feed primarily on roots, but some also consume aerial portions. The root-knot nematodes (RKNs; *Meloidogyne* spp.) are the most yield-limiting type of PPNs responsible for the majority of economic losses. RKNs are obligatory stationary endoparasites that may readily proliferate in the roots of over 3,000 plant species. They are found all over the world and their population in the soil grows quickly when conditions are favourable.

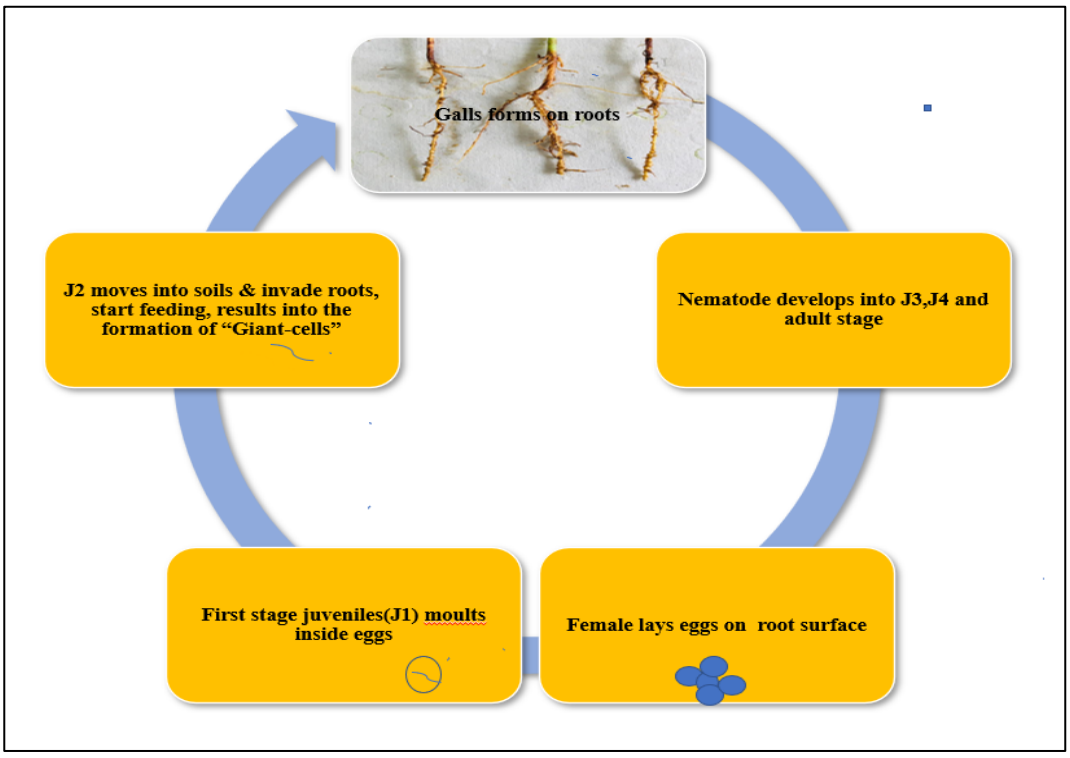
Because of their economic relevance, there is a growing need to create long-term management techniques and treatments for RKN control. Although cultural controls are widely utilised, they have additional restrictions due to the wide host range of *Meloidogyne* spp. and the occurrence of mixed populations of several RKN species in the field. Meloidogyne-resistant cultivars have proven to be an efficient RKN management tool; however, few resistant cultivars are commercially available, and resistance may be overcome by new developing RKN species such as *M. enterolobii* (Hajihassani *et al.*, 2020; Xiang *et al.*, 2018). Certain chemicals are employed as nematicides for short-term control, but they are currently limited due to their negative effects on humans and the environment. There is a need to create some sustainable ways of controlling root knot nematode infection.

Nematode life cycles:

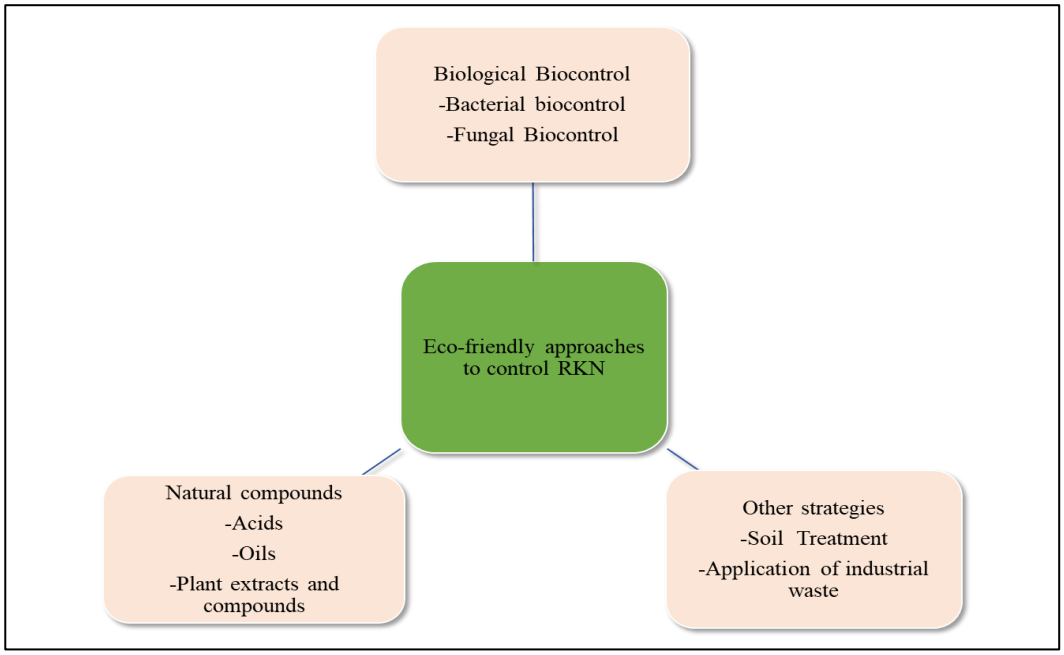
All nematodes moult four times during their life span, from juvenile to adult. A plant-parasitic nematode has six life stages: egg, four juvenile stages, and adult. Male and female nematodes are found in most species, however reproduction without males is common, and some species are hermaphroditic. The cycle is completed when the individual produces eggs. Most species generate between 50-500 eggs per female, depending on the nematode species and their surroundings, though many can produce more than 1,000 eggs. The length of the life cycle varies greatly depending on the nematode species, host plant, and environment temperature. Many

plant nematodes finish their life cycle in about four months during the summer months when soil temperatures range from 80 to 90°F.

The infective-stage juveniles (J2s) pierce the roots and migrate intercellularly to the vascular cylinder, where they produce a unique hypermetabolic and hypertrophic long-term feeding structure from which nutrients are withdrawn, resulting in the production of numerous coenocytic giant cells (Kyndt *et al.*, 2017). Following induction, the nematodes become inactive and rely entirely on a specialised feeding mechanism for nourishment for several weeks.



Life cycle of root knot nematodes



Symptoms:

1. The presence of noticeable areas of underdeveloped plants in the rows of the field.
2. The plants appear underdeveloped because the roots' ability to absorb nutrients has been decreased.
3. Extremely poor production, both qualitatively and quantitatively.
4. Increased vulnerability to bacteria, fungus, and viruses.
5. A poorly developed or deformed root system (galls and exaggerated secondary root formations)
6. Yellowing of the leaves as well as leaves wilt, and even an early death.

Eco-friendly approaches to control RKN:

The use of nematicides has remained the most common short-term management strategy against RKN. However, several chemicals, including methyl bromide and aldicarb, have been withdrawn from the market in recent decades due to environmental and human health concerns, as well as toxicity to non-target organisms (Kim *et al.,* 2018; Xiang *et al.,* 2018). Although substitute compounds have been produced, they have yet to achieve the same levels of efficiency (Desaeger *et al.,* 2017). Given the magnitude of the economic losses caused by RKN, as well as the increasing restrictions on the use of chemical nematicides, it is evident that new environment friendly solutions are required. It is also critical to keep enhancing current green methods in order to make them more efficient. Researchers all around the world have been working to develop new environmental friendly RKN management solutions such as biological control, the use of diverse natural substances, and a variety of other ways such as soil modification, the use of industrial waste, and so on.

Biological control:

Biological control mechanisms are divided into two categories: antagonism against PPN and plant growth stimulating substances. They can be caused by the biological agents themselves or by their metabolites. Although viruses, mites, collembola, turbellarians, oligochaetes, predaceous nematodes, and protozoans have been examined, they have not been demonstrated to be as effective or investigated as bacterial or fungal organisms. Bacteria and fungi continue to be the primary adversaries of PPN biocontrol (Xiang *et al.,* 2018).

1. Bacterial Biocontrol:

The use of *B. cereus* strain BCM2 to inhibit *M. incognita* in tomato has revealed that it colonised at root exudates and served as a second-stage juvenile (J2) repellent, resulting in less nematode infection (Li *et al.,* 2019). Nematode-infected tomato plants treated with BCM2 had 67.1% less J2 than the control. Another pot study with tomato found that agrobacteria also increase plant protection against RKN (Lamovšek *et al.,* 2017).

In a greenhouse research, *B. subtilis*, *B. pumilus,* and *P. fluorescens* were found to be effective against *M. incognita* on cowpea (Abd-El-Khair *et al.,* 2019). The best reduction in nematode counts was achieved with *P. fluorescens* (89%), followed by a combination of *P. fluorescens* and *B. subtilis* (88.50%). The combination treatment produced the maximum yield increase (70.2%), followed by *B. pumilus* at 49.3%. *Bacillus* has been used successfully for RKN management in the past, either alone or in combination with *Streptomyces rubrogriseus* and the nematicide Fosthiazate (Yue *et al.,* 2019). These biocontrol agents appear promising, but their effectiveness needs be tested in the field.

Rhizobacteria increase soil texture, and the substances they release are valuable biostimulants that help plants to deal with stress. Signal exchanges between plant roots and microorganisms govern these interactions. It has been demonstrated that biological growth factors can increase plant growth and prevent the harmful effects of RKN in seedlings. (Viljoen *et al.,* 2019) examined the effectiveness of 27 strains of plant growth-promoting rhizobacteria against *M. incognita* on 6 week old seedlings of carrots. Out of which five strains found effective resulting in reduced gall counts. In the greenhouse, *P. alvei* T30 and *B. aryabhattai* A08 demonstrated potential as biological control agents of *M. incognita* on carrot and tomato, respectively.

2. Fungal Biocontrol:

Nematophagous fungi use a variety of mechanisms to control nematode populations, which include: nematode-trapping (predatory) fungi that produce extensive hyphal networks and constricting rings as trapping devices to catch nematodes; endoparasitic fungi that infect nematodes by adhering to their surface or through direct ingestion, followed by germination, growth, and nematode killing. In addition to producing poisons that immobilise worms before hyphal penetration through the nematode cuticle, egg- and female-parasitic fungus also grow on and parasitize the sedentary stages of nematodes, such as eggs and cysts (Zhang *et al.,* 2020).

For decades, researchers have used fungal microbes to control RKN and other agricultural pests. *Actylellina, Arthrobotrys, Aspergillus, Catenaria, Dactylellina, Hirsutella, Pochonia, Purpureocillium,* and *Trichoderma* are some of the most prominent fungal species for RKN management. RKN J2s can be trapped in the soil by *Arthrobotrys* and *Dactylellina* using their hyphal structures, reducing the nematode's invasion capabilities. Plant colonisation by specific endophytic fungi can boost plant protection against PPN. The mechanisms involved appear to be multifactorial, include repellent activity that reduces J2 attraction to roots, adult female development that is slowed or delayed, and decreased fecundity.

Endophytes like *Paecilomyces* and *Trichoderma* can trap and kill RKN in the soil or root systems. They may function at various phases of nematode life (i.e. eggs, juveniles, or adults). Many in vitro and in vivo studies have also demonstrated the production and release of nematicidal metabolites. Some endophytic fungi exploit two more mechanisms: the generation of plant-like hormones such as cytokinins and gibberellins, as well as the mobilisation and enhancement of plant defence (Redman *et al.,* 2011; Sikder & Vestergård, 2020). A recent study found that *P. chlamydosporia* can develop plant-dependent systemic resistance to *M. incognita* (Ghahremani *et al.,* 2019).

Arbuscular mycorrhizal fungi (AMF) are obligate root symbionts that aid in the defence of their hosts against biotic stressors such as PPN infection. This is accomplished through a variety of methods, including increased nutrient uptake, changed root architecture, competition for space and nutrition with PPN, and induction of plant systemic resistance (Schouteden *et al.,* 2015). *Richoderma* spp. may decrease RKN infections by activating host defence. A group of researchers studied whether *Trichoderma* alters the host's hormone signalling network to promote nematode resistance (Martínez-Medina *et al.,* 2017). They discovered that root colonisation by *Trichoderma* reduced nematode performance at various stages, including invasion, gall development, and reproduction, using *M. incognita*. *Trichoderma* first primed salicylic acid-regulated defences, which limited nematode root penetration. Then, it boosted

jasmonic acid (JA) regulated defences, antagonising nematode deregulation of JA-dependent immunity and compromising galling and fertility.

Biocontrol agents: **Bacteria**	**RKN species**	**Host**	**References**
Bacillus cereus	*M. incognita*	Tomato	(Colagiero *et al.,* 2018)
Agrobactrium tumefaciens	*M. ethiopica*	Tomato	(Lamovšek *et al.,* 2017)
Pseudomonas fluorescens	*M. incognita*	Cowpea	(Abd-El-Khair *et al.,* 2019)
Pseudomonas luorescens, P. putida, Bacillus amyloliquifaciens, and B. megaterium,	*M. incognita*	Bottle Guard	(Rani *et al.,* 2022)
Pseudomonas fluorescens	*Meloidogyne javanica*	Tomato and Cucumber	(Sahebani & Gholamrezaee, 2021)
Bacillus altitudinis	*M. incognita*	Ginger	(Wang *et al.,* 2021)
Pseudomonas simiae	*M. incognita*	Tomato	(Sun *et al.,* 2021)
Fungi			
Arthrobotrys oligospora	*M. incognita*	Tomato	(Soliman *et al.,* 2021)
Pleurotus ostreatus)	*M. incognita*	Cowpea	(Youssef & El-Nagdi, 2021)
Fusarium oxysporum	*M. incognita*	Cucumber	(Patil *et al.,* 2021)
Trichoderma asperellum	*M. incognita*	Pineapple	(Kiriga *et al.,* 2018)
Mortierella globalpina	*M. chitwoodi*	Pepper	(DiLegge *et al.,* 2019)

Nematocidal Natural compounds

1. Application of acids

Organic acids like as amino, acetic, butyric, formic, and propionic acids have been shown to be poisonous to specific species of PPN. They are either the consequence of microbial breakdown of various chemicals in the soil or metabolites generated by microorganisms. Many acids, including heptalic acid and hydroxamic acids, have been shown to be effective against nematodes (Abd-Elgawad & Askary, 2018; Zasada *et al.,* 2005). However, the efficacy of acid to kill nematodes is strongly controlled by soil conditions. The nematocidal efficacy of 5-aminolevulinic acid (ALA) on *M. incognita* and other PPN was studied in vitro and in the greenhouse, and it was observed that ALA displayed a high anti-RKN impact and reduced egg hatching of *M. incognita* by 90%. The number of egg masses per root was reduced from approximately 180 to 50. It also had a substantial effect on nematode metabolism, such as total protein production, malondialdehyde concentration, and oxidase activities, indicating that ALA is a promising biodegradable bionematicide (Cheng *et al.,* 2017).

The anti-RKN potential of acetic acid and other natural compounds was assessed against *M. incognita* in a research on plant secondary metabolites (Ntalli *et al.,* 2017). Electron microscope analysis of the acid-treated J2s revealed that the acid damaged the cuticle, destroyed the pseudocoel cells' nuclei, vacuolized the cytoplasm, and caused nematodes death. In order to

prevent *M. incognita* from infecting bananas, humic acid was tested (Seenivasan & Senthilnathan, 2018). In vitro, humic acid suppressed egg hatching between 50 and 100% at concentrations of 0.08 to 2.0%. Parallel to that, it decreased J2's mobility in a concentration-dependent way. Soil humic acid treatment decreased root galling in pot tests. After humic acid treatment, nematode density in soil was reduced by 53.5-56.7%, and egg population decrease was 61.9-63.8%. Another advantage of humic acid treatment was that it improved plant development.

2. Application of Oils

Essential oils (EOs) have been studied as biopesticides for RKN control by researcher and industry. EOs were tested as soil biofumigants for *M. incognita* control on tomato in greenhouses (Laquale *et al.,* 2015). EOs from five different plants were examined at three different concentrations. At all concentrations, EOs of *Eucalyptus globulus* and *Pelargonium asperum* significantly decreased nematode multiplication and gall development on roots while increasing shoot and root biomass.

In another study, 29 plant-based EOs were tested in vitro and in vivo against *M. incognita* on tomato and it was found that EO of mexican tea (*Dysphania ambrosioides*) had the greatest impact, reducing the amount of galls and eggs by 99.5 and 100%, respectively (Barros, Campos, de Paula, *et al.,* 2019). Oils from three Brazilian plants (*Astronium graveolens, Hyptis suaveolens, and Piptadenia viridiflora*) were screened in vitro and in vivo against *M. incognita.* Only *P. viridiflora* shown toxicity against *M. incognita* and was examined further. The primary component of *P. viridiflora*, benzaldehyde, was discovered using gas chromatography-mass spectrometry and tested against *M. incognita*. It was able to reduce the quantity of eggs by up to 65%, while its oxime compound was capable of lowering both galls (up to 84%) and eggs (up to 89%) on tomato (Barros, Campos, de Oliveira, *et al.,* 2019).

3. Plant extracts and compounds

Wen et al. investigated the nematocidal activity of *Camellia oleifera* and *Paenoia rockii* extracts against *M. incognita* in vitro. It was found that extracts were nematotoxic to J2 as well as inhibit the egg hatching. After seven days, the *P. rockii* extract at 5 mg/ml completely inactivated *M. incognita* J2 (Wen *et al.,* 2019).

The inhibitory effect of allicin , garlic's main natural antibacterial component, was tested against *M. incognita* (Ji *et al.,* 2019). Allicin inhibited *M. incognita* and increased tomato yield in greenhouse tests. In addition, compared to the untreated control, it boosted superoxide dismutase, catalase, and peroxidase activity in tomato leaves. As a result, allicin may be a viable alternative to synthetic chemical substances for RKN regulation.

Bioactive saponins isolated from five Medicago species were tested in vitro against a variety of PPN, including *M. incognita* (D'Addabbo *et al.,* 2020). Generally, saponin potency differed depending on the plant species employed. J2 mortality was above 90% after 8-16 h contact time at 500 g/ml, whereas egg hatching ranged from 18 to 39% compared to the water control at 1,000 g/ml. The findings showed that saponin-rich extracts and plant biomasses from *M. heyniana, M. hybrida, M. lupulina, M. murex*, and *M. truncatula* might be strongly RKN suppressive.

Other strategies

1. Soil Treatments

For decades, soil solarization has been employed as a pest management strategy, and it has also been studied against RKN. Soil solarization and its combination with soil steaming have been studied since 2015 in order to produce an economical, yet efficient technique for the management of RKN in floriculture crops such as larkspur, snapdragon, and sunflowers (Kokalis-Burelle *et al.,* 2017). For comparison, the nematicide methyl bromide was utilised. The severity of root galling on all three crops was lower in steam treatments than in solarization alone. Steam treatment also resulted in *M. arenaria* control comparable to or greater than methyl bromide. Finally, it was proposed that steaming followed by solarization would be an effective strategy for replacing dangerous chemicals.

Ozonated water (O_3wat) is a well-known agricultural treatment for pathogen inactivation. It has recently been demonstrated that it is safe for plant irrigation (Martinez-Sanchez & Aguayo, 2020). The potential of O_3wat to control *M. incognita* was examined in quest of new anti-RKN medicines, indicating that O_3wat helped protect tomato against *M. incognita* through the modification of basal defensive mechanisms. The application of O_3wat to tomatoes caused no phytotoxicity harm. The root galling index was much lower in O_3wat-treated plants than in untreated controls. ROS and H_2O_2 in galls were increased after O_3wat therapy. The beneficial effect is most likely due to the altered antioxidant systems, which raise the levels of ROS, H_2O_2, and malondialdehyde in nematode feeding sites. Because of its composition, O_3wat degrades quickly to water, giving the various advantages for RKN management. O_3wat treatment, for example, can be used as an early treatment in an integrated RKN management approach without changing soil qualities (Veronico *et al.,* 2017).

2. Application of industrial wastes

With global industrialization and mass manufacturing, new methods of converting industrial waste into value-added products are required. Before now, there have been few attempts to apply this idea to RKN management. In one of these researches, agro-industrial wastes such as rice husk, common bean hull, soybean hull, orange bagasse, chicken litter, and a mixture of these materials were tested against *M. javanica* in the greenhouse utilising pot studies (Brito *et al.,* 2020). In summary, the most efficient wastes were powdered bean hulls, soybean hulls, orange bagasse, and waste combinations, with RKN control ranging from 55 to 100%.

Manipueira is the colloquial term for a liquid residue rich in nutrients and cyanogenic glycosides that is discharged from cassava (*Manihot esculenta*) starch manufacturers. In a tomato field, its nematicidal action against *M. incognita* was tested (Nasu *et al.,* 2015). Manipueria demonstrated nematicidal and plant growth enhancement activities at 50% concentration. Sisal, a native plant of Mexico, is becoming more popular as a fibre source in some regions of the world. The effect of sisal liquid residue (fresh and fermented) produced from its industrial processing on *M. javanica* was studied (Damasceno *et al.,* 2015). In vitro, J2 was found to have a 100% death rate when exposed to liquid residue at a concentration of 20%. It also reduced the amount of galls and egg masses per gram of tomato roots in the greenhouse, as well as the final population of *M. javanica* in the soil. In contrast to the fresh liquid residue, the fermented liquid residue inhibited the helpful bacteria.

Conclusion:

As compared to conventional chemical techniques, environmentally friendly solutions are now largely unable to effectively protect plants against RKN. As a result, it is important to consider about the establishment and enhancement of interdisciplinary management techniques for RKN, such as integrating microbial approaches utilising both bacterial and fungal agents with other cultural control procedures or host resistance. Although both biocontrol and the application of soil amendments have been partially investigated against PPN, there is still opportunity for more research on how these two techniques might work together. Finally, future research should concentrate on environmentally friendly approaches based on multidisciplinary strategies that can fill the gaps left by single-sided management methods.

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DISEASES OF BAEL AND THEIR MANAGEMENT

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Introduction:

One of India's most significant native fruit crops is bael. It is a member of the family Rutaceae and is botanically known as *Aegle marmelos* (L.) Corr. It is mostly found in Southeast Asia and has a diverse spectrum of biodiversity. In North-East India, bael is grown up to a height of 500 m in the Indo-Gangetic lowlands and Sub-Himalayan tracts. (Sharma *et al.*, 2007). It is also found in dry and deciduous forest of central and Southern India (Zeven and De Wet, 1982). It is among the earliest known fruits. In various regions of India, Bael is also known as Bengal quince, Bilva, Indian quince, Golden apple, Holy fruit, Belwa, Sriphal, Stone apple, and Maredo (John and Stevenson, 1979). There are several uses for the bael tree. The bael tree's wood is used to make tools for farming. Bael leaves are used as fodder, while the stem yields gum. It has significance in mythology as sacred tree whose unblemished triplet leaves are offered to Lord Shiva during worship. The bael fruit has a globular, rigid, yellowish-gray woody shell. Soft, yellow-orange, mucilaginous pulp with many seeds is enclosed in the woody shell. The fruit pulp is high in carbohydrates and includes 8 mg of vitamin C per 100 grams of pulp, along with 55 g of beta-carotene, 0.13 mg of thiamine, 1.19 mg of riboflavin, and 1.1 mg of niacin.

Marmelosin derived from bael is a medicinally important molecule and found in all parts of the plant. Other important ingredients present in bael are alkaloids, terpenoids, steroids, phenols glycosides and tannins (Venkatesan *et al.,* 2009). Its medicinal properties have been described extensively in the ancient Sanskrit literature 'Charaka Samhita' (Aiyer, 1956). In Ayurvedic and Siddha systems of medicines it is widely used to treat different ailments *viz.* diarrhea, astringent diabetes, liver toxicity, fungal infection, microbial infection, inflammation, pyrexia and to relieve pain, it is used for making sherbet and syrup (Kaushik *et al.,* 2008). Farmers in arid and semi-arid locations have been encouraged to cultivate commercial bael thanks to the creation of new, improved varieties and modernised scientific production techniques. Bael being a hardy crop, there is no serious disease as of now but sometimes the leaves are inhabited by *Sarcinella fumosu*. *Cercospora* sp. are reported to cause a leaf spot disease in Kanpur (Uttar Pradesh), *Cercospora aeglicola* and *Fusarium roseum* infect the leaves in Maharastra and *Stenella aegliues* in Muzaffarpur (Bihar). The fruits are subject to a dry rot by *Aspergillus* sp; *A. nidulans* produces a green rot and *A. niger*, a black rot. *Fusarium solani* is reported to cause a soft fruit rot, whereas *Botrytis cinerea* produces a red rot. The important diseases which seriously affect the bael production *i.e.* bacterial canker, black leaf spot, fruit drop, and internal rot In nursery, collar rot, root rot, wilt, die back and fungal foliar diseases caused by *Myrothecium roridum* Tode (1970), *Alternaria alternata* (Fr.) Keissler and *Fusarium pallidoroseum* (Cooke) Sacc. (1886) were observed. Generally *Myrothecium* leaf spot occurs during rainy season (July-August) whereas *Alternaria* leaf spot and *Fusarium* leaf spot symptoms occur after *Myrothecium* during September. *Alternaria* leaf spot causes much vegetative losses in the nursery stage are discussed further with their management practices.

Alternaria leaf spot/blight

Economic importance

Alternaria leaf blight of bael (*Aegle marmelos* L.) was reported for the first time from the Eastern Plateau and Hilly region of India (Maurya *et al.,* 2016). In Rajasthan's Chomu region (Jaipur district), this disease was noted. Moreover, some districts of Uttar Pradesh have reported it. Both nurseries and orchards exhibit the disease symptoms. The disease is much more severe in nursery stock than it is in existing orchards.

Symptom

The symptoms appear on leaves as small (4-8 mm), light brown or dark spots with rings which enlarge and become prominent with reddish brown lesions (fig1) . Eventually, these lesions combine to form irregular patches. Moreover, concentric rings can be visible around places. Additional diseased leaves become blighted, dry up, and eventually fall to the ground. Infected plants eventually die in the later stages of the disease.

Fig. 1: Alternaria Leaf Spot/Blight

Causal organism

Alternaria Leaf spot/Blight of bael is caused by *Alternaria alternata.*

Epidemiology

High rainfall and high relative humidity over 90% encourage the transmission of disease. The fungus thrives in the 23°C to 30°C temperature range.

Integrated management

- Ploughing/field sanitation to rid fields of wild species is needed to destroy the pathogen load.
- Prune the plant to ensure proper supply of sunlight and air to overcome the risk of disease.
- Two sprays of Mancozeb (0.2 per cent) at 15 days regular interval can check the disease in initial stage.
- It can also be successfully managed by foliar application of Mancozeb 75% WP @ 0.2% followed by a spray of Copper oxychloride 50% WP @ 0.2% and Propiconazole 25 EC @ 0.1% at 15 days interval.

Shell soft rot

Economic importance

Northern India has a high prevalence of shell soft rot. It has been seen on ripe, harvested, and stored fruits with an incidence of between 10% and 15%. Fruits affected by the disease

quickly decay and become unfit for ingestion because the entire fruit pulp becomes distasteful (Misra *et al.*, 2016).

Symptom

On fully developed fruits, lesions can be seen as rapidly growing, water-soaked, light brown rot spots with dark brown edges and a gelatinous texture (fig 2). Gelatinous layer removal is simple. The rotting intensifies swiftly on the harmed area. Inside, the rot spreads into the pulp where white to black fungal mycelium has taken up residence. The fruit that is impacted emits a foul odour that is often associated with deterioration or decay (Mishra *et al.*, 2016).

Fig. 2: Shell soft rot

Causal organism

The fungus *Syncephalastrum racemosum* Schr. is responsible for causing shell soft rot of Bael (Pitt and Hocking, 2009).

Epidemiology

The fungus grows best at temperatures between 17 and 40^{o} C and relative humidity levels between 75 and 80%. As a source of secondary infection, the fungus's spores, which are often airborne, soil-borne, and plant-borne, may also be spread by people.

Integrated management

- Sanitation of orchard.
- Pluck and discard affected fruits from the tree.
- Prevent unnecessary wounding during harvest and handling, and sorting out wounded/damaged fruits.
- Spray Carbendazim (0.1 per cent) before harvesting of fruits

Powdery Mildew

Economic importance

Powdry mildew on bael fruit is reported from West Bengal (Giri *et al*.,1989), from Kerala (Preetha *et al.*, 2007), Himanchal Pradesh (Gautam, 2015) and from Gujrat during winter season. The fungal infection can be seen on leaves, twigs and small fruits.

Disease symptom

Preetha and co-workers in 2007, observed the symptoms on the nursery plants. Throughout the months of November and December, disease signs are frequently seen. The young, green stems and leaves of the plants displayed significant chlorosis, which was followed by the development of white, powdery mycelium and persistent spots on the young leaves and

twigs (fig 3). Within 7–10 days of first appearing, these patches grow in size and quickly cover the entire lamina. Dusty grey or white patches on either side of the leaves are the first signs of powdery mildew infection on bael trees. Afterwards, the colony's color changes to a light grey or pink hue. Yellowing of the infected leaves is followed by tissue necrosis.

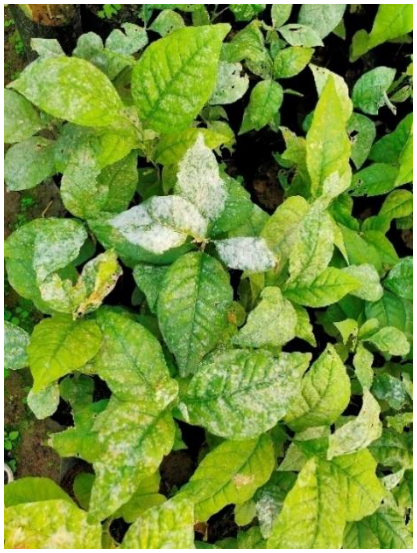

Fig. 3 : Powdery mildew

Causal Organism

The fungus *Oidium* species (Preetha *et al.,* 2007) is responsible for causing Powdery mildew in Bael.

Epidemiology

In leaf buds and other plant waste, fungus spores overwinter. The spores have been seen to spread to neighboring plants by wind, water, and insects. High humidity, moderate temperatures, and low soil moisture all contribute to the disease's favoring growth and development.

Integrated management

- Remove and dispose off diseased foliage from the plant and debris.
- In the initial stages of the disease a spray of wettable sulphur (0.2 per cent) is found to be useful. During warm weather and flowering period, the application of sulphur should be avoided.
- Sulphur containing fungicides are generally effective against powdery mildew that can be used at the first evidence of disease and repeat applications as necessary.
- A Spray with Carbendazim (0.1 per cent) or Karathane or Morestan (0.2 per cent) gives effective management of the disease.

Anthracnose

Economic importance

In just a few days, anthracnose may turn a fine, abundant harvest into rotten garbage. Lesions typically start to form after the fruit has been harvested, which has a negative impact on fruit quality and causes a significant loss.

Symptoms

Very few, erratic, light-brown to dark-brown dots on the underside of leaves are the symptoms. Where the fungus grows, on leaves closest to the soil's surface, the disease first

manifests itself. Disease Infected plants produce spherical, dark brown-black lesions that are water-soaked and surrounded by pink gelatinous masses of spores that appear to have a yellow halo. On occasion, shot holes can be noticed on leaves, especially in humid, warm weather. The symptoms first show up on fruits as small, depressed circular, dark brown spots that later turn black, quickly enlarge in size, and cover the majority of the pericarp surface (fig 4). Only fruit that has been damaged by other factors, such as sunburn, chemical burn, pest damage, bruising, or prolonged exposure to an insecticide, is typically affected by anthracnose.

Fig. 4: Anthracnose

Causal organism

The disease is caused by the fungus *Colletotrichum* sp.

Epidemiology

In infected, defoliated branch terminals, mature leaves, and other plant tissue, the disease persists between seasons. The pathogen reproduces even under poor circumstances by growing on the dead tissue of the host. When the spores germinate, they create a resting structure that enables them to wait until there is an injury before becoming active. The best conditions for the development of illness are cool (20°C) temperatures and extended periods of high relative humidity (>80%) with mists. On the field, the inoculum was dispersed by raindrops. Quiescent or latent infection results from field infection in developing fruit.

Integrated management

- Spray with Carbendazim (0.1 per cent) or copper oxychoride (0.3 per cent) or mancozeb (0.2 per cent) are helpful in controlling the disease.
- Infected fruits should be removed from orchards or culled from storage cartons since the disease spread fast in stored fruits.
- For post-harvest anthrcanose management, fruits should be dipped in hot water solution with 0.05 per cent Carbendazim at 50^{o}C for 30 minute.

Stalk end rot and fusarium root rot or wilt disease

Stalk end rot

Economic importance

Stalk end rot (Bhargava, 1977) is a serious disease of the bael. There are significant financial losses as a result of the impacted immature fruit dropping.

Disease symptoms

Softening of the rind and underlying pulp are the signs. Fruit does not lose its shape or shrink until it is squeezed. Afterwards, the fruit's rind turns dark brown, and the entire fruit

becomes pulpy and squishy (fig 5). When it rains or there is a strong breeze, fruit drops are more common. (Srivastava and Mehra, 2004).

Fig. 5: Stalk End Rot

Causal organism

Earlier the pathogen of disease had been reportedly identified as *Fusarium seritectrum* var. *najus* (Mitra, 1935) later on it was reported that *Fusarium solani* (Mart) Sacc causes stalk end rot of bael disease.

Root rot or wilt disease

Economic importance

Since 2007, the potential threat posed by bael tree decline brought on by root rot has increased in Haryana and Rajasthan. Also, the Chomu sector of the Jaipur district has had instances of wilting and dryness. (Maheshwari and Haldhar, 2018).

Disease symptom

Yellowing and necrotic spots on the leaves are the initial signs of root rot or wilt of bael. Root pruning, root degradation, root lesions of all sizes and colors—from reddish to brown to black—and root browning and softening of the root tips can all be seen. The disease's defining symptoms include vascular bundle blockage and brown discolouration. Occasionally, entire plants may perish from a lack of water and nutrients.

Causal organism

This disease is caused by the fungus *Fusarium* spp.

Epidemiology

The fungi *Fusarium spp.* are found in soil and associated with the roots of plants as deep in the ground up to 80 cm where its chlamydospores overwinter on plant tissue/seed or as mycelium in the soil. At the onset of favorable conditions spore produces asexual macro and microconidia which are dispersed through wind and rain as source of secondary infection.

Integrated management

- Carefully harvesting fruits with pedicle by using secateurs so as not to bruise them.
- Pruning and timely removal of infected plant parts.
- Moderately affected trees can sometimes be saved early on by pruning out the infected roots.
- Pre-harvest sprays of Carbendazim (0.1 per cent) at regular interval can effectively check the rotting during storage.
- In nursery, seed treatment with fungicides before sowing and later on drenching of Carbendazim (0.1 per cent) at regular intervals can check the disease.

Aspergillus fruit rot

Economic importance

A severe issue with bael fruits is internal rotting, which is primarily brought about by damage to the fruit during harvest, transit, and storage. Up to 100% of storage losses during the hot season were caused by disease infection.

Disease symptom

Large discoloured lesions on the fruit surface where it touches the container and bruises from the storage container's bottom or walls during storage or transportation are symptoms of the disease (fig 6).

Fig. 6: Aspergillus fruit rot

Causal organism

Dry rot is caused by *Aspergillus* sp; green rot by *A. nidulans* and *A. niger* causes black rot.

Epidemiology

The severity of *Aspergillus* spp. was discovered to be higher in a hot climate at 30 °C and 100% relative humidity. Time, temperature, and relative humidity all boosted the germination of spores and the development of diseases.

Integrated management

- Avoiding injuries to the rind during harvesting, transport and storage.
- Wrap the fruits with phenol paper/newspaper and internally cushion the containers with newspaper. Packing in baskets and plastic crates are better for transport purpose.
- Ensure proper ventilation during storage.
- Daily inspection of the storage fruits in container.
- Pre-harvest spray of Carbendazim (0.05 per cent) to control the disease.

***Myrothecium* leaf spot of bael**

Economic importance

Myrothecium leaf spot of bael (*Aegle marmelos* L.) was fist time reported by Kumar and Singh (2021) from Horticulture field situated at main campus of Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya (U.P.), India this disease was noted. Moreover, some districts of Uttar Pradesh have reported it. Both nurseries and orchards exhibit the disease symptoms. The disease is much more severe in nursery stock than it is in existing orchards.

Disease symptom

The initial symptoms were found on leaves as small, circular in shape which are yellowish brown in colour but later on these spots enlarges and cover the more area up to 40 mm in diameter and later become dark brown with grayish centre (fig 7). Chlorosis around the lesions may be seen and concentric rings appears in the middle of the spot are characteristic symptoms. Disease symptoms recorded first during rainy season (July- August), 15-20 days after symptom production concentric rings are produced. Characteristic symptoms of this disease are formation of shot hole due to shedding of necrotic tissues of the leaves. In severe condition number and size of lesions increases, black sporodochia with white and marginal mycelia tuffs bearing black in colour spores masses were observed in the older lesions

(A) **(B)**

(C) **(D)**

Fig. 7: (A) Myrothecium leaf spot of bael (B) Sporodochia produce in lower surface of the Myrothecium infected leaves (C) Myrothecium spot on mid rib of the leaves (D) Myrothecium present on midrib covered whole surface of the leaves and produces necrosis symptom

Causal organism

Myrothecium leaf spot of bael (*Aegle marmelos* L.) is caused by *Myrothecium roridum*

Epidemiology

High rainfall and high relative humidity encourage the transmission of disease. The fungus thrives in the 20°C to 35°C temperature range.

Integrated management

- Ploughing/Field Sanitation to rid fields of wild species is needed to destroy the pathogen load.

- Prune the plant to ensure proper supply of sunlight and air to overcome the risk of disease.
- At nursery condition, two sprays of Propiconazole @ (0.1%) at 15 days regular interval can check the disease in initial stage.

Die back

Economic importance

Dieback is the term used to describe the gradual death of twigs and branches, which often starts at the top of a plant and moves down. The new, green twigs initially begin to wither at the base, and then they eventually dry out, wilt, and fall off. The central U.P. suffers the most significant yield losses as a result.

Symptoms

Particularly in older trees, it is characterised by the drying back of twigs from the top down, followed by the drying of leaves, which creates the impression of fire scorch. On the branches, tip dieback disease manifests itself. Diseased trees' twigs and branches have interior discoloration and brown striations in the vascular tissue. Infested tree trunks initially begin to dry out slowly, but suddenly the limbs start to entirely dry up. On the tree, gummy material oozes may be seen hanging.

Causal organism

Dieback of bael is caused by the fungus *Botryodiplodia theobromae.*

Epidemiology

The pathogen can grow and develop more easily in the temperature range of 25 to 30º C and relative humidity levels of 75 to 80%. The fungus overwinters on the outside of damaged twigs, tree bark, and dropped fruits as pycnidia.

Integrated management

- While multiplying the planting materials scion wood/bud selected for propagation should be free from infection.
- Pruning (5-7cm below the infection site) followed by spraying of Bordeaux mixture (5: 5: 50) or copper oxychloride (0.3 per cent) gives effective disease management.
- If thicker branches are cut, pasting of cow dung may be done at cut ends.

Fusarium leaf spot and die back of bael

Economic importance

Fusarium leaf spot of bael (*Aegle marmelos* L.) was fist time reported by Kumar, *et al.* (2021) from Horticulture field situated at main campus of Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya (U.P.), India this disease was noted. Moreover, some districts of Uttar Pradesh have reported it. Both nurseries and orchards exhibit the disease symptoms. The disease is much more severe in nursery stock than it is in existing orchards.

Symptom

Much vegetative loss have been recorded in the nursery with this disease Die-back of branches caused by *Fusarium pallidoroserum* are seen, the disease appeared during post rainy season (September) as irregular pustules, which was brown in color, increases very fast cover the most of the leaf area (fig 8). After severe infection, affected leave become dry and fall off the disease progresses downward causing dieback symptoms but root remain healthy.

Fig. 8: Fusarium symptom produce in a leaf at initial stage

Causal organism

Fusarium leaf spot of bael is caused by *Fusarium pallidoroserum*

Epidemiology

High rainfall and high relative humidity encourage the transmission of disease. The fungus thrives in the 20°C to 30°C temperature range.

Integrated management

- Ploughing/Field Sanitation to rid fields of wild species is needed to destroy the pathogen load.
- Prune the plant to ensure proper supply of sunlight and air to overcome the risk of disease.

Sooty mould

Economic importance and symptom:

The disease is common in the orchards where mealy bug, scale insect and hopper are not controlled efficiently. The disease in the field is recognize by the presence of a black velvety coating, i.e., sooty mould on the leaf surface (fig 9). In severe cases the trees turn completely black due to the presence of mould over the entire surface of twigs and leaves. The severity of infection depends on the honey dew secretion by the above said insects. Honey dew secretions from insects sticks to the leaf surface and provide necessary medium for fungal growth.

Fig. 9: Sooty mould of Bael

Causal organism

Fungi that most commonly cause sooty mold on garden and landscape plants are in the genera *Capnodium*, *Fumago*, and *Scorias*.

Epidemiology

High rainfall and high relative humidity encourage the transmission of disease. The fungus thrives in the 20°C to 30°C temperature range.

Integrated management

- Spraying the leaves with insecticidal soap can help soften the sooty coating. Spray late in the day so the soap remains moist for as long as possible. If you can spray a few hours before a heavy rain is forecast the rain will be better able to remove the sooty mold.
- Neem oil is a great choice for getting rid of sooty mold organically. It works great to eradicate both the fungus and the pests that cause it.

Bacterial shot hole and fruit canker

Economic importance

Pune, Maharashtra has seen and reported the first cases of bacterial shot holes and bael fruit cankers (Patel *et al.*, 1953). It was later noted in Udaipur, Rajasthan. Serious infections result in defoliation, imperfect fruit, early fruit drop, twig death, and general tree weakness.

Symptom

Disease symptoms are visible on all plant parts, including leaves, twigs, thorns, and fruits. On leaves, symptoms take the form of erratic, water-soaked patches encircled by a yellow hole. Subsequently, these spots combine, causing the infected tissue to dry out and eventually turn necrotic. A round to slightly irregular perforation or shot hole is left on the lamina as the dead tissue of the leaf spot begins to fall off. These necrotic dead leaf tissues might occasionally remain attached to healthy tissue in separate form rather than falling out (fig 10). Little to large lesions, sometimes in the shape of irregular tissue ruptures with crater-like appearances in the middle, are frequently observed on twigs and thorns. Moreover, the bacteria cause the usual circular, elevated water-soaked areas on fruits. These spots also exhibit crater like depressions in their centers surrounded by irregular oily, raised margin. Later on, they became corky and regular in shape. Chocolate brown bacterial excludes is commonly found associated with all the infected plant parts. The diagnostic characteristic of the disease is presence of shining beads and scale of bacterial ooze on both sides of the leaf spot.

Fig. 10: Bacterial Shot Hole

Causal organism

Bacterial shot hole and fruit canker is caused by the bacterium *Xanthomonas compestris* pv. *bilvae* (Chakraborty *et al.*, 1984). This isolate was previously reported by Patel *et al.* (1953)

Epidemiology

The disease quickly multiplies as lesions in leaves, stems, and fruit and thrives in warm, humid climate. Bacterial exudates from lesions are spread by wind-driven rain sprinkles inside trees or to nearby trees, as well as by windblown showers to medium-long ranges. Secondary infections may develop in wounds from pruning and bug bites.

Integrated management

- Using canker-free nursery stock.
- Keep orchard free of wild species that harbor the pathogen.
- Remove severely affected plants and prune off all the infected twigs before monsoon and burn them.
- Removal of affected twigs followed by spray with Bordeaux mixture has been recommended to manage it. It can be easily managed by 2 to 3 spray of streptocycline (500 ppm) at 15-day interval during pre- and post-monsoon (Mohapatra and Sahoo, 2008; Hiwale, 2015).
- Spraying once or twice with streptomycin sulphate 250 ppm or Bordeaux mixture 1 per cent at 12-15 days interval effectively controls the disease.

Gummosis in bael plant

Economic importance and symptom

Like other rutaceous plants of citrus family, oozing of gum is common in bael orchards. The disease is characterized by oozing out of pale or amber colored gummy substance initially from bark of lower portion of trunk and later on other branches also. The gum oozing takes place from vertical splits in bark which turns dark from outside at the point of oozing but from inside other surrounding bark tissues turn light brown or white and very soft and sticky when touched with fingers. Because of gummosis, the vigor of tree is severely affected and in severely affected twigs defoliation and dieback occurs (fig 11).

Fig. 11: Gummosis in bael plant

Integrated management

- To manage the disease it is suggested to scrap off the infected portion of bark with the help of a sharp knife, which should be followed by application of Bordeaux paste.
- Spray with Copper fungicides (Bordeaux mixture 1% or copper oxychloride (0.3%) are also suggested to be applied at monthly interval during and after rainy season.
- Removal of highly infected twigs and incorporation of *Trichoderma viridae* propagules in the soil of rhizosphere of bael were found helpful to control the disease

Loranthus (*Dendrophthoe falcata*)

Economic importance and symptom

Loranthus is a genus of parasitic plants that grow on the branches of woody trees. It belongs to the family Loranthaceae, the showy mistletoe family. It is semi-parasitic plant because of growing on various host trees and shrubs and absorbing mineral nutrition and water from respective host (fig 12).

Fig. 12: Bael plant infected with Loranthus

Causal organism

Loranthus is caused by *Dendrophthoe falcata*

Integrated management

- To manage the disease it is suggested to scrap off the infected portion of bark with the help of a sharp knife, which should be followed by application of Bordeaux paste.
- Keep orchard free of wild species that harbor the pathogen.
- Remove severely affected plants and prune off all the infected twigs before monsoon and burn them.

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GREEN CHEMISTRY FOR SUSTAINABLE AGRICULTURE AND SOIL HEALTH

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Introduction:

Green chemistry entails the formation of goods and procedures that lessen or do away with the usage and production of chemicals that are harmful to both the environment and people. The creation of green catalysts and the use of non-toxic reagents are central to the principles of green chemistry. It places a focus on the use of processes with increased atom efficiency, solvent-free or ecologically friendly recyclable solvent systems, and renewable resource usage. Green chemistry has become the new paradigm in agriculture today (Becer, 2021). Green chemistry and sustainable agriculture are related and revolutionary topics. In recent years, the utilization of renewable biomass resources has increased in agriculture for sustainable production in order to provide bio-based food items with low inputs, zero waste, significant social values, and minimal environmental effect.

Principles of green chemistry

The principles of green chemistry involve the development of green catalysts and use of non-toxic reagents (Bhandari & Kasana, 2018). Avoiding garbage altogether is preferable to treating or cleaning up waste after it has already created. Maximizing the assimilation of all components utilized during the process into the finished product should be a goal of synthetic approaches. When possible, synthetic procedures should be created to use and produce materials with low or no toxicity to the environment and human health. When feasible, it is best to avoid using auxiliary substances like solvents and separation agents, or to use them sparingly. Energy needs should be limited because of their negative effects on the environment and the economy. Conducting synthetic procedures at room temperature and pressure is recommended. When it is technically and economically feasible, a raw material or feedstock should be renewable rather than finite. Derivatization should be avoided whenever possible, including unnecessary blocking group protection/deprotection and temporary change of physical/chemical processes. Stoichiometric reagents are inferior to (as selective as possible) catalytic reagents. Chemical goods should be made with the intention of degrading into harmless byproducts rather than remaining in the environment after serving their purpose. It is necessary to improve analytical approaches to enable real-time in-process monitoring and control before the creation of hazardous compounds. To reduce the possibility of chemical accidents, such as releases, explosions, and fires, substances and the form of a material should be employed in chemical processes. Ionic liquids have recently replaced organic solvents with significant volatility and intrinsic toxicity (ILs). Ionic liquids are organic salts that typically melt below 1000 degrees Celsius, have great thermal stability, are practically non-volatile under normal circumstances, and dissolve non-polar and polar organic and inorganic substances. Ionic liquids are referred to be "designer solvents" for this reason.

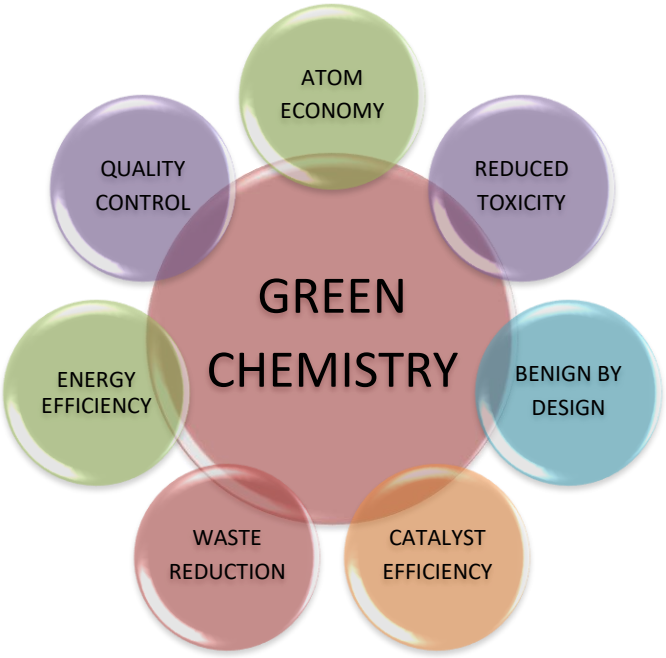

Fig. 1: Principles of Green Chemistry

Green chemistry for sustainable agriculture

Synthetic pesticide production has increased over the past few years, and contemporary farming practices have significantly increased greenhouse gas emissions. Animals and human health are negatively impacted by contamination in agricultural fields caused by incorrect pesticide usage, either indirectly or directly exposed. Pesticides are any chemicals that are used to prevent or kill pests, however due to the food chain, biomagnification, and bioaccumulation, these chemicals have negative impacts on many aspects of human and animal existence. In order to reduce these negative impacts, organic farming practices should be used more frequently in place of synthetic pesticides, which also contaminate groundwater, cause rivers and lakes to become eutrophic, and allow dangerous chemicals to enter living things.

The sustainable use of fertilizers and pesticides in agriculture is the primary sector that calls for green chemistry solutions in the agrochemical industry. Intensive production techniques based on non-sustainable green revolution technologies still underpin modern agricultural operations. The agrichemical sector will need a second "green revolution" based on green chemistry concepts to continue producing goods relevant to agricultural operations as consumer focus switches to creating a sustainable and secure food supply. In order to replace carbon-containing sources and lessen global warming emissions, renewable energy sources such as solar, wind, hydroelectric, biomass, biorefineries, geothermal, and ocean energy are key resources for future sustainable development. Due to Green Chemistry, Agriculture will evolve to sustainable development.

Green chemistry for soil health

A major factor in determining sustainable agriculture is soil quality, which is influenced by a variety of physical and chemical characteristics while taking the impact of the climate and outside inputs into account. The design and management of a healthy soil ecosystem includes, but is not limited to, the biological control of biotic and abiotic elements, the regulation of water and air supply, and the maintenance of a healthy and sustainable ecosystem. An emerging theory

holds that using carbon sequestration will help to promote a healthy soil environment. In addition to improving soil quality and encouraging plant growth, it can also make more water available to plants, lessen the negative effects of contaminants, and boost soil and plant resistance to environmental changes. Soil is "the most complex biomaterial on the planet" (Young and Crawford, 2004), comprising heterogeneous bio-physico-chemical reaction pathways that are critical for human, ecological, and planetary health (Duvlworth *et al.,* 2022).

Establishing preventive actions to stop additional soil degradation is also crucial. In order to implement soil-friendly practices on a worldwide basis, a thorough action plan is required. Stakeholders from various socioeconomic sectors, including farmers, researchers, trade associations, businesspeople, and the general public, must be involved in this. A significant step toward preserving soil health is the development of qualitative and quantitative indicators that can be used to continuously monitor soil health and aid in the creation or modification of an intervention plan to improve the nutrient content, soil microbiota, access to water, and air.

The probable environmental degradation that had led to crops having less access to nutrients and being more vulnerable to infections and environmental pressures. Additionally essential to building sustainable farming methods is the function of soil microorganisms. Establishing a healthy soil microbial population that may aid in increasing plant development and yield while also aiding in the maintenance of healthy soil can be accomplished by treating the soil ecosystem as a diverse food web and controlling the proliferation of helpful microorganisms and plant diseases. Because soil microbiota is frequently employed as a measure of soil health, choosing growth promoters, plant protection products, and microbial inoculants that are biologically derived rather than chemically derived has less of an impact on the soil ecosystem.

Conclusion:

Thus, sustainable agriculture, soil health and green chemistry are revolutionary fields and intertwined. Green chemists needs that the farmers used green technology for sustainable agriculture. Also, Farmers can choose agricultural inputs that don't harm the soil while replenishing it via tax incentives, financial aid, and legislation that promote the adoption and long-term application of soil-friendly techniques.

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SIGNIFICANCE AND APPLICATION OF BIOFERTILIZERS TOWARDS SUSTAINABLE AGRICULTURE: A REVIEW

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Abstract:

Agriculture and agricultural resources have been the solitary component in human livelihood. Majority of the population in globe rely on agriculture for food, feed and few other things like fiber, gums, wood and products of medicinal importance for replenishing healthful lifestyle. The expanding human population drifts represent the need of occurrence of worthy methods to reach the craving requisite of increasing population. The concepts of Sustainable agriculture direct to grow crops to their greatest limitation and making sure the environmental quality along with the natural resource on which agriculture depends. Present world population is 7.8 billion as of Feb 2023 as per the most recent United Nations estimate. About one billion population have scarcity of enough and healthy food. It is a big difficulty to feed the expanding population. Nevertheless, country has become self-reliant in satisfying bulk amount of food supply with the extensive utilization of agrochemicals, simultaneously harms the environment to a greater extent inducing unfavorable effects on living things. Taking into consideration all the deleterious effects of the continued application of agrochemicals and pesticides, organic farming has emerged as a powerful alternative for safe food supply. Biofertilizers and biopesticides act as ecofriendly substitute for harmful agro chemicals and acts as one of the best practices for nutrient regulation system.

Keywords: Sustainable**,** agriculture, Biofertilizers, Organic farming, agrochemicals

Introduction:

Organic farming plays a major role in feeding the increasing population of humans which has also resulted in growing dependency onagrochemicals. By contaminating air, water and soil indiscriminate use of agrochemicals pose a great threat to nature. Since plants cannot take up these toxic chemicals, they begin to accumulate in the ground water and some of which may also result in eutrophication of water bodies. Such chemicals have a detrimental effect on soil by depleting its water holding capacity, soil fertility, increase salinity and soil nutrient disparity. These chemicals adversely affect soil in terms of depletion of water holding capacity, soil fertility, increased salinity, and disparity in soil nutrients (Savci, 2012). The results of use of excess chemical addition have made crops more sensitive to diseases and reduced fertility of the soil (Tilman *et al.*, 2002; Aktar *et al.*, 2009). Organic farming is one of the best practices that ensures food security as well as Come up with soil biodiversity (Megali *et al.*, 2013). The additional benefits of organic farming include longer shelf life and no harm to the environment (Sahoo *et al.*, 2014). Organic farming is mainly dependent on soil's normal, natural microflora including all types of beneficial bacteria which are known as plant growth promoting rhizobacteria (PGPR). Biofertilizers is one such component of organic farming that maintains the soil environment enriched with all kinds of micro- and macro-nutrients through nitrogen fixation, phosphate and potassium solubilization or mineralization, release of plant growth

regulating substances, production of antibiotics and biodegradation of organic matter in the soil (Sinha *et al.,* 2014). In general, 60% to 90% of the total applied fertilizer is lost and the remaining 10% to 40% is taken up by plants. Biofertilizers improve soil fertility by fixing the atmospheric nitrogen and solubilizing insoluble phosphates and produce plant growth-promoting substances in the soil (Mazid and Khan, 2015). These are preparations are efficient living microorganisms that help crop plants uptake of nutrients by their interactions in the rhizosphere when applied through seed or soil. They enhance certain microbial processes in the soil which increase the range of availability of nutrients in a form easily taken up by plants (Raja, 2013).

Nitrogen fixing biofertilizer (NBF):

Nitrogen (N) is one of the most essential nutrients for growth and yield of crops. Although it is present in the atmosphere at 78 percent, it is unavailable for plant use. Nitrogen must be converted to utilizable form that is ammonia by plants through the biological Nitrogen fixation process (BNF) (Tairo and Ndakidemi, 2013). Nitrogen fixers are classified as symbiotic and non-symbiotic. The members of family Rhizobiaceae that establish symbiotic relationships with leguminous plants are types of symbiotic organisms (Ahmad and Khan, 2012). On the other hand, free-living and endophytic forms of microorganisms such as *Cyanobacteria*, *Azospirillum*, *Azotobacter* etc. are types of non-symbiotic microorganisms (Bhattacharyya and Jha, 2012).

Phosphate solubilizers:

The second important mineral nutrient required in considerable quantities by plants is Phosphorus (P) (Kaiser *et al.,* 2016). There is a large amount of Phosphorous present in the soil, but it is normally fixed at the point of application and thus, becomes insoluble and is not available to plants. The phosphorus that is insoluble is present as inorganic substances such as apatite or as one of many organic forms like inositol phosphate (soil phytate), phosphomonoesters, and phosphotriesters (Mahdi *et al.,* 2012). Again, after it is added to the field, much of the soluble inorganic phosphorus used as chemical fertilizers becomes immobilized making it unavailable to plants and is therefore wasted (Feng *et al.,* 2004; Angus, 2012). The application of Phosphorus solubilizing biofertilizer (PSB) is a remedy and make Phosphorous more bioavailable and bio accessible to promote growth and development of plants. The solubilization effect is achieved by the phospho-bacterin synthesis of organic acids that decreases the soil pH leading to the breakdown of phosphate compounds and the release of ample Phosphorous for plant use (Arun, 2007; Glick, 2012; Mahanty *et al.,* 2017). The examples of bacteria with the capacity to solubilize and mobilize Phosphorous are *Pseudomonas, Bacillus, Rhizobium, Burkholderia, Achromobacter, Agrobacterium, Micrococcus, Aerobacter, Flavobacterium, Aspergillus,* and *Erwinia* (Mahdi *et al.,* 2010; Mahanty *et al.,* 2017).

Iron sequestration:

Iron (Fe), an essential micronutrient, important in chlorophyll formation, photosynthesis, respiration and various enzymatic reactions. Iron is absorbed by plants either Fe2+ (ferrous cation) or Fe3+ (ferric cation) forms. All living beings need iron. Under anaerobic conditions, it forms insoluble hydroxides and oxyhydroxides and predominantly occurs as Fe3+ (Mahdi *et al.,* 2010). Most of the Fe is not available to both bacteria and plants. In general, bacteria obtain Fe by producing iron chelators known as siderophores. (Rajkumar *et al.,* 2010). Majority of the siderophores both intracellular and extracellular siderophores have low molecular weights, soluble in water and high affinity for complex Fe (Mahdi *et al.,* 2010; Hider and Kong, 2010; Rajkumar *et al.,* 2010). Fe3+ is converted into Fe2+ in the bacterial membrane by both Gram-

positive and Gram-negative bacteria. Siderophores then releases Fe2+ molecules into the cell through a gating channel that connects the inner and outer membranes (Mahanty *et al.,* 2017).

Plant growth promoting rhizobacteria (PGPR):

Kloepper first defined Plant Growth Promoting Rhizobacteria (PGPR) as those soil bacteria that colonize plant roots after seed inoculation and enhance growth of plants. These PGPR include *Actinoplanes, Agrobacterium, Alcaligenes, Amorphosporangium, Arthrobacter, Azotobacter, Bacillus, Cellulomonas, Enterobacter, Erwinia, Flavobacterium, Pseudomonas, Rhizobium, Bradyrhizobium, Streptomyces and Xanthomonas.* The inoculation of these microorganisms increases the growth of plants via various mechanisms including plant disease suppression (bioprotectants), improved acquisition of nutrients (biofertilizers) and production of phytohormones (bio stimulants). These PGPR strains produce growth promoting hormones like Indol acetic acid, gibberellins and cytokinins thereby acting as bio stimulants and facilitate plant growth by increasing the surface of absorption for the uptake of nutrients and water (Adesemoye *et al.,* 2009; Gholami *et al.,* 2009).

Biofertilizer in remediation of pesticides:

For control of plant diseases, insecticides, herbicides, fungicides and nematicides are employed on large scale. A noteworthy fact of modern agriculture is the use of pesticides for cost-effective pest management. Excessive and persistent use of pesticides is harmful to the ecosystem and possesses a potential burden to both plants as well as mankind due to bioaccumulation (Aktar *et al.,* 2009; Kumar and Puri 2012). However, due to their eco-friendliness, cost-effectiveness and considerable removal from environment, bioremediation methodologies have gained notable attention (Nawaz *et al.,* 2011). Presently, research on pesticide degrading strains of bacteria is emerging as a promising way to neutralize the adverse effect of pesticides. The role of PGPR in the bioremediation of pesticides was therefore subjected to a series of investigations. The ability to reduce pesticide toxicity has been reported from micro-organisms such as *Azospirillum, Azotobacter, Bacillus, Enterobacter, Gordonia, Klebsiella, Paenibacillus, Pseudomonas, Serratia,* etc. (Shaheen and Sundari 2013). In addition to these strains, Actinomycetes also have a huge potential for pesticide biotransformation and biodegradation. The primary mechanism used by micro-organisms for the degradation of pesticides is the enzymatic degradation. Based on numerous reports, it can be concluded that PGPR holds a promising approach to reduce the contamination of pesticides in soil in a sustainable manner.

Effect of biofertilizers on sustainable agroecosystem:

The mechanism involved in the interaction of biofertilizers with plant and resident microbial community is still a matter of concern in people. The presence of indigenous microflora in rhizosphere is one of the important factors deciding the biofertilizer effectiveness in the natural environment. The survival and plant growth promoting properties of the biofertilizers are influenced by the presence of the highly competitive community with diverse species in rhizosphere (Hibbing *et al.,* 2010). In addition, bacterization of seeds and seedlings or soil amendments can alter the structure of indigenous microflora that are important to consider regarding the safety of bacterial introduction in environment (Dey *et al.,* 2012). It is very important to estimate the non-target effects of biofertilizers on the population of resident microflora and on the ecosystem prior to their release in the environment. It has been reported that the extent of impact of biofertilizer introduction into the residential community depends on

many factors viz. mode of application of biofertilizers, soil characteristics, different environmental conditions etc. (Dey *et al.,* 2012).

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INFLUENCE OF BIOCHAR ON GREEN HOUSE GAS EMISSION FROM SOIL

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Abstract:

Worldwide, there is an increasing interest in using Biochar production and application is effective counter measure to mitigate climate change through increasing soil carbon storage by reducing direct GHGs emission from agricultural soils. Biochar, a co-product of a controlled pyrolysis process, Use of biochar in agricultural systems is a viable option that can enhance natural rates of carbon sequestration in the soil, reduce farm waste and improve the soil quality

Introduction:

Climate change has an adverse impact on agricultural production and environmental quality. Rising emission of GHGs from agricultural lands, due to anthropogenic activities like burning fossil fuels and land-use changes (deforestation), are major contributors to global warming. Agricultural activities collectively contribute between 5 and 6 pg carbon dioxide (CO_2) equivalents (approximately 10% – 12%) of the total global GHG emissions. This is primarily due to nitrous oxide (N_2O) emissions from mineral and organic fertilizers applied in soils. Additionally, burning of fossil fuels and deforestation has increased as a consequence of global surface temperature up to 0.8^0C during the 20th century, which likely will increase to 1.4 ^{0}C–5.8 ^{0}C in the 21st century. This increase in global temperature is attributed to the greenhouse effect caused by CO_2 as the major source of GHG. However, the global warming potential (GWP) of N_2O is 298 times greater than CO_2 so the former can trap more heat in the atmosphere compared to the latter. Among all the sectors that are responsible for GHG production, agriculture contributes a significantly large share (Mandal *et al*., 2016).

Table 1: Concentration of different greenhouse gases in atmosphere

Compound	Formula	Concentration in atmosphere
Water vapourand clouds	H_2O	10–50,000 (ppm)
Carbon dioxide	CO_2	~400 (ppm)
Methane	CH_4	~1.8 (ppm)
Ozone	O_3	2–8 (ppm)
Nitrous oxide	N_20	326 (ppb)

Global Emissions by Economic Sector

Global greenhouse gas emissions can also be broken down by the economic activities that lead to their production (IPCC)

Electricity and Heat Production: The burning of coal, natural gas, and oil for electricity and heat is the largest single source of global greenhouse gas emissions.

- **Industry:** Fossil fuels used for electricity on-site at facilities account for the majority of greenhouse gas emissions from industry. This sector also contains emissions from waste management operations as well as emissions from non-energy-related chemical, metallurgical, and mineral transformation processes.
- **Agriculture, Forestry, and Other Land Use**: Agriculture (the cultivation of crops and animals) and deforestation account for the majority of the greenhouse gas emissions from this industry. This number excludes the carbon dioxide (CO_2) that ecosystems remove from the atmosphere by storing it in biomass, decomposing organic matter, and soils, which makes up about 20% of this sector's emissions.
- **Transportation:** Fossil fuels used in land, air, sea, and train transportation account for the majority of this industry's greenhouse gas emissions. The majority of petroleum-based fuels, such as gasoline and diesel, account for 95% of the world's transit energy.
- **Buildings:** In this industry, greenhouse gas emissions are produced on-site and come from burning fuels for cooking or heating structures.
- **Other Energy:** This category of greenhouse gas emissions includes all emissions from energy-related activities such as fuel extraction, refinement, processing, and transportation that are not immediately related to the production of electricity or heat.

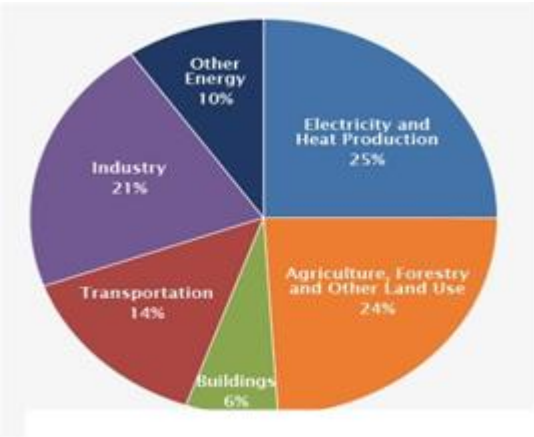


Fig 1: Global Emissions by Economic Sector

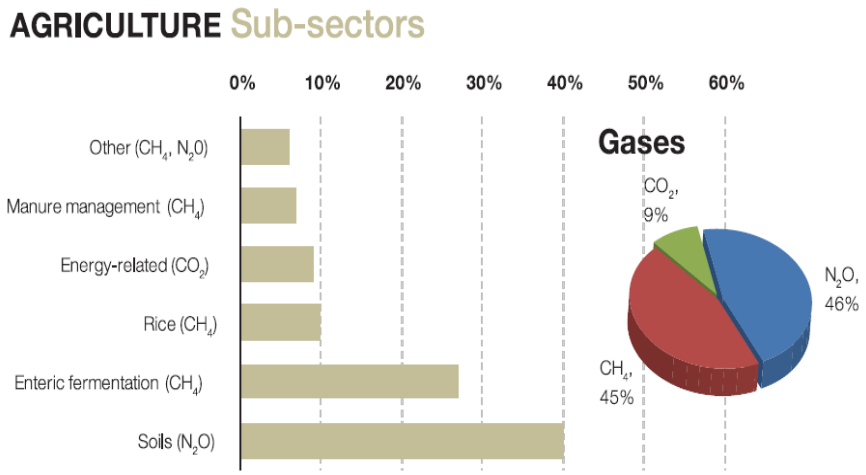


Fig. 2: Greenhouse gas emissions from agriculture

Greenhouse gas emissions - main sources

- **Carbon Dioxide:** There are both natural and human sources of carbon dioxide (CO_2) emissions. Natural sources include decomposition, ocean release, respiration and volcanoes. Human sources come from activities like cement production, deforestation and the burning of fossil fuels. Other important natural CO_2 sources include plant and animal respiration as well as soil respiration and decomposition.
- **Methane:** While there are both natural and human sources of methane (CH_4), humans create the majority of total emissions. The main natural sources include wetlands, termites and the oceans. Important human sources come from landfills, livestock farming, as well as the production, transportation and use of fossil fuels.
- **Nitrous Oxide:** Nitrous oxide (N_2O) emissions are also produced by both natural and human sources. The main natural sources are soils under natural vegetation and the oceans. Important human sources come from agriculture, fossil fuel combustion and industrial processes.

Due to ineffective crop residue management methods, there are enormous amounts of extra and unused crop and agroforestry residues in India, which is a concern. The current availability of biomass in India (2010-2011) is estimated at about 500 million tons/year. Studies sponsored by the Ministry of new and Renewable Energy (MNRE), the government of India has calculated that there are 120–150 million tonnes of excess biomass available annually. About 93 million tonnes of agricultural residues are burned annually out of this total. Crop residue production is at its greatest level in Uttar Pradesh (60 million t) followed by Punjab (51 million t) and Maharashtra (46 million t). Maharashtra contributes maximum to the generation of residues of pulses (3 million t) while residues from fiber crop is dominant in Andhra Pradesh (14 million t). Gujarat and Rajasthan cause about 6 million tons each of residues from oilseed crops. Application of organic amendments in agricultural soils can alter GHG emissions while improving physical, chemical, and biological properties of soil. To mitigate GHG emission, carbonized biomass such as biochar, graphite, charcoal, and activated carbon (AC) can be used.

Table 2: Generation and crop leftovers in excess in different states of India

State	**Crop leftovers generation (MNRE, 2009)**	**Crop leftovers surplus (MNRE, 2009)**	**Crop leftovers burned (IPCC coeff.)**
	Million t/ year		
Uttar Pradesh	59.97	13.53	0.58
Punjab	50.75	24.83	8.94
Maharashtra	46.45	14.67	6.27
Andhra Pradesh	43.89	6.96	5.73
West Bengal	35.93	4.29	10.82
Karnataka	33.94	8.98	2.85
India	**501.76**	**140.84**	**83.66**

IARI (2012)

Biochar

Biochar is a fine-grained, carbon-rich, porous product remaining after plant biomass has been subjected to thermo-chemical conversion process (pyrolysis) at low temperatures (~350–600°C) in an oxygen poor atmosphere (Amonette and Joseph, 2009).

Biochar production

Biochar is a high-carbon, fine-grained residue that today is produced through modern pyrolysis processes; It occurs when biomass directly thermally decomposes without the presence of oxygen. (Preventing combustion), which produces a mixture of solids (the biochar proper), liquid (bio-oil) and gas (syngas) products. The specific yield from the pyrolysis is dependent on process condition, such as temperature, and can be optimized to produce either energy or biochar.

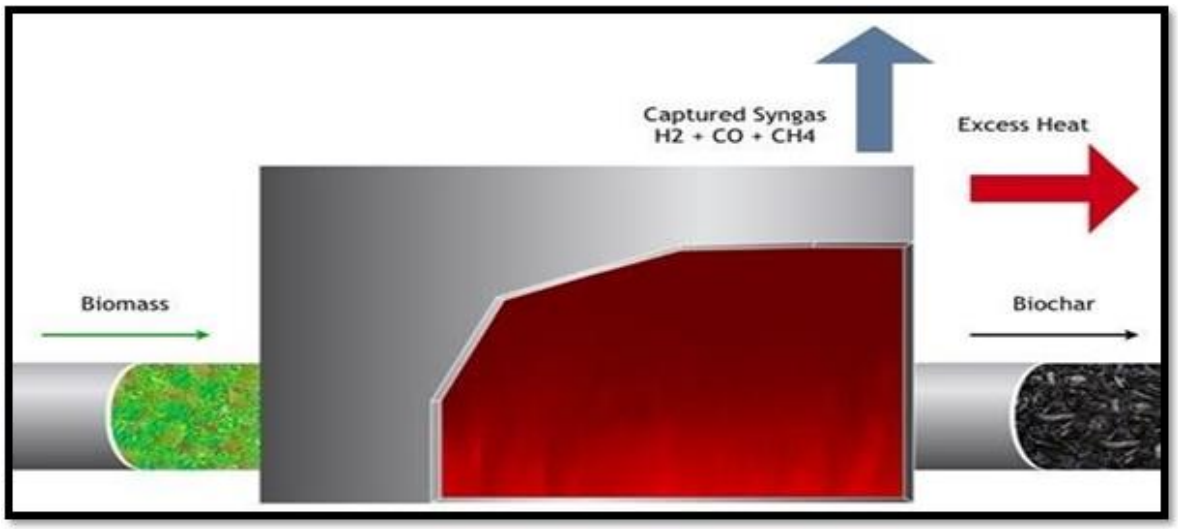


Fig 3: Biochar production

Methods of biochar production

Heap method

Charcoal making is one of the traditional practices to generate income in various parts of India. In traditional method, a heap of pyramid like structure (earth kiln) is prepared by keeping wood logs and roots of plants for making charcoal. To allow the combustion products to escape, vents are opened starting from the top and working downwards. When smoke production is stopped, the cooling process is started by covering stack with a layer of moist earth. The cooling process takes several days before the earth is removed and the biochar produced is separated from the surrounding carbonized portions. Earth-mound kilns equipped with a chimney are most advanced among earth kilns. The ability to alter the chimney diameter according to the oxygen demand, and precise control of the draft of the chimney, which is dependent on height, results in better control of the pyrolysis process

Drum method

Kilns that are built in place, typically are constructed from soil or other local materials, are located close to biomass resources and are small. They are economically viable if the cost of construction and transportation of biochar is lower than the cost of transporting and processing of biomass. In a modified method, char production is done by pyrolysis kiln. A cylindrical metal oil drum (200 L capacity) with both sides intact was procured from local market and was modified for use as charring kiln. A square shaped hole of 16 cm x 16 cm was made on the

center of top side of the drum for loading the crop residues. On the opposite side (bottom) of the oil drum, a total of 36 holes each measuring 4 cm^2 were made in concentric circles with a 5 cm2 hole at the center covering 20% of the total surface area of the bottom portion of the oil drum to facilitate uniform circulation of air from below.

Pyrolyser

The biomass pyrolysis plant is mainly used for large scale production for sale.

Difference between Carbon and Biochar cycles

- ✓ **Carbon cycle:** Green plants remove CO_2 from the atmosphere via photosynthesis and converts it into biomass. Virtually all of that carbon is returned into the atmosphere when the plant die and decay, or immediately if the biomass is burned as a renewable substitute for fossil fuels.
- ✓ **Biochar cycles:** Green plants reduce atmospheric carbon dioxide levels via photosynthesis and converts it into biomass. Up to half of that carbon is removed and sequestered as biochar, while the other half is converted to renewable energy co-products before being return.

Characteristics of biochar

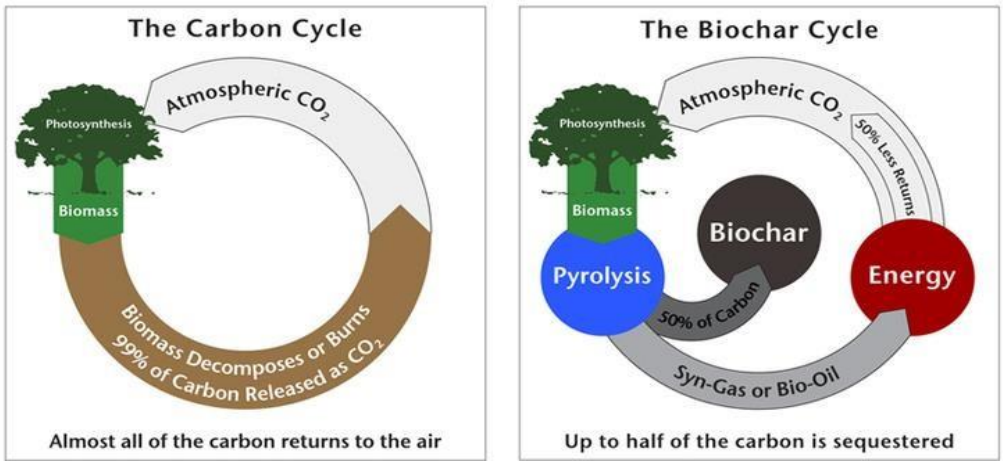
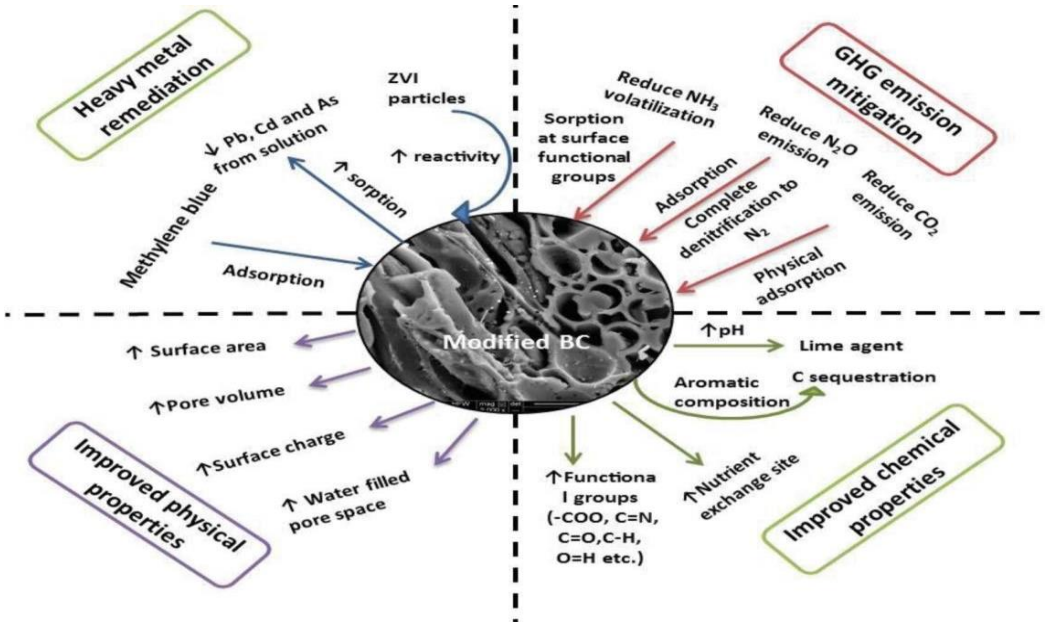


Fig 4: Difference between Carbon and Biochar cycles

Climate Smart Benefits of Biochar

- ❖ **Soil Fertility:** Biochar can improve soil fertility, stimulating plant growth, which then consumes more CO_2 in a positive feedback effect.
- ❖ **Reduced fertilizer inputs:** Biochar can reduce the need for chemical fertilizers, resulting in reduced emissions of greenhouse gases from fertilizer manufacture.
- ❖ **Reduced N2O and CH4 emissions:** Biochar can reduce emissions of nitrous oxide (N_2O) and methane (CH_4) two potent greenhouse gases from agricultural soils.
- ❖ **Improved soil microbial activity:** Biochar can boost soil microbial life, which increases soil carbon storage.
- ❖ **Reduced emissions from feedstock's:** Converting agricultural and forestry waste into biochar can avoid CO_2 and CH_4 emissions otherwise generated by the natural decomposition or burning of the waste.
- ❖ **Energy generation:** The heat energy and also the bio-oils and synthesis gases - generated during biochar production can be used to displace carbon positive energy from fossil fuels.

Characteristics of biochar



- Biochar is produced through pyrolysis. It is carbon-rich and is both highly chemically and biologically stable, with a large proportion of aromatic C.
- Generally speaking, biochar has a high cation exchange capacity, a large specific surface area, and a high porosity. The majority of the time, biochar has an elemental makeup that is more than 60% C, N, H, along with smaller amounts of K, Ca, Na, and Mg.
- The types of pyrolysis temperature and feedstock material have the most effects on the properties of biochar. In general, compared to biochar formed from feedstocks with greater amounts of lignocelluloses, such as wood, biochar derived from manures, seaweeds, and agricultural wastes has a higher pH, a higher concentration of nutrients, and less stable C.
- Additionally, compared to biochar made from grass biomass, biochar made from wood biomass often has a larger surface area.
- According to research on the effects of pyrolysis temperature, biochar formed at relatively high temperatures (600–700 °C) contains higher proportions of aromatic C and lower proportions of H and O functional groups, which results in a decreased cation exchange capacity.
- The CEC of biochar produced at relatively low (300–400 °C) pyrolysis temperatures are higher due to the increased fraction of C–O and C–H functional groups.

Measurement emissions of greenhouse gases from Crop Fields (Srinivasarao *et al*., 2013)

Material: Rigid plastic or acrylic sheets.

- Chamber is placed on the aluminium channel inserted 10 cm inside the soil and the channels filled with water to make the system air-tight.
- One silicon septum is fitted at the top of chamber to collect gas samples.
- Gas samples are collected from headspace immediately after sealing (Not exceeding 2hrs)
- The air inside the chamber should be homogenized with the help of fan.
- Gas samples can be drawn with 20-50 ml syringe
- Analysis: Gas chromatograph (GC)

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HERBICIDE RESISTANCE AND PHOSPHATASE ENZYME ACTIVITY OF PHOSPHATE SOLUBILIZING BACTERIA IN RHIZOSPHERE SOIL

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Abstract:

Phosphate solubilizing microbes plays an important role in plant nutrition through increase in phosphate uptake by plants and used as biofertilizers of agricultural crops. Phosphate is one of the most vital macronutrient required for the growth and development of plants. A large number of microorganisms present in the rhizosphere are known to solubilizer and make available the insoluble phosphorus in the available form to the plants. Plant growth promoting rhizobacteria (PGPR) mediate the soil processes such as decomposition, nutrient mobilization, mineralization, solubilization, nitrogen fixation and growth hormone production. Rhizosphere soil of *Oryza sativa* (paddy plant) was collected from cultivated crop field and enumerated for total heterotrophic and phosphate solubilizing bacterial population. In this study, twenty five strains of phosphate solubilizing bacteria (PSB) were isolated by plate assay and characterized biochemically. Mineral phosphate solubilizing (MPS) activities of all isolates were tested on tricalcium phosphate of Pikovskaya's agar medium by analyzing the soluble phosphate content after 24 hours of incubation at 37^0C. The aim of the present study was to screen the phosphatase enzymes of phosphate solubilizing bacteria and to access their potential tolerance to herbicides (one shot). The heterotrophic bacterial load was found in the order of 10 CFU/gm and the phosphate solubilizer of 10 CFU/gm. The phosphate solubilizing bacteria was composed of *Bacillus* sp. and *Micrococcus* sp. with a lone of *Pseudomonas* sp. Both *Bacillus* sp. and *Micrococcus* sp. were noted to produce phosphatase at neutral pH and at 45^0C with the exception of *Bacillus* sp. phosphate solubilizer (PS) 25. Probably this bacterium is secreting alkaline phosphatase that has temperature optima at 35°C. None of the three representative bacterial strains were found to tolerate the most commonly used herbicide (one shot) at the concentration of 20 ppm. Only *Pseudomonas* sp. was recorded to tolerate this herbicide at 10 ppm. Further understandings on the nature of the enzyme, optimization on its activity would provide basic data for the commercial production of these enzymes.

Keywords: Rhizosphere soil, Pikovskaya's agar medium, Griseofulvin, Phosphatase enzyme, Phenolphthalein diphosphate and Herbicide.

Introduction:

Soil microorganisms have great potential in providing soil phosphates for plant growth. Phosphorus biofertilizers can help to increase the accessibility of accumulated phosphates for plant growth by solubilization (Goldstein, 1986; Gyaneshwar *et al.*, 2002). Rhizosphere is the soil zone surrounding the plant roots while rhizoplane is directly in contact with the roots (Kennedy, 2005). Plant roots exuded the organic contents in the rhizosphere and subsequently increased the microbial activity and termed as "rhizosphere effect" by Hiltner (1904). Microorganisms exerted beneficial effect on plant growth and development through different means is termed as plant growth promoting rhizobacteria (PGPR) (Vessey, 2003). Plant growth

promoting rhizobacteria accounts for about 2-5% of total the rhizobacteria involved in plant growth promotion (Antoun and Kloepper, 2001).

Phosphate solubilizing microorganisms (PSM) have attracted the researchers to exploit their potential to utilize phosphate reserves in semi-arid regions and to enhance the crop yields (Goldstein *et al.,* 1993; Fasim *et al.,* 2002; Khan *et al.,* 2006). Phosphate solubilizing microorganisms have established their role for optimum growth of plants under nutrient imbalance conditions (Glick, 1995; Iguala *et al.,* 2001; Wu *et al.,* 2005).

Phosphorus (P) is the second most important plant nutrient after nitrogen (Donahue *et al.,* 1990). Phosphorus is an essential macronutrient for growth and development of plants involved in important metabolic pathways like photosynthesis, biological oxidation, nutrient uptake and cell division (Illmer and Schinner, 1992). Worldwide soils are supplemented with inorganic phosphate as chemical fertilizers to support crop production but repeated use of fertilizers deteriorates soil quality (Tewari *et al.,* 2004).

The problem of phosphate fertilization may become serious in the coming years because of the fact that manufacture of phosphatic fertilizers requires the use of non-renewable resources such as high grade rock phosphate which are getting depleted progressively and becoming costlier. It is estimated that about 98% of Indian soils contain insufficient amounts of available phosphorous to support maximum plant growth. So to increase the soil fertility and agricultural productivity phosphatic fertilizers are widely used.

Rock phosphates are the only commercial raw material for the production of phosphatic fertilizers. The total world reserves of rock phosphate are estimated to be about 27,000 million tones, of which 80% are located in USA, USSR and Morocco. In India, rock phosphate deposits are of low grade (contain less than 25% of the P_2O_5) and do not satisfy the requisite specification of fertilizer industry. Beneficiation of such low grade rock phosphates is successful only in acidic soils and in neutral and an alkaline soils, rock phosphate turns out to be rather poor source of phosphorus (Neelima Garg *et al.,* 1989).

Many soil microorganisms including bacteria, fungi, yeast and actinomycetes are capable of solubilizing the insoluble inorganic phosphates (Skujins, 1967). Soil microorganisms play a very significant role in mobilizing phosphorous for the use of plants in bringing about changes in the pH of the soil microenvironment leading to the solubilization of native as well as added insoluble phosphorous.

Sen and Paul (1957) observed the solubilization of calcium and iron phosphate in liquid medium by *Bacillus subtilis*, *Bacillus megaterium* and *Bacillus mesentericus* with ammonium sulphate as the nitrogen source. They also isolated the bacteria capable of solubilizing tricalcium phosphate from the glands of *Cassia occidentals*.

The soil environment surrounding the plant roots is the zone of intense microbial activity. Swaby and Sperber (1959) reported that population of phosphate dissolving microorganisms are more in the rhizosphere (20–40% of the total population) compared to non-rhizosphere (10–15% of the total population). Solubilization of phosphorous by phosphate solubilizing microorganisms (PSM) is attributed to excretion of organic acids (Sperber, 1957).

Artidave and Patel (1999) worked on inorganic phosphate solubilizing soil Pseudomonads and isolated thirty eight *Pseudomonas* sp. which were examined for their tricalcium phosphate solubilizing activity in Pikovskaya's broth for twenty one days? Though all cultures solubilizer tricalcium phosphate in liquid medium only thirteen cultures showed zone of

phosphate dissolution on Pikovskaya's agar medium. The decomposition and mineralization of organic phosphate occurs under the influence of specific enzymes of phosphatase. Hence, it has an essential role to play in the phosphorous cycle in the environment.

Chemical pesticides are commonly used by farmers and corporate in agriculture to protect the crops, but these activities are depleting the soil fertility and soil health. The use of chemical pesticides in Indian agriculture drastically increased in recent years. The word pesticides include a heterogeneous group of chemicals developed to control a variety of pests. Pesticides are generally categorized as insecticides, herbicides and fungicides according to the type of pest which they have shown efficacious action (Miligi *et al.,* 2006).

As microorganisms play an important role in many soil biological processes, including nitrogen transformations, organic matter decomposition, nutrient release and their availability, as well as stabilize the soil structure and affect its fertility, investigated by (Vyas, 1988; Edwards and Bater, 1990; Khan and Sculion, 2000). However, soil is the most important site of biological interactions. The indiscriminate use of pesticides disturbs the soil environment by affecting flora and fauna including soil microbial flora, soil enzymes and also the physicochemical properties of soil like pH, salinity, alkalinity leading to infertility of soil. The important micro flora, beneficial for the growth of plants includes nitrogen fixing bacteria and phosphate solubilizing bacteria, present in the rhizosphere of the plant.

Various agrochemicals e.g. herbicides when applied intensively and erratically on herbicide resistant non-transgenic and transgenic crops to control the noxious weeds leads to their accumulation in soil to a dangerous level that affects growth, survival, efficiency and quality of beneficial microbial communities of soil (Srinivas *et al.,* 2008; Pereira *et al.,* 2008; Ahemad *et al.,* 2009). The naturally abundant plant growth promoting rhizobacteria are also metabolically inactivated through the uptake of herbicides applied in excess to the soil (Barriuso *et al.,* 2010). In contrast, a few microorganisms found to be tolerant or resistant towards specific herbicides. It is of great concern that how to reduce the effect of herbicides on the beneficial microorganisms and at the same time it is of great interest to screen out microorganisms which are tolerant / resistant to herbicides. Herbicides are applied in modern agricultural practices to offset the plant growth restricting weeds and subsequently to augment the productivity of crops (Ahmed *et al.,* 2009). The intensive application of herbicides leads to their accumulation in soils to a level that adversely affects both the quality and biological composition of soils (Srinivas *et al.,* 2008), the naturally abundant plant growth promoting rhizobacteria (PGPR) are metabolically inactivated by taking up excessive herbicides (Ahemad and Khan, 2010; Bellinaso *et al.,* 2003).

Many pesticides showed no detectable effects on soil microorganisms at the recommended application rates. However, application at increased rates is often reported as most of the farmers decide based on their own experience of the effective pest control. Repeated and overuse of pesticides in agriculture is a matter of concern because these chemicals are recognized as a source of potential adverse impacts on the metabolic activities of soil microorganisms as well as their plant growth promoting characteristics (Wani *et al.,* 2005; Ahemad *et al.,* 2009). Some microorganisms (called as phosphate solubilizing microorganisms - PSMs) perform phosphate solubilization. Their growth and phosphate solubilizing activity may also be affected by the pesticides leading to the imbalance phosphorous nutrition for the crop plants. However, the effect of pesticides on microbial growth and their activity especially

phosphate solubilization can only be assessed using microorganisms which are tolerant to the pesticide of concern (Oves *et al.*, 2009).

Scope and aim of the study

The present study falls on the following lines:

Bacteriological examination

- To study, the enumeration of total heterotrophic bacterial population (THBP) in rhizosphere soil sample by total viable count.
- To study, the isolation and enumeration of phosphate solubilizing bacteria (PSB) from the rhizosphere soil sample by spread plate technique.
- To maintain the phosphate solubilizing bacterial isolates on nutrient agar slants.
- To identify, the phosphate solubilizing bacterial isolates using the scheme of Aiso and Simidu (1962).

Experimental works

- To observe, the determination of phosphatase enzyme activity in phosphate solubilizing bacteria
    - Preparation of the soup of phosphatase enzyme source
    - Phosphatase enzyme assay by spectrophotometric method (Spectronic 20D)
- To study, the culture condition optimization for phosphatase enzyme production
    - Effect of pH on phosphatase enzyme production
    - Effect of temperature on phosphatase enzyme production
- To evaluate the herbicide resistance in phosphate solubilizer by plate assay method

Materials and Methods:

Sampling area

The sampling area is a crop field in the surroundings of Alwarkurichi village, Tirunelveli Kattabomman district. Here, the surroundings of this area are completely covered by crop field. Most of the people in this village are farmers. They cultivated various types of crops and vegetable plants. The crop field is enriched by applying phosphatic fertilizers and natural phosphate containing manures. So the plants rhizosphere root regions can be expected to load of phosphate solubilizing bacterial flora. Some pests are affected the rhizosphere root region of plants and to retard the growth of plants and to decent the crop yield. So pesticides are very important to needed for the control of insect pests.

Sampling

Rhizosphere acidic soil samples of *Oryza sativa* (paddy plant) was collected from cultivated crop field. The acidic soil samples were collected from various corners and middle regions of the paddy field very important. Because, the content of microbial load is variable from region to region of the paddy field. Where, good growth of paddy plant absorbs high amount of soluble and insoluble phosphates around their rhizosphere root regions. Hence, their rhizoplane of paddy plant root regions would have appreciable concentrations of phosphate solubilizing bacterial flora. So, these selected paddy plant root regions were chosen for the isolation of phosphate solubilizing bacterial flora. The rhizoplane root regions of the paddy plants having higher density of phosphate solubilizing bacterial species and the phosphate solubilizing capacity were highly greater.

During acidic soil sample collection from the paddy crop field, the following care was taken.

1. The rhizosphere acidic soil sample of paddy field were taken and transferred into the sterile conical flask with the help of sterile knife. The acidic soil samples from rhizosphere root regions of the paddy field were taken compulsorily.
2. The sterile conical flask was transferred in an ice pack (>4°C). Because the activity of bacterial flora would not be altered.
3. Then, the soil sample was brought to the laboratory subjected to microbial analysis within six hours to prevent the reduction of bacterial countings.

Bacteriological examination

Selective culture media employed for the isolation of phosphate solubilizing bacteria

1. Pikovskaya's agar medium (modified by Sundara Rao and Sinha, 1963).
2. Hydroxy apatite agar medium (modified by Ayyakkannu and Chandramohan, 1970).
3. Soil extract agar medium (modified by Katznelson and Bose, 1959).

Enumeration of total heterotrophic bacterial population (THBP) in rhizosphere soil sample by total viable count

The collected soil sample was allowed to attain room temperature. The soil sample is used to determine the total viable count and the density of phosphate solubilizing bacterial flora.

Steps in serial dilution technique

About ten gm of soil sample was taken from the conical flask and aseptically transferred into a conical flask containing 90 ml of sterile distilled water. It represents 10^{-1} dilution. One ml was pipette out from the conical flask and transferred into a test tube containing 9 ml of sterile distilled water. It represents 10^{-2} dilution. This is repeated up to 10^{-5} dilution. Care was taken to mix the sample solution of each dilution thoroughly in a vortex mixture prior to pipetting out.

Steps in pour plate technique

Petri dishes and pipettes were sterilized in hot-air oven (180°C - 2 hours). Then, the nutrient agar medium was prepared, autoclaved and cooled to 45°C. 15 µl stock solution of antifungal agent griseofulvin (1 mg / 1 ml) was added aseptically to the medium to suppress the growth of fungal flora. From the above serially diluted sample, one ml of three consecutive dilutions to be tested was pipette out on to the sterile Petri dishes in replicates. Then the cooled nutrient agar medium was added to the plates (approximately 20 ml) and mixed thoroughly with the inoculums by rotating the plate in clockwise and counterclockwise directions, taking care not to spill the medium. Care was taken to see that while mixing the nutrient agar medium and the inoculums, the media does not get solidify while rotating it clockwise or counter clockwise directions. The pour plated materials after solidification were inverted and incubated at 37°C for a period of 24-48 hours. After incubation, plates with colonies ranging from 30 to 300 were selected for counting.

Isolation and enumeration of phosphate solubilizing bacteria (PSB) from the rhizosphere soil sample by spread plate technique

About ten gm of soil sample was weighed aseptically and dissolved in 90 ml of sterile distilled water and mixed thoroughly in a rotator shaker for the maximum recovery of bacterial cells from the soil. This represents 10^{-1} dilution. From the above dilution, the sample was diluted up to 10^{-5} dilution. Then Pikovskaya's agar medium, hydroxy apatite agar medium and soil extract agar medium were prepared, sterilized and cooled, to this 15µ1 stock solution of

griseofulvin (1 mg / 1 ml), antifungal agent was added aseptically and dispensed the medium in sterile Petri dishes. From the above 10^{-3}, 10^{-4} and10^{-5} dilutions 0.1 ml of the sample was pipette out and inoculated on the air dried sterile Pikovskaya's agar medium, hydroxy apatite agar medium and soil extract agar medium, these were spread plated. The replicates and control were maintained. One set of plates were incubated at 37°C for 24 hours in an incubator and the another set of plates were incubated at room temperature for 48 hours. After sufficient incubation, the plates were observed for the presence of phosphate solubilizer on the basis of transparent clear zone formation surrounding the colony.

Maintenance of phosphate solubilizing bacterial isolates

Isolations were made at random from Pikovskaya's agar medium or other selective media plates containing countable number of colonies (30 -300 colonies). After recording the morphological characteristics of the colony and pigmentation, they were checked for their purity by repeated streaking on nutrient agar medium plates and were maintained on nutrient agar slants preserved in a refrigerator at 4°C after growth.

Identification of phosphate solubilizing bacterial isolates

The isolates were identified on the basis of their morphological, physiological and biochemical characters. The bacterial cultures from the stored nutrient agar slants were sub cultured in the nutrient broth and the broth cultures were subjected to various identification tests. The bacterial cultures were identified using the scheme of Aiso and Simidu (1962). The Bergey's manual (1984) was also referred in the identification procedure.

Biochemical tests employed for the identification of phosphate solubilizing bacteria

1) Gram's staining technique

A loopful of the broth fresh culture was subjected to Gram staining procedure and observed microscopically under oil immersion objectives.

2) Motility test

The inoculation needle was dipped into the bacterial culture and stabbed into the SIM medium to test for the motility after incubation at 37°C for 24 hours.

3) Penicillin sensitivity test (Kirby-Bouer test)

Overnight fresh cultures were streaked on the penicillin sensitive nutrient agar plates. The plates were incubated at 37°C for 24 hours. Then plates were observed for the presence of penicillin resistant isolates.

4) King's media for pigment production test (King's *et al.*, 1954)

From the overnight test bacterial culture, a loopful culture was streaked on the pigment production medium and incubated for 24 hours at 37°C. The results were observed for the pigment production of bacterial isolates.

5) Oxidation-fermentation (OF) test (Hugh and Leifson test)

The oxidation-fermentation basal medium is used for differentiating Gram-negative organisms on the basis of fermentative and oxidative metabolisms of carbohydrates. The oxidative-fermentative basal medium was incorporated with lactose. Culture tube with 2 ml of sterile medium was stabbed with the inoculation needle of dipped bacterial culture and the culture tubes were incubated at 37°C for 24-48 hours. Acid and gas production was observed from the change in the medium colouration and cracking of the medium in the culture tube.

6) Luminescence test

A loopful of test bacterial culture was inoculated into the *Photobacterium* broth and incubated at 37°C for 24 hours. The results were observed for the luminescence of bacterial isolates.

7) Kovac's oxidase test

To place the oxidase disc strips into an empty Petri dish and with a sterile platinum wire loop or sterile glass rod smear fresh bacterial culture cells were thoroughly into the oxidase disc. The oxidase test is positive if the transferred cells turn into dark purple colour within 5-10 seconds.

Growth of selective isolates on selective media

To confirm the identify and purity of the isolates they were streaked on air dried selective agar medium plates such as Pikovskaya's agar medium and hydroxy appetite agar medium and incubated appropriately. Their growth pattern was observed and recorded.

Experimental works

a) Determination of phosphatase enzyme activity in phosphate solubilizing bacteria

i) Preparation of the soup of phosphatase enzyme source

Overnight fresh cultures of phosphate solubilizing bacterial isolates were withdrawn in a 1.5 ml eppendorf and centrifuged at 10,000 rpm for 10 minutes. After centrifugation, the pellet was taken and the soup was discarded. The pellet was resuspended in 1.5 ml of sterile saline. Then this was centrifuged at 10,000 rpm for 10 minutes and the soup was discarded. The pellet was resuspended in 1.5 ml of sterile saline and vortexed well. The enzyme soup was stored at 4°C under refrigeration for the determination of phosphatase enzyme activity.

ii) Assay of phosphatase enzyme on diphosphate by spectrophotometric method

Phenolphthalein diphosphate (sodium salt), at 0.5% concentration was prepared and filter sterilized. Then, the nutrient broth was prepared and 10ml of this nutrient broth was transferred into test tubes. The nutrient broth test tubes were sterilized by autoclaving. After sterilization, one ml of filter sterilized phenolphthalein diphosphate stock solution was added into each 10 ml of nutrient broth containing test tubes under aseptically and then mixed well. To this, 10 µl of saline suspension of the phosphate solubilizing culture was inoculated aseptically. The test tubes were incubated at 35°C for 24-48 hours. After incubation, 1 ml of 1 N sodium hydroxide (NaOH) solution was added to this incubated test tube cultures. A bright pink red colouration was observed. This colouration indicates the liberation of phenolphthalein due to phosphatase enzymes. Then the optical density of this coloured solution was read at 420 nm in a spectrophotometer (Spectronic 20D).

b) Culture condition optimization for phosphatase enzyme production

i) Effect of pH on phosphatase enzyme production

The phosphate solubilizing bacterial culture that had exhibited maximum phosphatase activity was chosen and subjected to optimization experiments.

Phenolphthalein diphosphate nutrient broth was prepared and pH of the broth medium was adjusted to 5, 6, 7 and 8 using 1 N hydrochloric acid solution (HCl) or 1 N sodium hydroxide solution (NaOH). From each pH group, 10 ml of the broth was transferred into test tubes and sterilized by autoclaving. After sterilization, 100 µl of saline washed cultures were inoculated in all the test tubes aseptically. The tubes were incubated at 37°C for 24 hours and an uninoculated blank was also maintained. A bright pink red colouration was observed. This

colouration indicates the liberation of phenolphthalein due to phosphatase enzymes. At six hours interval, phosphatase enzyme activity in its broth culture at different pH was recorded at 420 nm in a spectrophotometer (Spectronic 20D).

ii) Effect of temperature on phosphatase enzyme production

The phosphate solubilizing bacterial isolates that have exhibited high degree of phosphatase activity was chosen and preceded for optimization experiments.

Phenolphthalein diphosphate nutrient broth was prepared and 10 ml of this broth was transferred into test tubes and sterilized by autoclaving. After sterilization, 100 µl of saline washed cultures were inoculated into the test tubes aseptically. Then, one set of culture inoculated broth tubes were incubated at room temperature for 24 hours. Another set of culture inoculated broth tubes were incubated at 35°C for 24 hours in an incubator. Another set of culture inoculated broth tubes were incubated at 45°C for 24 hours in rotator shaker. In each set blank was maintained. A bright pink red colouration was observed. This colouration indicates the liberation of phenolphthalein due to phosphatase enzymes. At six hours interval, broth culture was tested for phosphatase enzyme activity of this coloured solution was read at 420 nm in a spectrophotometer (Spectronic 20D).

c) Evaluation of herbicide resistance in phosphate solubilizer by plate assay method

To check the herbicide resistance profile of the phosphate solubilizing bacterial isolates, plate assay was carried out.

The nutrient agar medium was prepared and sterilized. The concentration herbicide, one shot (2, 4-D and anilofos) was poured into the sterile nutrient agar medium at different concentrations of 10 ppm and 50 ppm aseptically and mixed well. Then, the mixture of the contents were poured (approximately 15-20 ml) into the sterile Petri dishes and was allowed to solidify. After solidification, the saline washed cultures of phosphate solubilizing bacterial isolates were streaked on one shot nutrient agar plates. Then, the plates were incubated at 37°C for 24-48 hours. The control and replicates were maintained. After incubation, the herbicide resistant bacteria isolates were observed and stored for further analysis.

Results and Discussion:

Total heterotrophic bacterial population in the rhizosphere soil of *Oryza sativa* was enumerated and the results are presented in table - 1. Throughout the sampling period, total heterotrophic bacterial population was found to be in the range of 10 CFU/gm, similar distribution pattern was recorded in phosphate solubilizing bacteria also (table - 2). All the isolation of phosphate solubilizers was recorded to have comparable recovery of phosphate solubilizers. Comparing the data presented in table - 1 and table - 2, it can be understood that the majority of rhizosphere bacterial isolates were comprised of phosphate solubilizers. Since, the root zone represents high metabolic activity one can expect huge populations that have direct or indirect interaction with plant. Phosphate solubilizers are often found to predominate around rhizosphere region where they could utilize the plant exudates and in turn solubilizer phosphates and make it available for the plants.

Table 1: Total heterotrophic bacterial population in the rhizosphere soil of *Oryza sativa*

S. No.	Sampling Cycle	Bacterial Load (CFU/gm)	Method of Sampling
01	I	36×10^{-3}	Pour plate technique
02	II	25×10^{-3}	
03	III	32×10^{-3}	

Table 2: Phosphate solubilizing bacterial load in the rhizosphere soil of *Oryza sativa*

S. No.	Sampling Cycle	Bacterial Load (CFU/gm)			Method of Sampling
		Pikovskaya's Agar Medium	Hydroxy Apatite Agar Medium	Soil Extract Agar Medium	
01	I	50×10^{-2}	30×10^{-2}	20×10^{-2}	Spread plate technique
02	II	30×10^{-2}	20×10^{-2}	20×10^{-2}	
03	III	30×10^{-2}	30×10^{-2}	10×10^{-2}	

On the basis of phosphate solubilization, 25 bacterial isolates were characterized up to generic level and the data was presented in table - 3. From this, it can be noted that the major components are *Micrococcus* sp., *Bacillus* sp. and *Pseudomonas* sp. Among these, both *Micrococcus* sp. and *Bacillus* sp. were equally distributed (48%) and the only other isolate was *Pseudomonas* sp. (4%) presented in figure - 1.

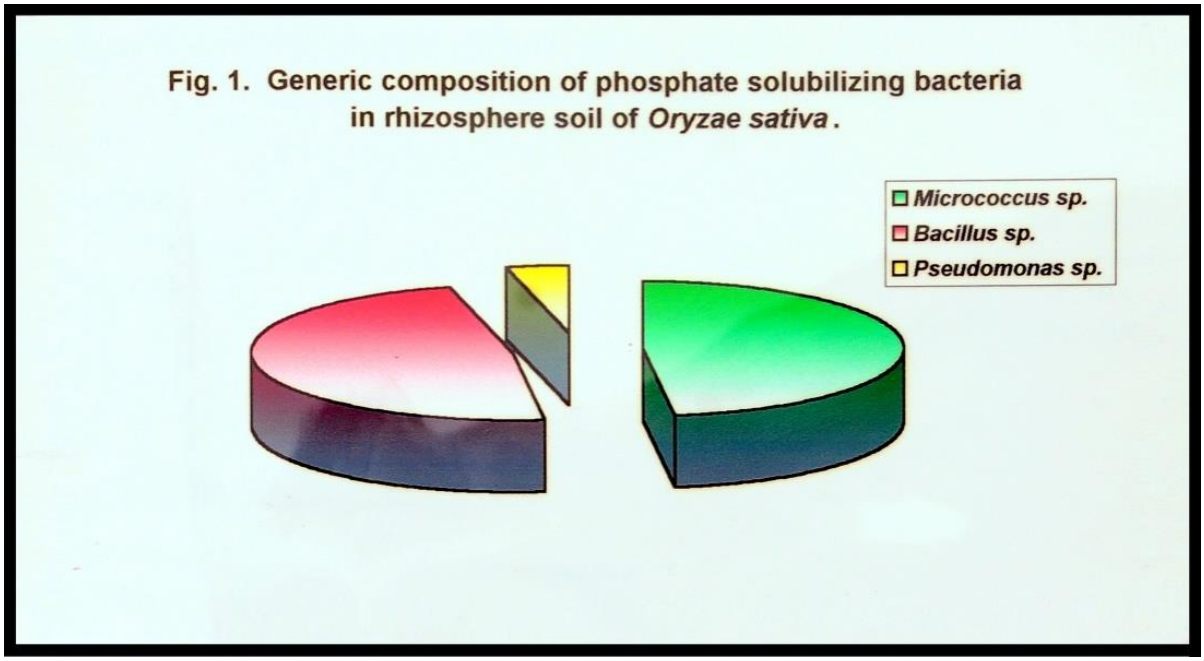

Figure 1: Generic composition of phosphate solubilizing bacteria in rhizosphere soil of *Oryza sativa*

Table 3: Biochemical characterization of phosphate solubilizing bacterial isolates

S. No.	Tests Performed	Phosphate Solubilizing Bacterial Isolates																								
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
01	Morphology	Small cocci	Rod shaped	Rod shaped	Rod shaped	Small cocci	Small cocci	Small cocci	Small cocci	Small cocci	Small cocci	Rod shaped	*Rod	Rod shaped	Small cocci	Rod shaped	Rod shaped	Rod shaped	Rod shaped	Small cocci	Rod shaped	Rod shaped	Small cocci	Small cocci	Small cocci	Rod shaped
02	Gram staining	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
03	Motility	-	+	+	+	-	-	-	-	-	-	+	+	+	-	+	+	+	+	-	+	+	-	-	-	+
04	Penicillin sensitivity test	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
05	Pigment production test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
06	Oxidation-fermentation test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
07	Luminescence test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
08	Kovac's oxidase test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

*Legend: Small cocci indicates Micrococcus sp., rod shaped indicates Bacillus sp., and *rod shaped indicates Pseudomonas sp.*

Growth of this organisms in media supplemented with inorganic phosphate indicates their ability to secrete organic acids that facilitate phosphate solubilization. Most phosphate solubilizing organisms have capabilities of elaborating enzyme namely phosphatase that plays a vital role in the catalysis of organic phosphates. To understand this phenomenon, 5 isolates were selected at random (*Bacillus* sp. PS 11, PS 16, PS 18, PS 25 and *Micrococcus* sp. PS 23) and examined for phosphatase activity with the help of an appropriate indicator. Photometric analysis of this experiment was presented in figure - 2. From this, it can be understood that *Bacillus* sp. PS 11, PS 18 and PS 25 were slightly more efficient in phosphatase production compared to the other two.

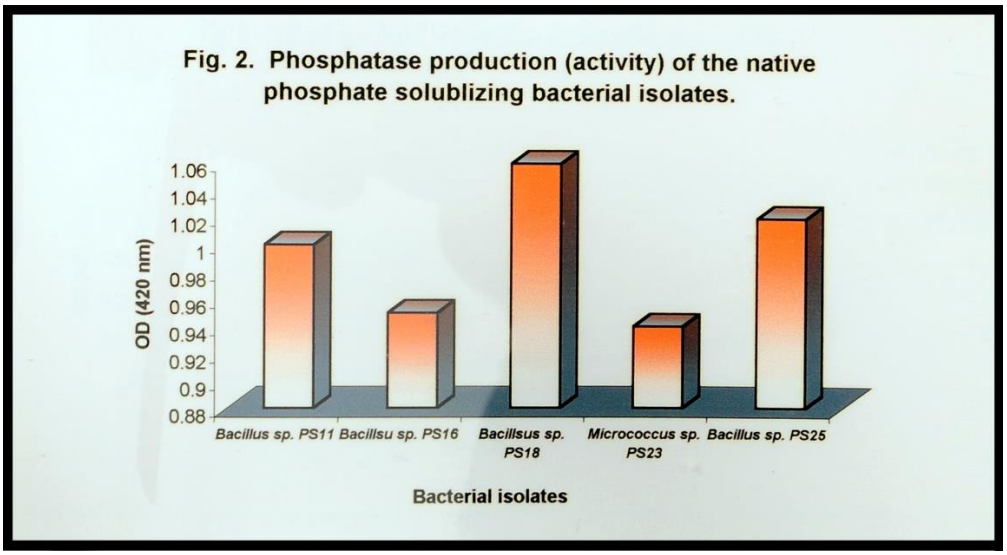

Figure 2: Photometric analysis of phosphatase enzyme production (activity) of the native phosphate solubilizing bacterial isolate

Further all the 5 strains were subjected to culture optimization experiments and the data are presented in figure 3-12. In the case of phosphate solubilizer (PS) 11, optimum pH for enzyme production was recorded to be 7.0 (fig. 3) and the temperature at 45°C (figure - 4).
Similar observations can be noted for *Bacillus* sp. PS 16, PS 18 and *Micrococcus* sp. PS 23 (figure - 5-10).

In the case of *Bacillus* sp. PS 25, the optimum pH for phosphatase production was recorded to be pH 8.0 and the temperature of 35°C.

Phosphatase production is an important property that enables the bacteria to flourish especially in the rhizosphere region. As plants are continuously amended with organic fertilizers, these organisms would generate phosphate by more than one mechanism. Furthermore, phosphatase producers would be of special value especially when organophosphate pesticides through phosphatase enzyme thereby ensure the faster disappearance in soil. Even though these organisms are expected to resist organophosphonates, resistance to other pesticides would be an added advantage as these organisms can be used both as biofertilizers and bioremedial agents. In this background, one isolate from each genera was exposed to a commonly used herbicide, ‘one shot’ (2, 4-D and anilofos) at two different concentrations (10 ppm and 50 ppm) and checked for their tolerance level, while *Micrococcus* sp. and *Bacillus* sp. were unable to tolerate this herbicide even at low concentration (10 ppm). *Pseudomonas* sp. was able to tolerate this farm chemical at lower concentration (table - 4).

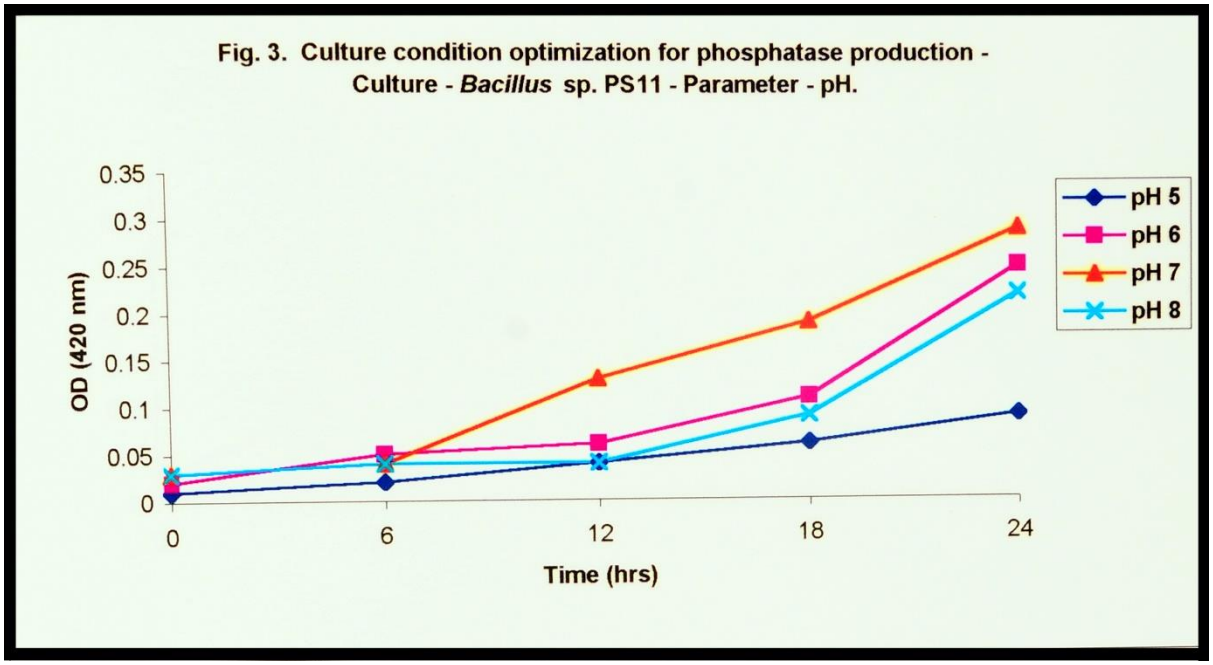

Figure 3: Culture condition optimization for phosphatase production of *Bacillus* sp. PS 11 in the parameter of pH

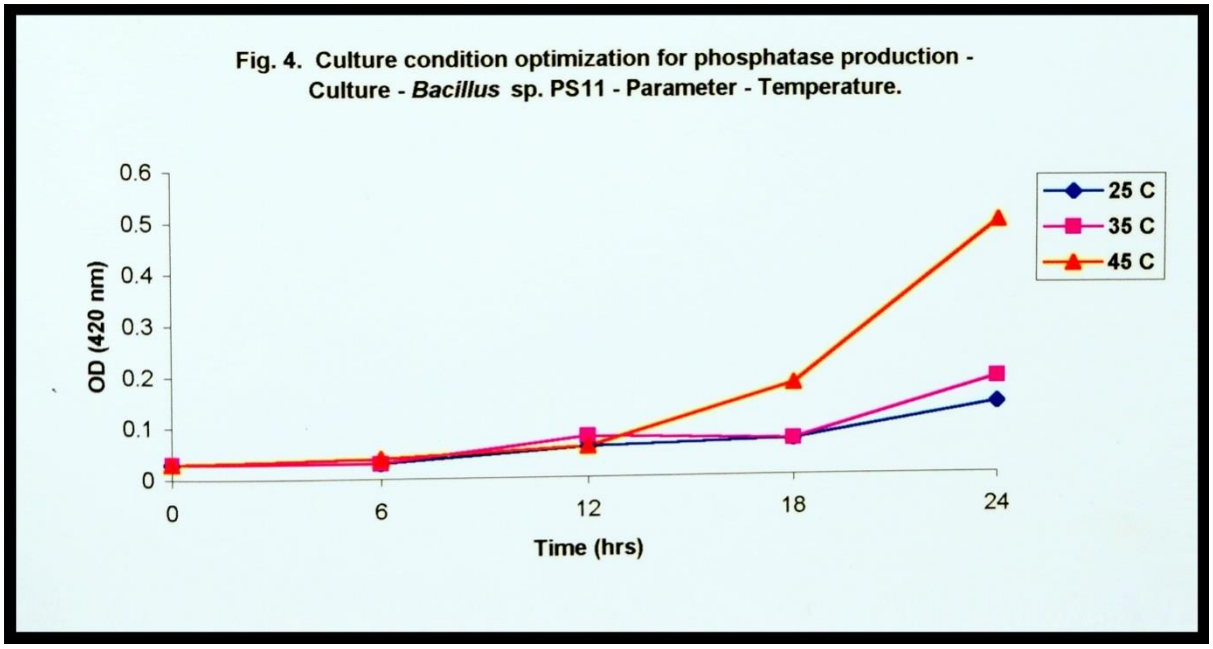

Figure 4: Culture condition optimization for phosphatase production of *Bacillus* sp. PS 11 in the parameter of temperature

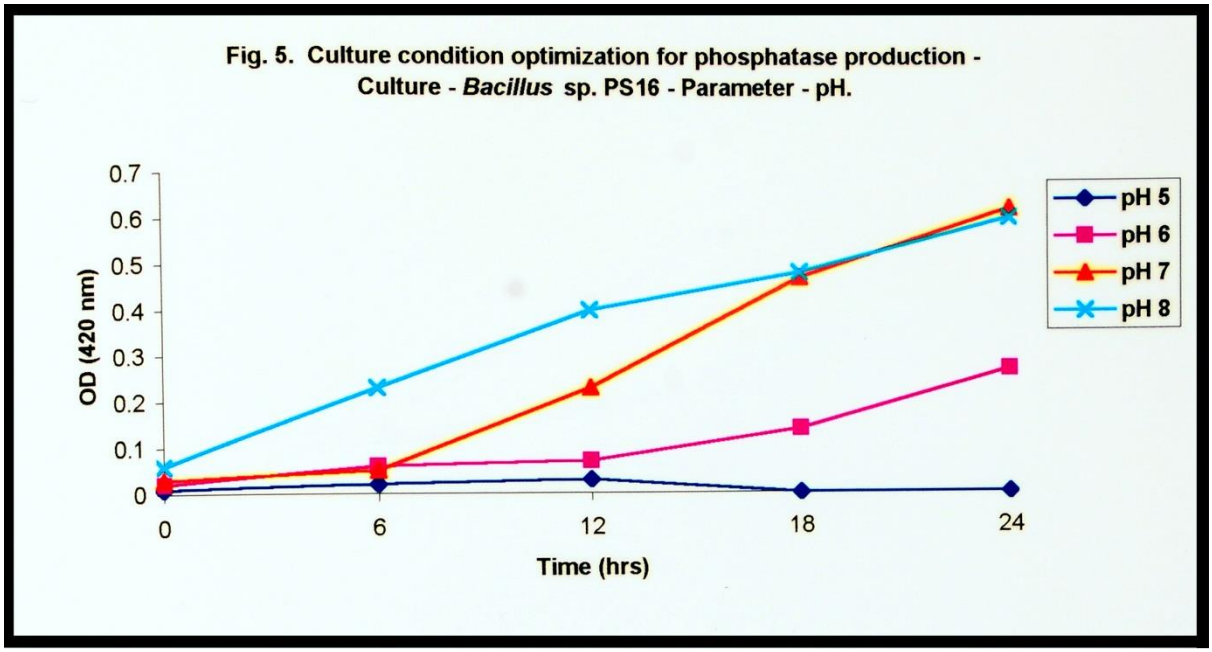

Figure 5: Culture condition optimization for phosphatase production of *Bacillus* sp. PS 16 in the parameter of pH

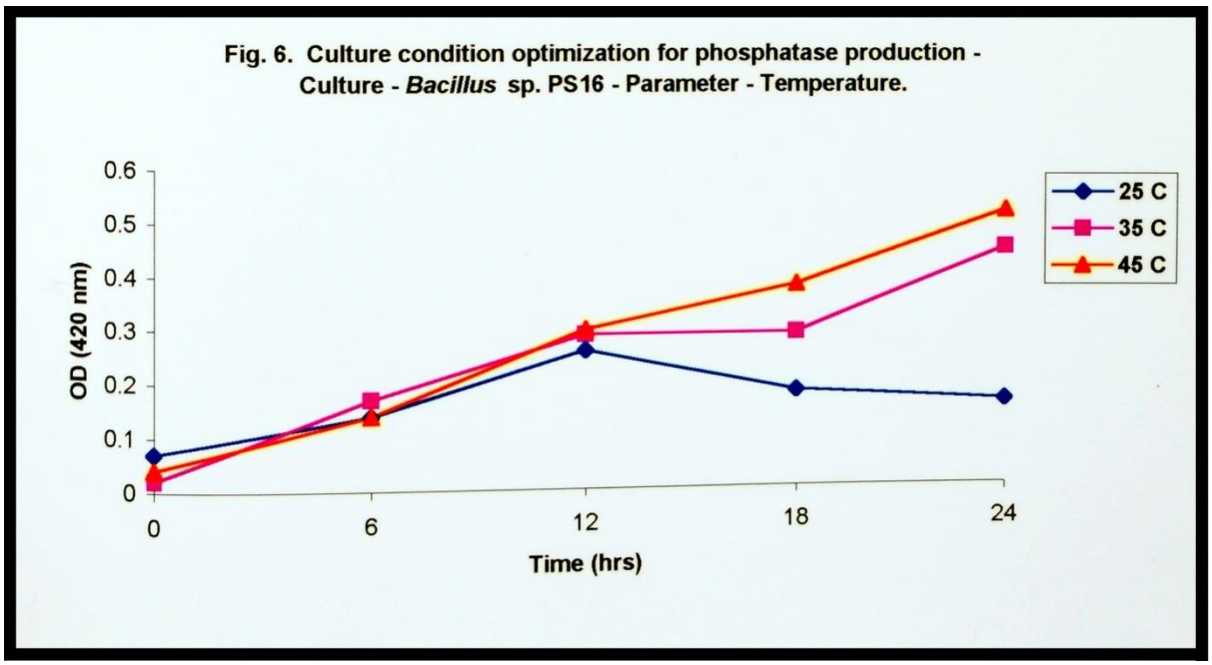

Figure 6: Culture condition optimization for phosphatase production of *Bacillus* sp. PS 16 in the parameter of temperature

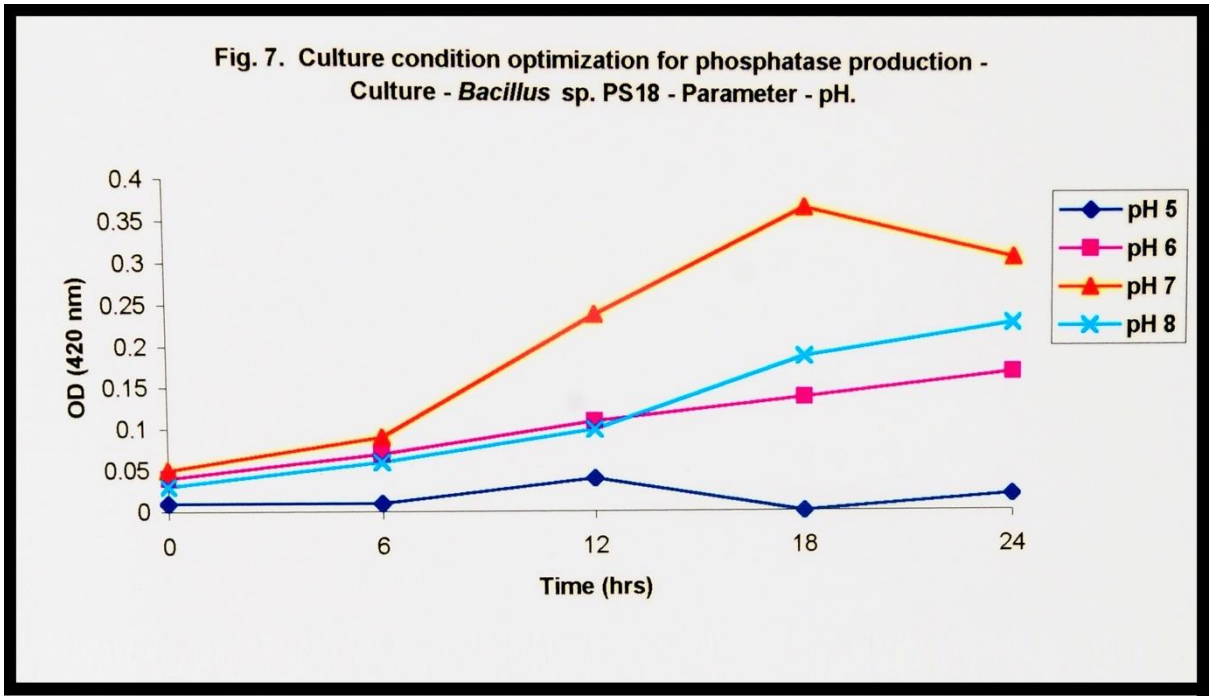

Figure 7: Culture condition optimization for phosphatase production of *Bacillus* sp. PS 18 in the parameter of pH

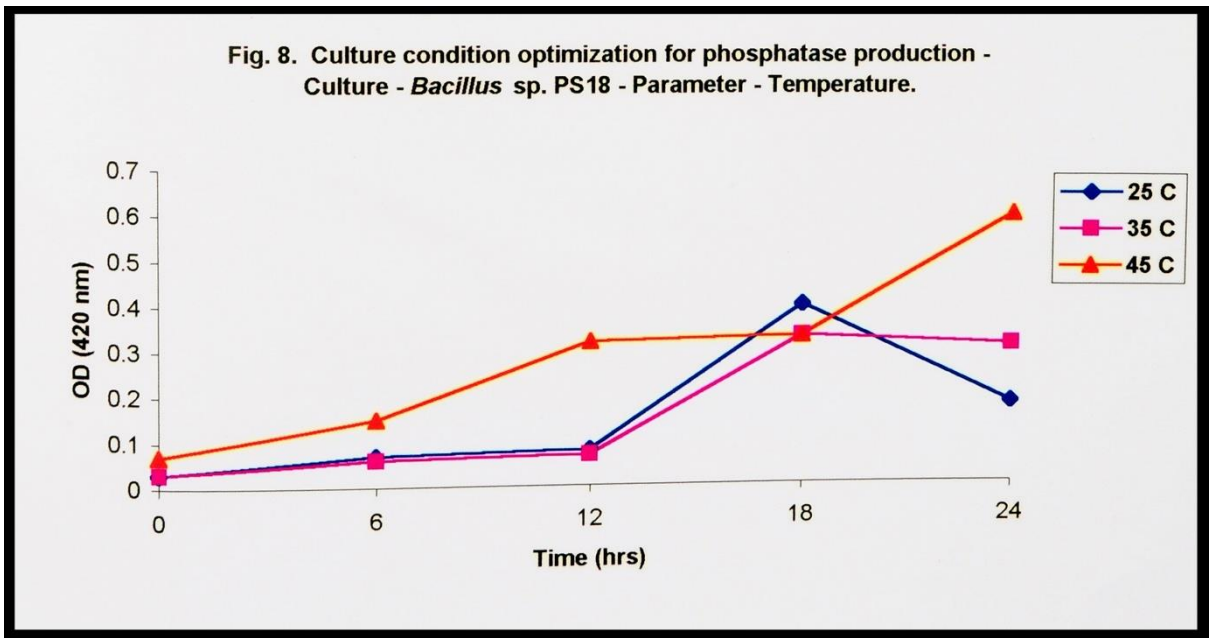

Figure 8: Culture condition optimization for phosphatase production of *Bacillus* sp. PS 18 in the parameter of temperature

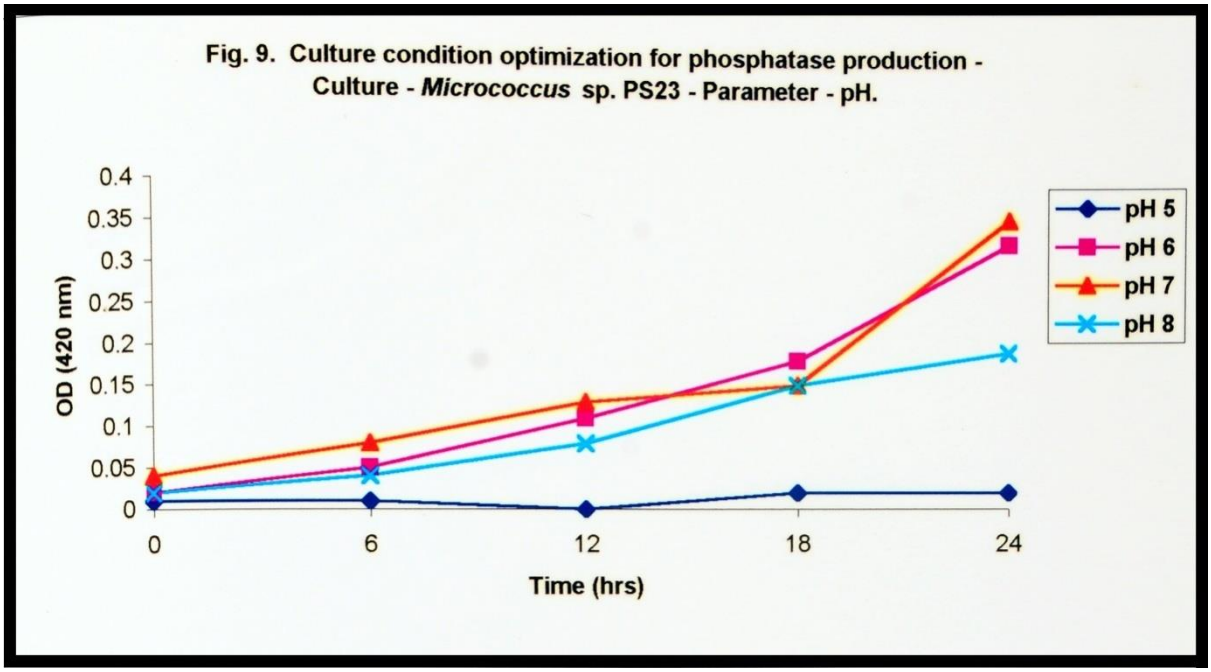

Figure 9: Culture condition optimization for phosphatase production of *Micrococcus* sp. PS 23 in the parameter of pH

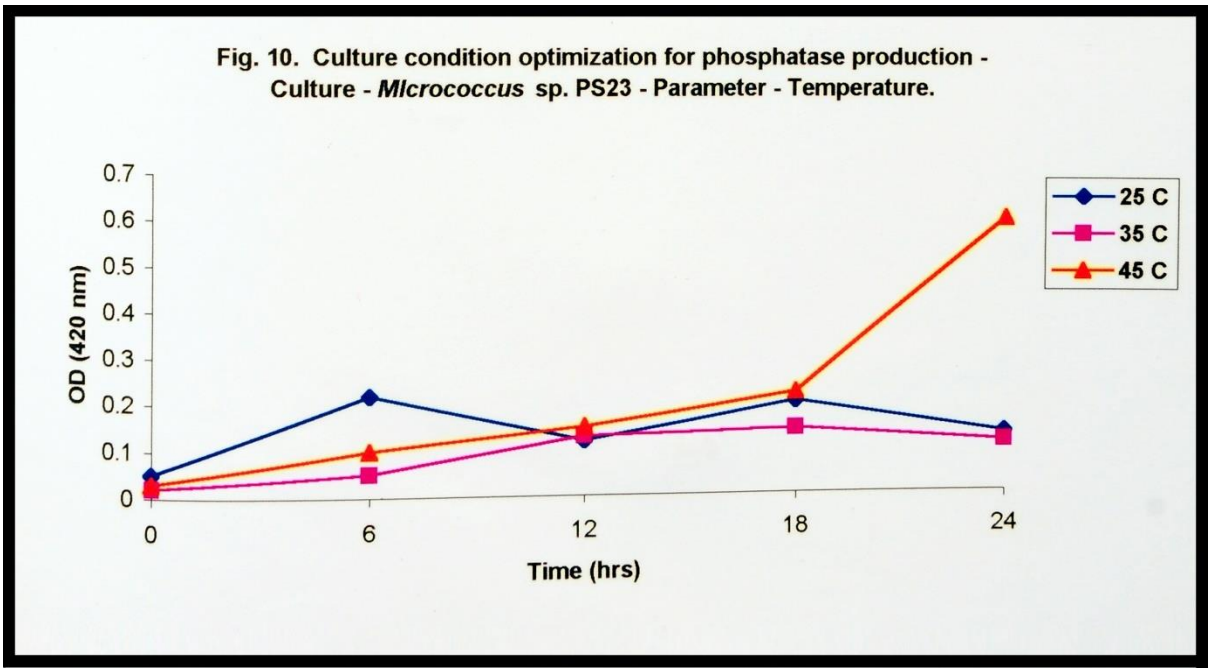

Figure 10: Culture condition optimization for phosphatase production of *Micrococcus* sp. PS 23 in the parameter of temperature

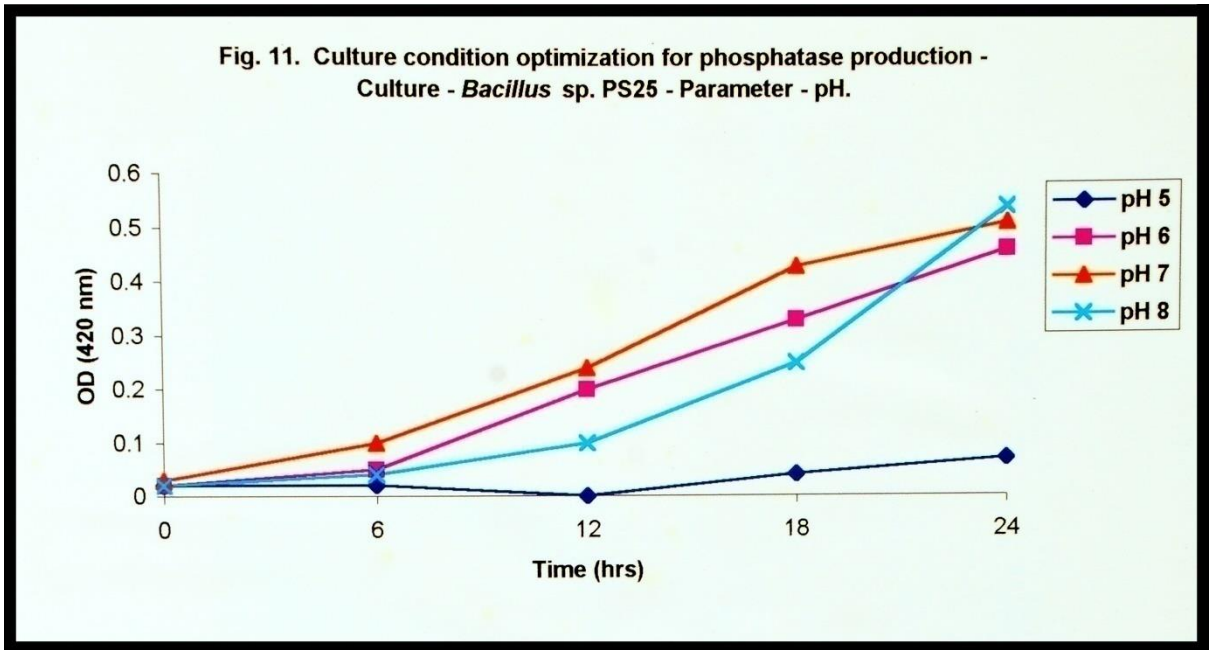

Figure 11: Culture condition optimization for phosphatase production of *Bacillus* sp. PS 25 in the parameter of pH

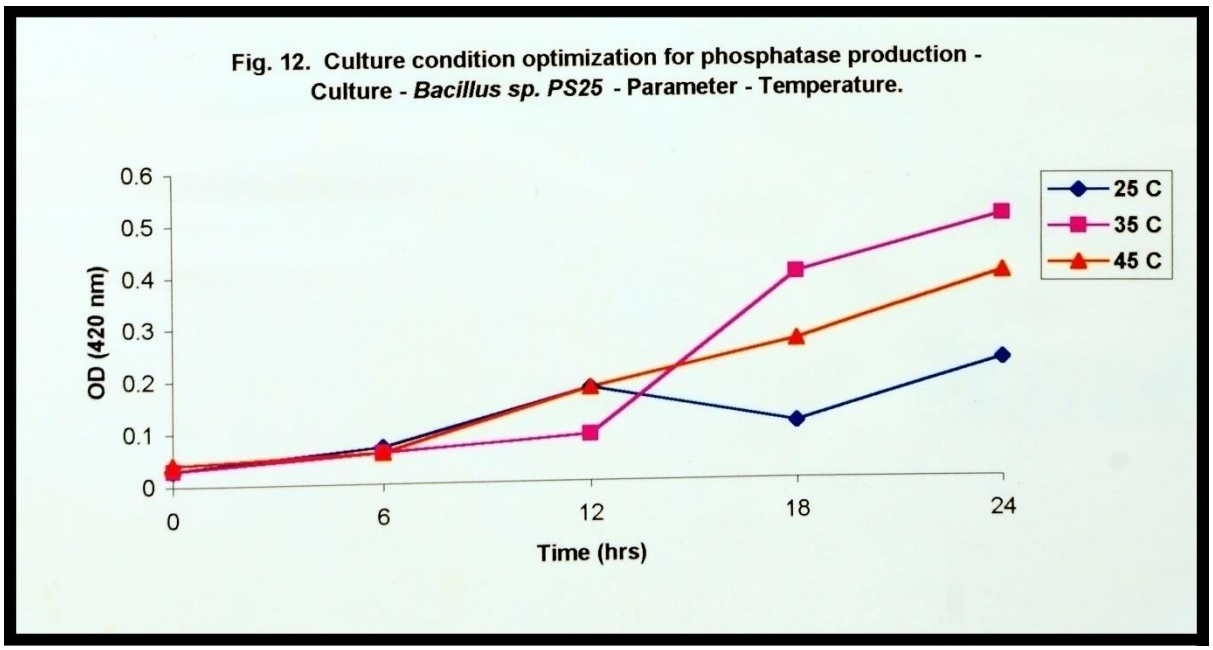

Figure 12: Culture condition optimization for phosphatase production of *Bacillus* sp. PS 25 in the parameter of temperature

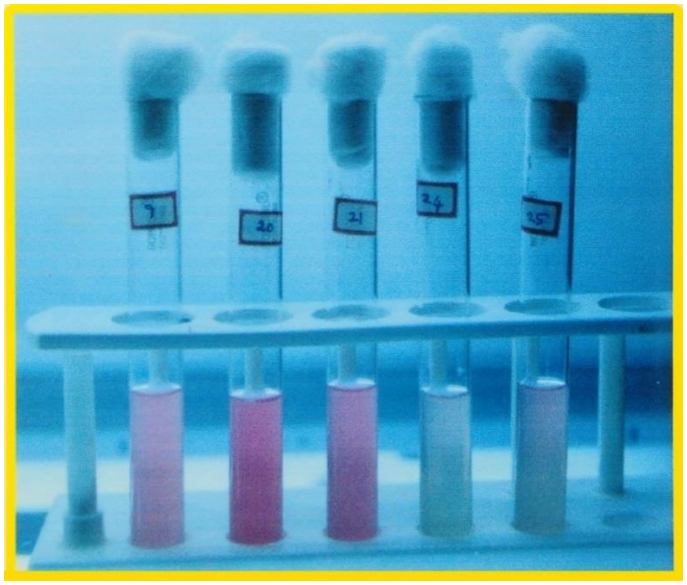

Figure 13: Phosphatase enzyme assay (before adding 1 N NaOH solution)
Legend: Light pink colour indicates the presence of phosphatase enzyme

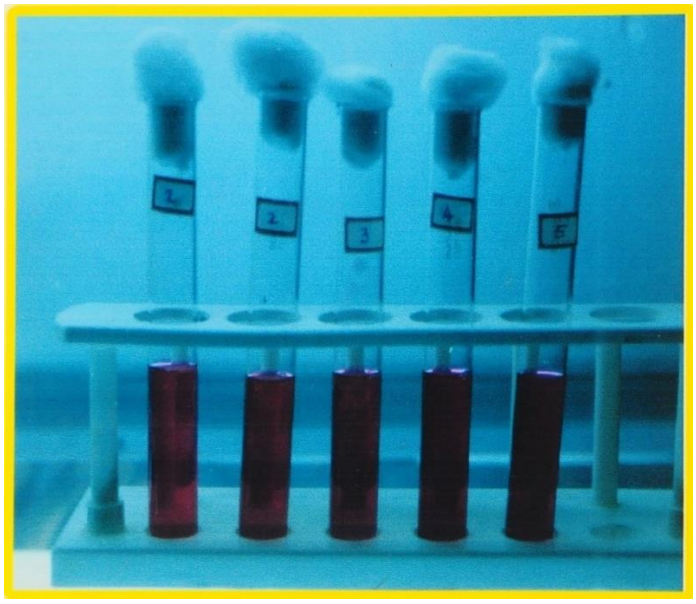

Figure 14: Phosphatase enzyme assay (after adding 1 N NaOH solution)
Legend: Bright pink colour indicates the presence of phosphatase enzyme

Table 4: Herbicide (one shot) resistance pattern of phosphate solubilizing bacterial isolates

S. No.	Culture Tested	Concentration of Herbicide	
		10 ppm	50 ppm
01	*Micrococcus* sp.	-	-
02	*Bacillus* sp.	-	-
03	*Pseudomonas* sp.	+	-

Legend: + sign indicates the appearance of bacterial growth and - sign indicates the absence of bacterial growth.

Conclusion:

This study indicates, the abundance of phosphate solubilizing bacteria in the rhizosphere region of *Oryza sativa*. Furthermore, their phosphatase activity would prove beneficial especially in inactivating residual organophosphate pesticides that would prevent the problem of pesticide pollution due to this chemical. Further research on the nature and production of phosphatase in these strains would be useful in their commercial applications.

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First and foremost of all, I thank the almighty for blessing me to complete my Master of Science course in Microbiology and the project work successfully. I am grateful to express my deep sense of gratitude to my profound research guide Dr. Gowrishankar Rajam M.Sc., Ph.D. I owe him personnel in debtness for giving valuable suggestion for problem, his guidance, wise council, unstinted encouragement both psychologically and academically. His keen interest and effort helped me in completing this project. I feel pleasure, immense pride and as most blessed person in presenting the work under his guidance.

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SOILS AND IODINE DEFICIENCY OF NORTH KASHMIR HIMALAYAS

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Abstract:

The present research work was carried out to analyze the concentration of Trace elements Iodine in soils of North Kashmir Himalayas. Analysis of data reveals that the soils of the study area have average concentration of iodine as 1.570mg/Kg with a considerable negative mean deviation in all the altitudinal zones. The concentration decreases from an average concentration of 1.617 mg/Kg in altitudinal zone A to an average concentration of 1.524 mg/Kg in altitudinal zone F. The reason could be that organic matter and pH influence the concentration of I in the soils because OM binds up I ions in the soil but the increasing slope and coarser texture cause I ions flow and translocate easily during rainfall. Extensive soil erosion in this mountainous region and water leaching of some acid arenaceous soils low in organic matter increases the magnitude of iodine losses. Suggestions have been made for the control of iodine losses in the sols.

Keywords: Trace elements, Iodine, Control sample, North Kashmir Himalayas.

Introduction:

Trace elements are increasingly becoming recognized as vital to human health. Each trace element has a standard requirement adequate for human health recommended by W.H.O (Fishbein, 1986). All essential trace elements either in imbalance states (Warren, 1991) or in deficiency states (Pyle, 1979) are known to create serious health problems particularly in the areas where these are regionally deficit (Lear month, 1988; Akhtar, 1991). Diseases due to trace element deficiencies as well as excess are known for iodine, copper, zinc, selenium, molybdenum, manganese iron, calcium, arsenic and cadmium (Lindh, 2005) and radon (Warren, 1991). Endemicity of many chronic or mild disorders such as goiter, dental fluorosis (Dissanayake, 1990) cancers (Akhtar, 1998), skin disorders, etc. is quite prevalent across India because of the variable concentrations of certain trace elements such as arsenic, fluoride, iodine, cerium, etc. (Dissanayake *et al.*, 2010).

The distribution of iodine is uneven in the biosphere. Both natural process such as leaching (Jeelani, 2010), volcanism (Buunnell *et al.*, 2007) and human activities such as mining and smelting (Keller, 1999) are responsible for redistribution of trace elements in the soils and water and organic environments (plants, animals and humans) through a network of pathways called as biogeochemical cycles (Furley and Newey, 1982). Its deficiency does not cause a mere enlargement of thyroid gland (endemic goiter), it can cause a variety of disorders called as iodine deficiency disorders (IDDs) or thyroid disorders consisting hypothyroidism, endemic cretinism, still-births, mental retardation, defects in vision, hearing and speech, and neuromuscular weakness. These disorders are mainly found in those people who live in mountainous areas, previously glaciated areas. Even people living in coastal areas and on islands suffer from IDDs because sea salt does not contain iodine content as much as required by the people and due to their unsuitable habits (UNICEF, 2002).

People living in hilly areas are more prone to thyroid disorders as iodine from the soil is washed away leaving behind iodine deficient soils which ultimately effects human as well as animal health (Akhtar, 1978). Near about 70 percent of the world populations living in mountainous soils are at risk of iodine deficiency with endemic goiter. W.H.O. estimated that about 20 to 60 % of the world's population is iodine deficient (Zimmermann, 2009) with most of the burden in developing countries. In India about 41% of the population suffers from iodine deficiency and in the state of Jammu & Kashmir nearly 33% of the population suffers from iodine deficiency (Zargar *et al.,* 1996)). According to recent research conducted in 2013 on school children of Kulgam district, it was found that 18.9 % suffer from Total Goiter Rate (TGR); 21.2 % boys and 16.7 % girls (Khan *et al.,* 2014).

Study area

North Kashmir Himalayas is a part of Great Kashmir Himalayas and lies between 34016”15”.09 – 34^0 12’41.35” North Latitude and 74048’00.00” – $75^0$05’09.58” East Longitude. The mountainous range has an average altitude of 2324 meters and stretches over an area of 5110.60 sq. Kms. It is at a point near Zojila that Great Kashmir Himalayas takes a blend towards the south west and is described as the North Kashmir range (fig. 1). North Kashmir Range acts as a water divide between Jhelum in Kashmir valley and Kishanganga of Gurez valley. The area experiences temperate climate with an average annual rainfall of about 1,230 mm (Raza *et al.,* 1978). Paleozoic sedimentary rocks, Triassic limestone, Karewa and Alluvium are the predominant geological formations of the area with limestone, shale, sandstone and unconsolidated sediments as the dominant litholog, the soils of the concerned area vary in origin from alluvial to lacustrine and glacial.

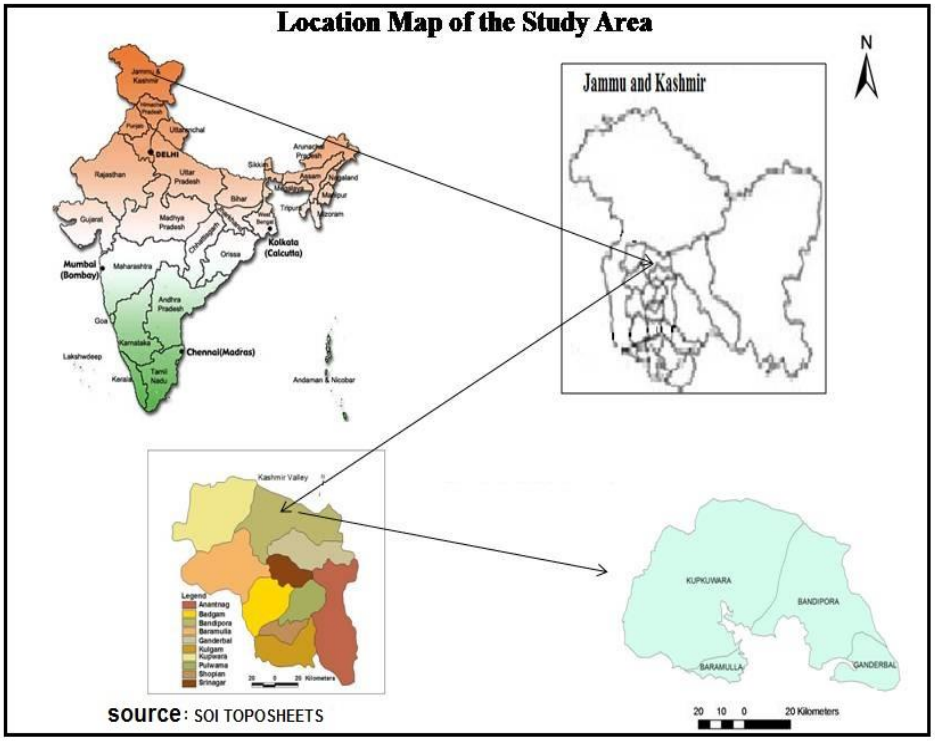


Figure 1: Location map (Generated from SOI toposheets, 1971)

Soils

The present study is based on the soil classification given by Indian Council of Agricultural Research (ICAR), Nagpur in 2008. The ICAR have divided soils of Jammu and

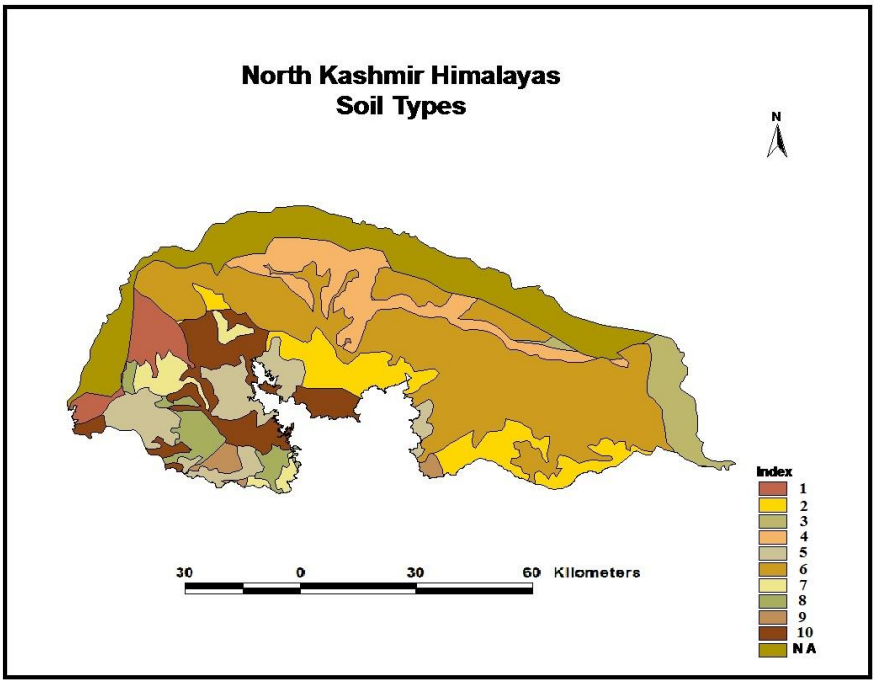
Kashmir state in 2008 into 140 classes plus one i.e., Glaciers, Water bodies, etc. on the basis of climatic conditions and physical character of the soils. In the present work, the soil map produced by ICAR, 2008 was a bit modified and nine soil types were identified. These are briefly explained in table 1

The soils of the study area very in origin from alluvial to lacustrine and glacial and have evolved through a long geomorphic history. These soils are deposited in their present sites by two main process-rivers and glaciers and are continuously subjected to tremendous transformation both by natural and human activities. Fertility of these soils in general decreases with altitude.

Mountain soils are young and immature soils, found usually along mountain slopes and are characterized by high content of pebbles and are shallow. . Mountain soils of steep slopes are less fertile and have high tendency of leaching. While as Mountain soils of gentle slopes have high organic content, available to these soils from forests. The moisture retaining capacity of the soil is poor as the upper layer has a high sand content. The highland soils are deficient in bases and become more and more acidic as altitude and vegetal cover increases. They have many differences on account of site, nature of slope and altitude. They are generally thin and immature soils. These soils have a higher tendency to leach (Raza *et al.,* 1978). Glacial soils are formed under glacial conditions and have origin from moraines. Glacial soils have high concentration of pebbles. Soils of side valleys and low altitude terraces are fertile soils with high content of nitrogen, Phosphorus, potassium and organic matter (figure .2)

Table 1: Major Soil types in North Kashmir Himalayas

Soil type	Description
1	Very shallow, excessively drained, loamy soils on steep slopes and severe erosion; associated with: Rock outcrops.
2	Medium deep, excessively drained, coarse-loamy, calcareous soils on steep slopes with loamy-surface, severe erosion and moderate stoniness; associated with: Rock outcrops.
3	Dominantly glaciers and rock outcrops; associated with: Shallow, extensively drained, sandy-skeletal soils on very steep slopes with sandy surface, severe erosion and strong stoniness.
4	Rock outcrops; associated with: Deep extensively drained, sandy-skeletal soils on very steep slopes with sandy surface, very severe erosion and moderate stoniness.
5	Medium deep, moderately well drained, mesic, fine-loamy soils on steep slopes and severe erosion, associated with: Deep somewhat extensively drained, fine soils on steep slopes with loamy surface, severe erosion and slight stoniness
6	Deep, well drained, coarse-loamy soils on gentle slopes with loamy-surface, moderate erosion and slight gravelliest; associated with: Deep, well drained, calcareous, coarse-loamy soils with loamy surface, moderate erosion and slight gravelliest.
7	Deep, moderately well drained, fine loamy soils and on very gentle slopes with loamy surface and slight erosion; associated with: Deep, moderately well drained, fine soils with loamy surface and very slight erosion.
9	Deep, well drained, fine soils on gentle slopes with loamy-surface, moderate erosion; associated with: Medium deep, well drained, fine loamy soils with loamy surface, moderate erosion and moderate stoniness.



Source: Modified from ICAR, Nagpur

Figure 2: Soil types

Data Base and Methodology

A comprehensive methodological framework has been adopted for the present research.

Data Base

The present research work is based mainly on primary data and partly on secondary sources of data. The primary data include the Survey of India toposheets on 1:50,000 scale of 1971 year, the data regarding concentration of essential trace element iodine in soils was obtained from analysis of soil samples collected from 32 sample sites and 4 control samples. The Soil map of the area was taken from the Soil Map of Jammu and Kashmir prepared by Indian Council of Agricultural Research (ICAR), Nagpur-2010.

Methods used

The methodology has been divided into many steps based on the materials and techniques used and described under the following headings.

Delineation of study area and Altitudinal zone mapping.

Table 2: Altitudinal zones in North Kashmir Himalayas.

Altitudinal Zone	Alt. in meters amsl	Area in Sq. Kms.	Area in % to total Area
A	1600-1750	499.18	9.76
B	1750-1900	308.43	6.04
C	1900-2050	269.87	5.28
D	2050-2200	286.23	5.61
E	2200-2350	275.50	5.39
F	2350-2500	293.19	5.74
G	2500 & Above	3178.20	62.18
	Total	5110.60	100

Source: SOI Topo-sheets.

The study area was delineated from thirteen SOI toposheets of 1:50,000 scale of 1971 with numbers as (43 series, j/2, j/3, j/6, j/7/ j/10 /j/11, j/14, j/15, N/3, N/4, N/7, N/8 and O/1. The toposheets were processed under GIS environment with the help of Arc View 3.2a software.

The area under study is fairly large characterized by large altitudinal variations; it was divided into seven altitudinal zones (table 2).

Selection of Sample Villages, Sample Households and Sample sites

Stratified Random Sampling technique was used for selection of around 15% of sample villages (32), in proportion to total number of villages and households from each altitudinal zone. From each sample village soil sample site was selected from major agricultural field, (table 3).

Table 3: Sample Frame

Altitude Zone	Altitude (meters)	Area (Sq. Kms)	Revenue villages			Soil	Control Samples
			Total Area	Sample	Sample (% age)		
A	1600-1750	499.18	32	4	12.5	4	Soil = 4
B	1750- 1900	308.43	43	5	11.63	5	
C	1900- 2050	269.87	51	8	15.68	8	
D	2050- 2200	286.23	42	6	14.28	6	
E	2200- 2350	275.5	31	6	19.35	6	
F	2350- 2500	293.19	18	3	16.67	3	
G	2500-6000	3178.2					
Total		**5110.6**	**217**	**32**	**14.74**	**32**	**4**

Sample collection and sample codification

An intensive and multi-faceted field work was undertaken to collect the soil samples. One soil sample was collected from each sample village making a total of thirty two (32) and one soil sample was collected from 4 control sites of Bandipora, Gander bal, Kupwara and Baramulla having high agricultural productivity, good crop health and good human health status to make standard for comparative analysis. Soil samples were collected in clean unused polythene bags and were labeled properly. Soil samples were taken only from surface soil (depth of 0-30 cm) for the reason that these soils are under the cultivation of paddy, vegetables and maize and this is the major zone of root development of crops (Brady, 1991). All the samples were properly packed, coded properly and reached to the lab for trace element-iodine, copper and zinc analysis.

Proper codification scheme was followed for identification of the samples.

Codes were used as:

1st alphabet for Macro region

2nd alphabet for Geo chemical component (S= Soil)

3rd alphabet as simply S for sample in all components

4th alphabet as a numerical number of the village (1,2,3.4 etc.)

In case of control samples alphabet C for control is added.

Data analysis and map work

Data obtained from the analysis of soil from the RCRQA Laboratory, SKUAST-K and data obtained after tabulation was statistically analyzed and interpreted. Map work has been carried out under GIS environment.

Results and Discussions

Concentration of Iodine

The soils of the study area have average concentration of iodine as 1.570mg/Kg with a considerable negative mean deviation of -0.030 mg/Kg from control samples. The concentration decreases from an average concentration of 1.617 mg/Kg in altitudinal zone A to an average concentration of 1.524 mg/Kg in altitudinal zone F. Average concentration of iodine shows positive deviations of + 0.017 mg/Kg from control sample in altitude zone A and decreases with altitude to a negative deviations of -0.038 in altitudinal zone B, – 0.012 mg/Kg in altitudinal zone C, - 0.054 mg/Kg in altitudinal zone D, -0.082 mg/Kg in altitudinal zone E and -0.076 mg/Kg in altitudinal zone F (table 4). The reason could be that organic matter and pH influence the concentration of I in the soils because OM binds up I ions in the soil but the increasing slope and coarser texture cause I ions flow and translocate easily during rainfall. The study area being formed during Orogensis of Himalayas and subjected to tremendous transformation both by natural and human activities. Extensive water leaching of some acid arenaceous soils low in organic matter increases the magnitude of iodine losses and reduces the intrinsic availability of soil selenium and zinc. High soil acidity strongly potentiates crop uptake of almonium, iron and manganese.

Conclusion / Suggestions

The present research work leads to the conclusion that concentration of trace elements Iodine of each sample villages was less than the mean of control samples with mostly negative deviation and generally decreases with altitude. It was also found that concentration of trace elements in soil has direct relationship with organic matter and clay and inverse relationship with pH, Leaching and run-off play an important role.

On the basis of inferences drawn from the present analysis, it is suggested that Soil tests should be carried out to assess the trace element status of soil for cultivation of crops. Soil management practices needs to be modified in order to minimize the risk of trace element iodine deficiency in the soils.

Table 4: Concentration of Iodine, Copper and Zinc in soils (Source: Analysis of soil samples in RCRQA Lab, SKUAST-2018)

Altitudinal Zone	Sample Village	Sample Site	Concentration of trace elements with deviation from the Mean of control sample *	EC (ms) with deviation from the Mean of control sample *	pH with deviation from the Mean of control sample *	Org. Matter with deviation from the Mean of control sample *
			I (mg/Kg)			
A 1600-1750	Chatragul	GSS3	1.640 (+0.040)	1.40	7.38	1.50
	Ajas	BSS1	1.600 (0.000)	1.14	7.55	1.50
	Shirhama	KSS2	1.600 (0.000)	1.93	7.65	0.98
	Nadihu	BL SS4	1.630 (+0.030)	1.80	7.29	1.73
	Average	**-**	**1.617 (+0.017)**	**1.567**	**7.47**	**1.42**
B 1750-1900	Gund	GSS1	1.600 (0.000)	1.42	6.61	1.50
	Aragam	BSS2	1.640 (+0.040)	1.05	5.81	0.90
	Aloosa	BSS9	1.560 (-0.040)	7.50	6.99	0.75
	Shiltra	KSS7	1.500 (-0.100)	1.84	7.51	1.58
	Kalamabad	KSS4	1.510 (-0.090)	2.15	7.00	1.73
	Average	**-**	**1.562 (-0.038)**	**2.79**	**6.78**	**1.29**
C 1900-2050	Khanan	GSS4	1.600 (0.000)	1.39	6.82	1.65
	Arin	BSS5	1.650 (+0.050)	1.66	7.11	0.75
	Sumlar	BSS4	1.810 (+0.210)	3.60	7.32	1.58
	Haril	KSS6	1.560 (-0.040)	1.04	6.41	1.88
	Mawar	KSS11	1.480 (-0.120)	1.65	7.29	1.50
	Indedeji	KSS12	1.490 (-0.110)	2.20	6.83	1.58
	Potwari	KSS13	1.480 (-0.120)	3.50	6.54	1.05
	Wanpur	BLSS3	1.640 (+0.040)	5.98	7.13	1.13
	Average	**8**	**1.588 (-0.012)**	**2.63**	**6.93**	**1.39**

D 2050-2200	Chitibandi	BSS3	1.600 (0.000)	1.48	7.04	1.73
	Malangam	BSS8	1.520 (-0.080)	12.60	7.46	0.75
	Waderbala	KSS5	1.570 (-0.030)	1.15	7.94	1.50
	Monbal	KSS9	1.510 (-0.090)	1.08	6.62	1.50
	Lache	KSS3	1.600 (0.000)	1.50	6.20	1.88
	Potus	BLSS1	1.480 (-0.120)	3.65	6.91	1.50
	Average	**6**	**1.546 (-0.054)**	**1.69**	**7.03**	**1.47**
E 2200-2350	Wangat	GSS5	1.780 (+0.180)	1.00	6.36	1.65
	Waniarm	GSS6	1.710 (+0.110)	1.18	6.39	1.50
	Mukam	BSS7	1.450 (-0.060)	1.34	6.82	1.73
	Rishwari	KSS10	1.500 (-0.100)	1.40	6.77	0.90
	Kandi	KSS1	1.540 (-0.060)	3.24	7.46	1.73
	Walthur	BLSS2	1.660 (+0.060)	2.12	6.89	0.75
	Average	**6**	**1.518 (-0.082)**	**1.71**	**5.64**	**1.38**
F 2350-2500	Chont waliwar	GSS2	1.500 (-0.100)	2.30	7.85	1.65
	Kitsan	BSS6	1.550 (-0.050)	1.01	6.54	1.65
	Zachaldara	KSS8	1.520 (-0.080)	1.90	6.62	0.75
	Average	**3**	**1.524 (-0.076)**	**1.73**	**7.00**	**1.35**
G 2500 & Above	Uninhabited					
Total	**32**	**32**	**1.570**	**2.01**	**6.8**	**1.38**

* Concentration of I in Control Soil Samples

Control Soil Sample	Con. of I (mg/Kg)	Ecms	pH	OC
GCSS	1.550	1.10	6.86	1.73
BCSS	1.680	0.80	7.62	0.90
KCSS	1.680	1.11	7.57	1.13
BLCSS	1.490	0.80	7.34	1.05
Average	**1.600**	**0.95**	**7.34**	**1.20**

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VERMIWASH IS IMPLEMENTED IN SUSTAINABLE FARMING TO BOOST CROP PRODUCTIVITY, AND ITS BIOACTIVE CONSTITUENTS ARE PRECIOUS

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Abstract:

Earthworms are God's most very kind creatures, considering that they are naked, eyeless, lungless, tubular cylinders that perform miracles on the biosphere. Recent days plenty of them have utilized an inorganic fertilizers to improve crop production. It causes a variety of diseases in humans and has an impact on soil fertility and erosion, so instead of using biofertilizer, vermicompost and vermiwash have more nutrients, enzymes, vitamins and hormones needed for growth. The analysis of this vermiwash shows their high content of various macro nutrients and micro nutrients . It is a category of elimination widely recognized as vermicast. A complex process in which organic matter is partially decomposed and mixed with sludge and intestinal microbial flora such as earthworms that has the capacity to improve soil fertility. They can practice sustainable agriculture to benefit the environment by helping to maintain soil quality, reduce soil erosion and conserve water. Research on the effect of foliar spraying on plants when the prevalence of pathogens and pests is reduced. Now we can control pests without pesticides. This showed that the quality and quantity of the harvest improved significantly. The bioactive compound in the crops generally shows variations when both vermiwash drenching grown crop and soil based grown crop is compared.

Keywords: Earthworm, vermiwash, Bioactive compounds, soil fertility.

Introduction:

Earthworms are land-dwelling invertebrates that belong to the Phylum- Annelida, Chaetopoda, order - Oligochaeta and identified approximately 600 million years ago in the Precambrian time. Earthworms are found in various habitats and show effective activity in causing physical and chemical changes in the soil that improve soil fertility which was the important role of earthworms in soil development and soil fertility that is well documented. Charles Darwin describes earthworms as the armed forces of humanity and "they are the farmer's friend". Vermicast is described as black gold. They are used as a valuable organic fertilizer (Ansari and Ismail, 2012).

They play an significant role in plant growth. A quick change is quite possible for sustainable agriculture using brand new vermicomposting technology in the soil. in more recently, commercial pest farmers have begun promoting the product by name worm washing this vermiwash contains enzymes, an earthworm secretion that would stimulate the growth and yield of crops and even develop opposition in host plants to spray. Such a product would certainly contain soluble plant nutrients, with the exception of a few organic acids and mucus from earthworms and microbes (Sawant Konkan, 2019).

Vermiculture is a hybrid culture made up of useful earthworms and soil microbes. It is well known that earthworms have the capacity to biologically degrade organic wastes into

organic manure. The efficiency of earthworms allows them to eat trash from both industrial and agricultural sources that is rich in organic material (Varghese and Lakshmi Prabha, 2014).

Earthworm

Earthworms are a member of the Phylum Annelida, which also includes leeches and marine bristle worms. Long, essentially cylindrical body, internally and externally segmented body, have a nervous, gastrointestinal and cardiovascular system. Many have two appendages that resemble legs on each segment. The weight or biomass of invertebrates in soil is considerably increased by the presence of earthworms. The term "intestine of the earth" was first used by Aristotle to draw attention to their function in aerating the soil. In 1881, Charles Darwin assessed the function of earthworms in preserving soil structure, aeration and fertility by infringement of dead plant and animal matter in soil and forest litter. It is demonstrated in his well-known work, "Formation of Vegetable Mould through the Action of Worms," proves that “Earthworms have played most important part in the history of the world” (Abesekara *et al*. 2006).

Earthworms' impact on soil quality

Earthworms, which increase soil fertility and production, have a major impact on the structure of the soil. Due to the physical, chemical and biological changes they cause in soil, earthworms are known as soil manages (Ansari and Ismail, 2012).

Scientific Classification of *Eiseniafetia*

Phylum: Annellida
Class: Oligochaeta
Subclass: Clitelata
Order: Haplotaxia
Suborder: Lumbricina
Superfamily: Lumbricoidea
Family: Lumbricidae
Subfamily: Lumbricinae
Genus and species: ***Eisenia fetida.***

There are two types of worms that create humus: epigeal and anecic. The epigeal, which include *E. Fetida, Perionyx excavatus, E. andrei, P. Sansibaricus* and *Eudrilus eugenie*, are surface-dwelling organisms that feed on organic materials and debris. The anecic, like as *Lumbricus terrestris,* dwell in vertical burrows (Del Aguila Juárez *et al*. 2011)

Fig. 1: *Eisenia fetida*

Vermiwash

Vermiwash is a mixture of micronutrients from soil organic molecules and bedding material with earthworm digestive fluid, mucus secretions, and calcareous layer (P Abesekara *et al*., 2006). Vermiwash is a by-product or necessary liquid organic fertiliser that also functions as a moderate biocide (pale yellow fluid). Vermiculture and vermicomposting components in the form of waste are gathered after water flows through a column of worm action and is really helpful as a foliar spray (Jaysawal, 2020). Vermiwash is a liquid vermicompost solution is extracted in the company of earthworms and contains a variety of plant growth hormones, enzymes, vitamins and micro and macronutrients that improve crop development and production and raise the crop's resilience to various diseases. The sustainability of soil and water is greatly threatened by widespread application of inorganic fertilisers, pesticides, and herbicides in today's agriculture system. In such a worrying circumstance, it's important to hunt for eco-friendly and efficient solutions (Prasanth and Vijaya Lakshmi, 2020).

Vermiwash act as liquid organic fertilizer

Such products would definitely contain the soluble plant nutrients, with the possible exception of few more enzymes, organic acids, growth hormones and earthworm mucus. It looks to have an essential quality that serves as both a moderate biocide and a liquid organic fertiliser. Vermiwash is a mix of earthworm secretion and washing. It is a drainage-like organic fertiliser made from vermiculture/vermicomposting units. The only unique equipment needed to collect the vermiwash is a tap that is installed at the base of the containers used for growing earthworms. Even during the period of routine vermiculture management, water is sprayed often to ensure proper moisture and surplus water that includes some crucial plant nutrients-is drained (Kumar Das *et al*., 2014). It includes excretory byproducts of earthworm secretions, coelomic fluid released by the worm through its dorsal pores, mucus, and enzymes. microbes, plant nutrients, vitamins, and chemicals that promote plant development. Vermicasts are a collection of excrement. They are rich in nutrients, especially soluble K, Ca, and Mg, which are incorporated into vermiwash. Auxin and cytokinin, as well as nitrate-fixing and phosphorus-solubilizing bacteria, are also present (Prasanth and Vijaya Lakshmi, 2020). It carries a high amount of enzymes, amino acids, Heterotrophic bacteria, fungi, actinomycetes including nitrogen fixers and phosphate solubilizers, Vitamins and hormones like Cytokinins, auxins and gibberellins, etc., Along with macro and micronutrients, Vermiwash used as a foliar spray Soluble Nitrogen, Phosphorus and Potassium source (https://www.agrifarming.in/vermiwash-preparation-process-benefits-cost)

Principle of vermiwash

The premise of Vermiwash is the idea that soils with worm infestations have burrows in them. These burrows are inhabited by drilospheres, which are tiny creatures resembling bacteria. The nutrients that are added to the water that flows through these tunnels are later absorbed by the plants. For the creation of vermiwash, this idea is utilised. By allowing water to trickle through earthworm tunnels made of substrate like leaf of coconut and cow dung stored in a plastic barrel and then the vermiwash may be created. The water is then collected in the vermicomposting system, where it is dripped into a barrel from a pot that is suspended above it. Both barrels and buckets may be used to build up vermiwash systems (Kaur and Kaur, 2017).

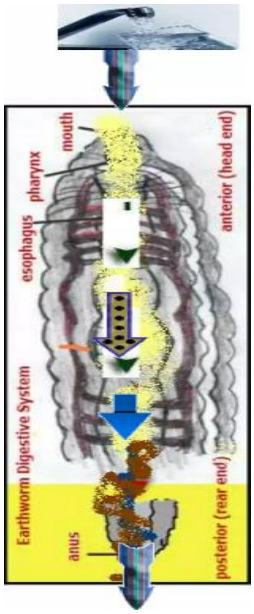

Fig. 2: Sprinkling of water to earthworm and passage
Source:(https://www.slideshare.net/ChidambaraManickam/final-vermiwashppt)

Worms species used

Species of red earthworms are used for profit composting or worm farming, due to their relatively high acceptance of environmental variations: Red wiggler – *Eisenia foetida.*

Preparation of vermiwash

To maintain the moisture level of the organic waste use for vermicompost preparation, waster is sprinkled to the vermicompost heap. The earthworms eat up the wet organic waste and thus some amount of water absorb by the earthworms. Later on the earthworms along with organic waste release this water. This yellowish liquid released by the earthworms is known as vermiwash. In general, vermiwash can be collected using following methods like ECO-Science research foundation, Ismail's method, Karunas method, Economic technique, KAU's method, Plastic drum method (1000 litre), Household device, Kales method and Fluid method

Among all these methods of vermiwash collection Eco-Science research foundation and Ismail's method are most commonly and commercially used.

Approach of Eco-Science Research Foundation (ERF)

This technique is based on the foliar spray principle the bacteria-inhabited burrows formed by the earthworm's movement in soils are known as drilospheres. Water movement through these burrows washes nutrients present in these drilospheres to the plant roots, which are then absorbed by the plant roots. This fundamental principle is applied in the ERF method of preparing vermiwash. In case of ERF method either barrels or buckets are used for setting up of vermiwash units. In these barrels, there is a 25 cm layer of pebbles or broken bricks, a 25 cm layer of coarse sand, and another layer of pebbles. These layers function as basic filter units. Above these layers a 30 to 45cm layer of moistened soil is placed and 50 numbers each of epigeic (surface) and anecic (sub-surface) earthworms are introduced. At the top of this layer cattle dung and straw is placed and gently moistened.

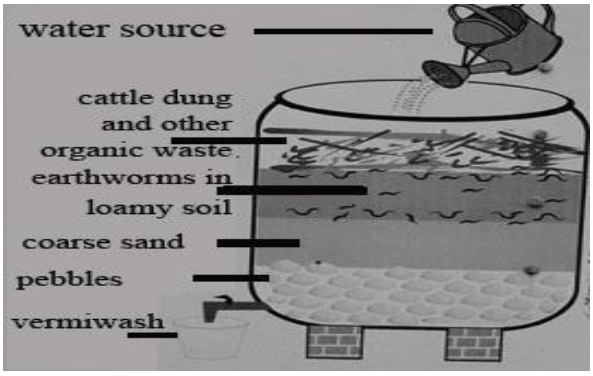

Fig. 3: Setting-up of vermiwash unit (ShiraniBidabadi, 2018)

Fig. 4: Collection of Vermiwash

The material is moistened on a daily basis, and the barrel's bottom tap is kept open. This procedure is repeated for up to 15 days, allowing the earthworms to decompose the organic material. On the 16th day, the bottom tap is closed and an earthen pot containing 5 litres of water with holes at the bottom is placed on top of the barrel, and the water is sprinkled overnight.

Collection of vermiwash

The bottom tap is opened in the morning, and vermiwash is collected. The cattle dung and straw are replaced on a regular basis, and this procedure is repeated every day for 10 to 12 months. Before being applied to the field, the collected vermiwash is diluted with water (10%). Another pot is kept under the stop cork and collect 3-4 liter of vermiwash each day (Meena and Karwal, 2021).

Heat stress method

Earthworms *Eisenia foetida* that were fully grown adults were removed from casting materials by putting them in a plastic tub for a few hours. After being carefully removed from the casting materials, the earth worms were placed in a glass beaker with 500 ml of warm (40℃ temperature) distilled water and stirred for 5–6 minutes. Warms weighing around 30 g were extracted. They were then quickly taken out and put into another presterilized plastic container that was already filled with room-temperature water. Here, the worms were carefully washed to remove any leftover excretory and secretory products that had adhered to the worm bodies. After that, the earthworms were returned to the stock culture container. The components of the glass beaker and the plastic beaker were combined, and the solution was maintained at 4℃ in a sanitized gloomy glass bottle for the purpose of the experiment (Karuna *et al*.1999).

KAU'S method

The system comprises of a plastic basin with a capacity of 20 litres, a plastic perforated waste paper basket, and a 30 cm long PVC pipe with a diameter of 5 cm. The waste paper basket is wrapped in a nylon net and set upside down in the centre of the basin. A hole is drilled at the bottom of the waste paper basket so that a 5 cm diameter PVC pipe may be inserted into the basin through 13 holes, with one end touching the basin. The PVC pipe is perforated so that leachate from the basin seeps through the basket and gathers in the PVC pipe, where it may be extracted using a kerosene pump. A pile of brick fragments in the basin outside the waste paper basket a layer of brick fragments is laid down, followed by a 2-3 cm layer of coconut fibre. After moistening these layers, 2 kg of worms (about 2000) are placed into it, followed by 4 kg of kitchen garbage. After one week, the kitchen waste has decomposed into a dark, well-

decomposed manure. After 24 hours, the accumulated leachate in the PVC pipe is evacuated by syphoning, a process known as vermiwash (www.kau.edu/pop/verm.compost.html) (Abesekara *et al*. 2006) .

Fluid method

Mature earthworms (1 kg) are gathered and agitated for 2 minutes in a trough containing 500 ml of luke warm water (37-40 ℃) to release adequate mucus and body fluid. Earthworms are removed and cleaned in 500 cc of room temperature water to remove mucus from their body surfaces before being returned to tanks. This also aids earthworms in recovering from shock. It is feasible to gather solely bodily fluid in this manner without injuring earthworms (Gupta, 2004) (source:https://www.vkgroupindia.in/blog/vermiwash)

Composition of vermiwash

The principal nutrients in vermiwash are soluble plant nutrients such as Nitrogen, Phosphorus, Potassium, Calcium, and micronutrients. Different types of hormones such as cytokinins, auxin, different amino acids, vitamins, enzyme cocktails of proteases, amylases, urease and phosphatase, some other secretions and many useful microbes such as heterotrophic bacteria, fungi, actinomycetes including nitrogen fixing bacteria like *Azotobacter spp.*, *Agrobacterium spp.*, *Rhizobium spp.*, phosphate solubilizers are present in the vermiwash. It is also a non-toxic and environmentally beneficial substance that inhibits bacterial development and produces a protective barrier to ensure their life and growth Vermiwash contains dissolved minerals and amino acids that are easily absorbed by plants. (Kumar Das *et al*. 2014).

Analysis of vermiwash and its units. (Source: https//www.erfindia.org/vermiwash.asp)

Table 1: Nutrient analysis of vermiwash

S. No	Parameter	Value	Unit
1	PH	7.39-7.5	-
2	Electrical conductivity	0.008 $\pm$0.001	(dSm-1)
3	Organic carbon	0.25 ± 0.03	(%)
4	Nitrogen	0.01-0.001	(%)
5	Phosphorous	1.70	(%)
6	Potassium	26	(%)
7	Sodium	8	(ppm)
8	Calcium	3	(ppm)
9	Copper	0.01	(ppm)
10	Iron	0.06	(ppm)
11	Magnesium	160	(ppm)
12	Manganese	0.60	(ppm)
13	Zinc	0.02	(ppm)

Microbial Composition of Vermiwash

Table 2: Microbial composition of vermiwash (Nayak IAS *et al*. 2019)

S. No	Microbes	Microbial count CFU/ml
1	Total heterotrophs	1.79×10^3
2	Nitrosomonas	1.01×10^3
3	Nitrobacter	1.12×10^3
4	Total fungi	1.46×10^3

Vermiwash's Influence on Soil Properties

Organic formulations could be an effective way to improve soil fertility. It also improves the number of microorganisms in the soil, which aids in the putrefaction of organic matter in the soil. Organic carbon, magnesium, calcium, and zinc levels were 0.73%, 1ppm, 5ppm, and 15.62ppm, respectively, in soil treated with vermiwash and vermicompost, followed by vermicompost alone organic carbon, magnesium, calcium, and zinc levels (0.64%, 0.64 ppm, 3.4 ppm, and 10.24 ppm, respectively) in soil treated with vermicompost alone. The effect of vermiwash prepared with leaf litter and cow dung as substrate and earthworm species *Lampito mauritii, Eisena foetida,* and *Eudrilus eugineae* on the chemical properties of rice soil was investigated (Ranjan and Prasad, 2021; Sachin and Indrayani, 2021).

Bioactive Compounds in Vermiwash

Bioactive metabolites are essential and non-essential molecules that occur naturally as component of the food cycle that have a therapeutic health effect on humans. Substances contained in tiny amounts throughout whole part of plant and foods such as fruits, vegetables, nuts, lubricants, oats, and millet are examples of bioactive compounds. Bioactive substances are secondary plant metabolites that are not needed for normal plant function but have a vital function in resistance, interest, communication, and competition and are hence usually referred to as secondary plant metabolites.

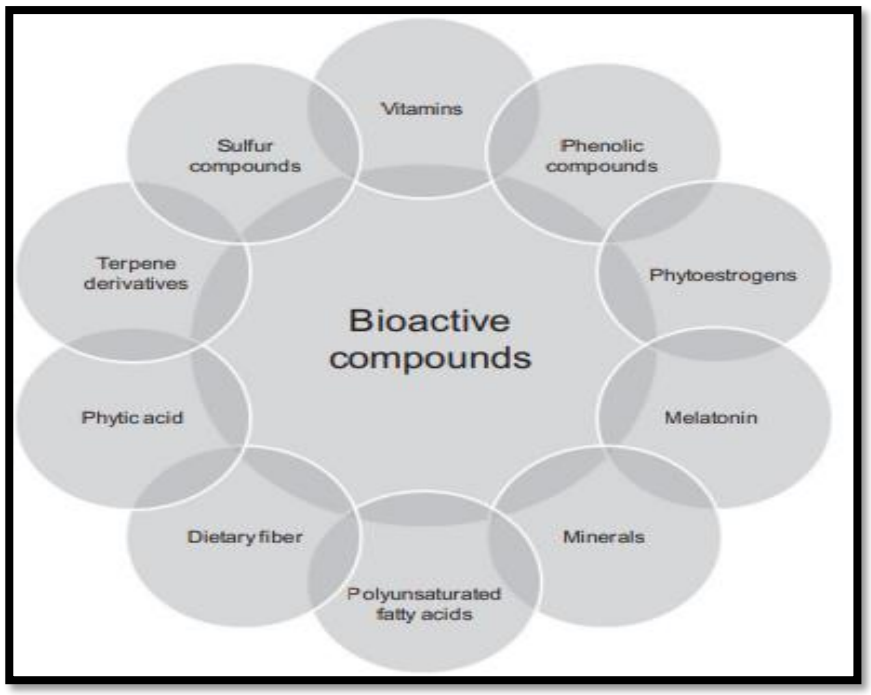

Fig. 5: Bioactive compounds found in leaf vegetable products (Barba *et al.*, 2014)

Variation between vermiwash drenching crop and soil based grown crop

During some variations are involves between vermiwash drenching and soil based grown crop such it can performing growth of crop and get more yield. Vermiwash is one of best liquid biofertilizer to plant it can be protect soil fertility and erosion .It could killed their pathogens and pests should be followed . The effectiveness of vermiwash on various growth factors of black pepper was demonstrated in a study. It has been observed that growthIn comparison to the control, vermiwash drenching had a significant influence on the parameters. Vermiwash is a

transparent pale yellow liquid bio fertiliser, according to chemical analysis. This bio-liquid contains a high concentration of nutrients and plant growth hormones.Vermiwash demonstrated potential application in agricultural sustainable development as a biopesticide and ecofriendly soil conditioner.(Sawant Konkan KrishiVidypeeth, 2019).As you might expect, the environments and innovative design for each of these investigations differ dramatically, and their creative choices have an implication on the analyses and their significance. Diverse research has demonstrated that leafy greens managed to grow with vermiwash drenching have decent efficiency than some of those grown with ordinary rhizosphere (P Abesekara*et al.*2006).

Useful Dosage

Root/stem dip: Plant seedlings are immersed in vermiwash solution for 15-20 minutes before transplanting. The solution must be diluted five times with water before being transplanted. Plant cuttings can also be immersed in the solution in this manner.

Foliar spray: Vermiwash is diluted with water five times before being sprayed over crops. It adds nutrients to the soil and aids in the treatment of plant disease.

Soil drench: Vermiwash is diluted roughly ten times with water and soaked into the soil to prevent some soil-borne infections due to its antimicrobial capabilities (Kaur and Kaur, 2017)

As an organic manure: Soil application increases nutrient absorption by plants, acting as a natural fertiliser for the crop.

As A growth promoter: Vermiwash is a fantastic growth booster that may also be used as a foliar spray. Excellent results require three to four applications of vermiwash.

Benefits of vermiwash

It is a biofertilizer or ecofriendly natural fertiliser that is fully organic and is obtained by decomposing organic waste. It has no chemicals.

- Vermiwash is a plant tonic that aids in the prevention of numerous plant diseases.
- 10% Vermiwash (100ml/1litr water) functions as a biopesticide as well as liquid manure. It increases soil physicochemical qualities such as soil texture and aeration. Because the organic matter content of the vermiwash is high, it improves the soil's water holding ability.
- Because it encourages cutting sprouting and root growth (Sachin and Indrayani, 2021)
- Vermiwash is a liquid fertiliser that has been shown to prevent pathogenic fungus mycelia development at 20-30% dilution.
- When diluted with 10% cow urine, neem extract, or garlic extract, it acts as a bio-pesticide. It has no negative effects on soil, plants, or the environment (Prasanth and Vijayalakshmi, 2020).

Conclusion:

Although chemically fertilised crops are widely utilised nowadays, people are becoming more conscious of the consequences of their use. These pollutants are causing serious health problems. As a result, organically grown fruits and vegetables are in high demand. Vermiwash is a non-toxic, ecologically friendly alternative. In crop cultivation, the wash is a brownish-red liquid that is both productive and protective. Vermiwash offers excellent 'growth stimulating' and 'pest killing' characteristics. It is a luxury, and its use in Utilized sustainability farming can result in an enormous enhancement in crop yield production. vermiwash is used for verticial system like Vermiponics and aquaponics, hydroponics systems may be installed wherever

possible to boost urbanised food production. It has the benefit of re-establishing sustainable activity without increasing land demand from urbanisation. The foremost component is lowering the distance between the food producer and customer. The current work using vermiponics provides an insight for future research on other agricultural species, which might aid in field application.

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CONCENTRATION OF TRACE ELEMENTS IN THE SOILS OF NORTH KASHMIR HIMALAYAS

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Abstract:

The present research work was an attempt to analyze the concentration of trace elements in soils and incidence of trace element related diseases in the mountain ecosystem of North Kashmir Himalayas. The study reveals that the soils of the study area have average concentration of iodine as 1.570mg/L with a considerable negative mean deviation of -0.030 mg/L from control samples. Average concentration of Zinc was 2.886mg/Kg with a high negative mean deviation of -0.864 mg/Kg from control samples. Average concentration of zinc shows negative deviations in all altitudinal zones with declining trend with altitude. Average concentration of copper in the soils of the study area was 5.439 mg/Kg with a negative mean deviation of -1.354mg/Kg from control samples. Average concentration of copper although shows negative deviations of -0.760 mg/kg from control sample in altitude zone A and a positive deviation of +0.240 from control samples in altitudinal zone B but again decreases with altitude with a negative deviation of – 2.205 mg/Kg in altitudinal zone C, - 2.349 mg/Kg in altitudinal zone D, -0.989 mg/Kg in altitudinal zone E and -3.123 mg/Kg in altitudinal zone F.

Keywords: Trace elements, Mean Deviation, Average concentration, Control sample North Kashmir Himalayas.

Introduction:

Trace elements are increasingly becoming recognized as vital to human health. Each trace element has a standard requirement adequate for human health recommended by W.H.O (Fishbein, 1986). All essential trace elements either in imbalance states (Warren, 1991) or in deficiency states (Pyle, 1979) are known to create serious health problems particularly in the areas where these are regionally deficit (Learmonth, 1988; Akhtar, 1991). Diseases due to trace element deficiencies as well as excess are known for iodine, copper, zinc, selenium, molybdenum, manganese iron, calcium, arsenic and cadmium (Lindh, 2005) and radon (Warren, 1991). Endemicity of many chronic or mild disorders such as goiter, dental fluorosis (Dissanayake, 1990) cancers (Akhtar, 1998), skin disorders, etc. is quite prevalent across India because of the variable concentrations of certain trace elements such as arsenic, fluoride, iodine, cerium, etc. (Dissanayake *et al.*, 2010).

The distribution of iodine is uneven in the biosphere. Both natural process such as leaching (Jeelani, 2010), volcanism (Buunnell *et al.*, 2007) and human activities such as mining and smelting (Keller, 1999) are responsible for redistribution of trace elements in the soils and water and organic environments (plants, animals and humans) through a network of pathways called as biogeochemical cycles (Furley and Newey, 1982). Its deficiency does not cause a mere enlargement of thyroid gland (endemic goiter), it can cause a variety of disorders called as iodine

deficiency disorders (IDDs) or thyroid disorders consisting hypothyroidism, endemic cretinism, still-births, mental retardation, defects in vision, hearing and speech, and neuromuscular weakness. These disorders are mainly found in those people who live in mountainous areas, previously glaciated areas. Even people living in coastal areas and on islands suffer from IDDs because sea salt does not contain iodine content as much as required by the people and due to their unsuitable habits (UNICEF, 2002).

People living in hilly areas are more prone to thyroid disorders as iodine from the soil is washed away leaving behind iodine deficient soils which ultimately effects human as well as animal health (Akhtar, 1978). Near about 70 percent of the world populations living in mountainous soils are at risk of iodine deficiency with endemic goiter. W.H.O. estimated that about 20 to 60 % of the world's population is iodine deficient (Zimmermann, 2009) with most of the burden in developing countries. In India about 41% of the population suffers from iodine deficiency and in the state of Jammu & Kashmir nearly 33% of the population suffers from iodine deficiency (Zargar *et al.*, 1996)). According to recent research conducted in 2013 on school children of Kulgam district, it was found that 18.9 % suffer from Total Goiter Rate (TGR); 21.2 % boys and 16.7 % girls (Khan *et al.*, 2014).

Study area

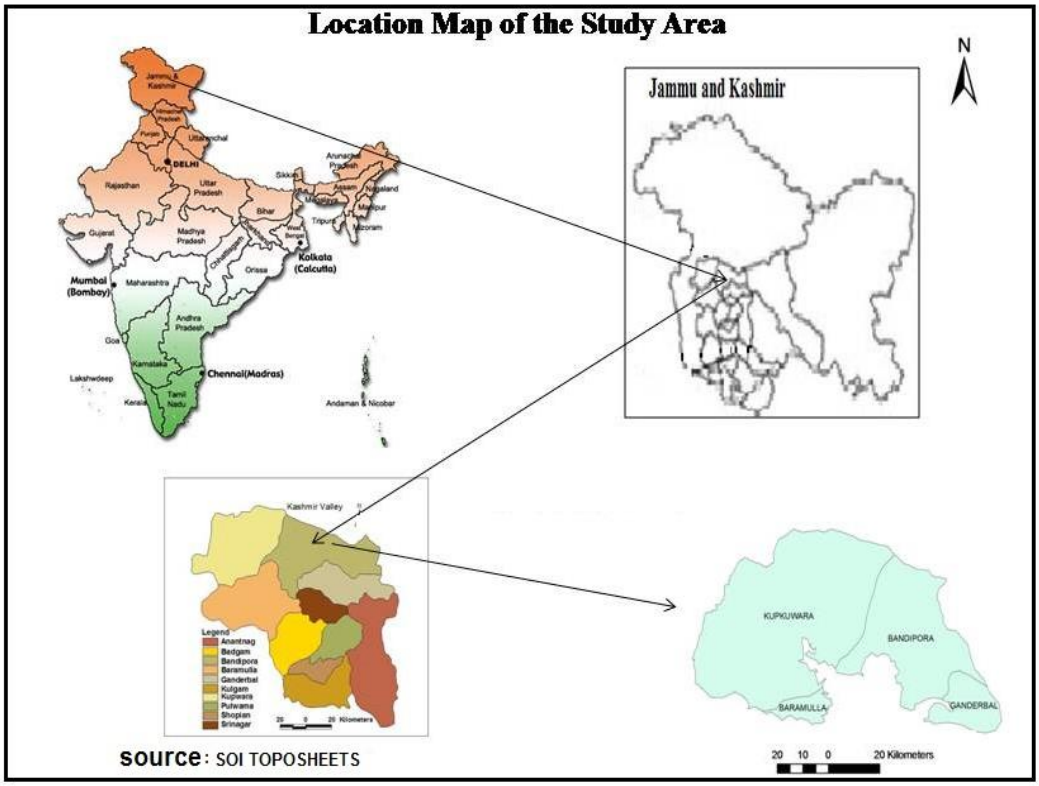

Figure 1: Location map (Generated from SOI toposheets, 1971)

North Kashmir Himalayas is a part of Great Kashmir Himalayas and lies between $34^0$16”15”.09 – 34^0 12’41.35” North Latitude and $74^0$48’00.00” – $75^0$05’09.58” East Longitude. The mountainous range has an average altitude of 2324 meters and stretches over an area of 5110.60 sq. Kms. It is at a point near Zojila that Great Kashmir Himalayas takes a blend towards the south west and is described as the North Kashmir range (fig.1). North Kashmir Range acts as

a water divide between Jhelum in Kashmir valley and Kishanganga of Gurez valley. The area experiences temperate climate with an average annual rainfall of about 1,230 mm (Raza *et al.,* 1978). Paleozoic sedimentary rocks, Triassic limestone, Karewa and Alluvium are the predominant geological formations of the area with limestone, shale, sandstone and unconsolidated sediments as the dominant lithology The soils of the concerned area vary in origin from alluvial to lacustrine and glacial.

Data base and methodology:

A comprehensive methodological framework has been adopted for the present research.

Data base

The present research work is based mainly on primary data and partly on secondary sources of data. The primary data include the Survey of India toposheets on 1:50,000 scale of 1971 year, the data regarding concentration of essential trace element iodine in soils was obtained from analysis of soil samples collected from 32 sample sites and 4 control samples. The Soil map of the area was taken from the Soil Map of Jammu and Kashmir prepared by Indian Council of Agricultural Research (ICAR), Nagpur-2010.

Methods used

The methodology has been divided into many steps based on the materials and techniques used and described under the following headings.

Delineation of study area Altitudinal zone mapping and Thematic Mapping

The study area was delineated from thirteen SOI toposheets of 1:50,000 scale of 1971 with numbers as (43 series, j/2, j/3, j/6, j/7/ j/10 /j/11, j/14, j/15, N/3, N/4, N/7, N/8 and O/1. The toposheets were processed under GIS environment with the help of Arc View 3.2a software.

The area under study is fairly large characterized by large altitudinal variations; it was divided into seven altitudinal zones (table 1).

Table 1: Altitudinal zones in North Kashmir Himalayas

Altitudinal Zone	Alt. in meters amsl	Area in Sq. Kms.	Area in % to total Area
A	1600-1750	499.18	9.76
B	1750-1900	308.43	6.04
C	1900-2050	269.87	5.28
D	2050-2200	286.23	5.61
E	2200-2350	275.50	5.39
F	2350-2500	293.19	5.74
G	2500 & Above	3178.20	62.18
	Total	5110.60	100

Source: SOI Topo-sheets.

Selection of sample villages, sample households and sample sites

Stratified Random Sampling technique was used for selection of around 15% of sample villages (32) and 20% of sample households (4000) in proportion to total number of villages and households from each altitudinal zone. From each sample village soil sample site was selected from major agricultural field (table 2).

Table 2: Sample Frame

Altitude Zone	Altitude (meters)	Area (Sq. Kms)	Revenue villages			Number of households			Soil	Control Samples
			Total Area	Sample	Sample (% age)	Total households	Sample	Sample (% age)		
A	1600-1750	499.18	32	4	12.5	3246	546	16.82	4	Soil = 4
B	1750- 1900	308.43	43	5	11.63	3422	678	19.81	5	
C	1900- 2050	269.87	51	8	15.68	5056	1026	20.3	8	
D	2050- 2200	286.23	42	6	14.28	3171	638	20.12	6	
E	2200- 2350	275.5	31	6	19.35	3534	782	22.13	6	
F	2350- 2500	293.19	18	3	16.67	1540	330	21.43	3	
G	2500-6000	3178.2								
Total		**5110.6**	**217**	**32**	**14.74**	**19989**	**4000**	**20.01**	**32**	**4**

Sample Collection and Sample Codification

An intensive and multi-faceted field work was undertaken to collect the soil water and staple food both cereals and vegetable samples. One soil sample was collected from each sample village making a total of thirty two (32) and one soil sample was collected from 4 control sites of Bandipora, Ganderbal, Kupwara and Baramulla having high agricultural productivity, good crop health and good human health status to make standard for comparative analysis. Soil samples were collected in clean unused polythene bags and were labeled properly. Soil samples were taken only from surface soil (depth of 0-30 cm) for the reason that these soils are under the cultivation of paddy, vegetables and maize and this is the major zone of root development of crops (Brady, 1991). All the samples were properly packed, coded properly and reached to the lab for trace element-iodine, copper and zinc analysis.

Proper codification scheme was followed for identification of the samples.

Codes were used as:

1st alphabet for Macro region; 2nd alphabet for Geo chemical component (S= Soil); 3rd alphabet as simply S for sample in all components

4th alphabet as a numerical number of the village (1,2,3.4 etc.); In case of control samples alphabet C for control is added.

Data Analysis and Map work

Data obtained from the analysis of soil from the RCRQA Laboratory, SKUAST-K and data obtained after tabulation of data from Questionnaire/ Schedule was statistically analyzed. Map work has been carried out under GIS environment.

Results and Discussions

Concentration of Iodine, Copper and Zinc in soils

The soils of the study area have average concentration of iodine as 1.570mg/L with a considerable negative mean deviation of -0.030 mg/L from control samples. The concentration decreases from an average concentration of 1.617 mg/L in altitudinal zone A to an average concentration of 1.524 mg/L in altitudinal zone F. Average concentration of iodine shows positive deviations of + 0.017 mg/L from control sample in altitude zone A and decreases with altitude to a negative deviations of -0.038 in altitudinal zone B, – 0.012 mg/L in altitudinal zone C, - 0.054 mg/L in altitudinal zone D, -0.082 mg/L in altitudinal zone E and -0.076 mg/Kg in altitudinal zone F (table 3 and figure 2). The reason could be that organic matter and pH influence the concentration of I in the soils because OM binds up I ions in the soil but the increasing slope and coarser texture cause I ions flow and translocate easily during rainfall. The study area being formed during Orogensis of Himalayas and subjected to tremendous transformation both by natural and human activities. Extensive water leaching of some acid arenaceous soils low in organic matter increases the magnitude of iodine losses and reduces the intrinsic availability of soil selenium and zinc. High soil acidity strongly potentiates crop uptake of almonium, iron and manganese (Mills, 1997).

Average concentration of Zinc in the soils of the study area was 2.886mg/Kg with a high negative mean deviation of -0.864 mg/Kg from control samples. The concentration decreases from an average concentration of 3.790 mg/Kg in altitudinal zone A to an average concentration of 1.160 mg/Kg in altitudinal zone F. Average concentration of zinc shows negative deviations in all altitudinal zones with declining trend with altitude. There was a positive deviations of +0.040 mg/kg from control sample in altitude zone A but decreases to a deviation of -0.513mg/Kg from control samples in altitudinal zone B,–0.913mg/Kg in altitudinal zone C, -1.010 mg/Kg in altitudinal zone D, - 0.196 mg/Kg in altitudinal zone E and -2.590 mg/Kg in altitudinal zone F (table 3). It is evident from the table 5.1 that the soils of the study area in all the elevations are deficient in Zn. The altitude zone A has high Zn content as compared to all other altitudinal zone because the former is characterized by relatively more total organic matter and favorable pH media than the latter. The reason could be altitudinal variation of OM and pH in the soils Since the soils at all the elevations are Zn deficit and the crops grown in these soils might be Zn deficient leading to its deficiency in human beings especially where people are more dependent on locally cultivated food items.

Average concentration of copper in the soils of the study area was 5.439 mg/Kg with a negative mean deviation of -1.354mg/Kg from control samples. The concentration decreases from an average concentration of 6.210 mg/Kg in altitudinal zone A to an average concentration of -3.847 mg/Kg in altitudinal zone F. Average concentration of copper although shows negative deviations of -0.760 mg/kg from control sample in altitude zone A and a positive deviation of +0.240 from control samples in altitudinal zone B but again decreases with altitude with a negative deviations of – 2.205 mg/Kg in altitudinal zone C, - 2.349 mg/Kg in altitudinal zone D, -0.989 mg/Kg in altitudinal zone E and -3.123 mg/Kg in altitudinal zone F (figure 2).

Table 3: Concentration of Iodine, Copper and Zinc in soils (*Concentration of I, Zn and Cu in control soil samples)

Altitudinal Zone	Sample Village	Sample Site	Concentration of trace elements with deviation from the Mean of control sample *			EC (ms) with deviation from the Mean of control sample *	pH with deviation from the Mean of control sample *	Org. Matter with deviation from the Mean of control sample *
			I (mg/L)	Zn (ppm)	Cu (ppm)			
A 1600-1750	Chatragul	GSS3	1.640 (+0.040)	2.730 (-1.020)	2.500 (-4.470)	1.40	7.38	1.50
	Ajas	BSS1	1.600 (0.000)	4.000 (+0.250)	4.300 (-2.670)	1.14	7.55	1.50
	Shirhama	KSS2	1.600 (0.000)	4.130 (+0.380)	8.780 (+1.810)	1.93	7.65	0.98
	Nadihu	BL SS4	1.630 (+0.030)	4.300 (+0.550)	9.270 (+2.300)	1.80	7.29	1.73
	Average	**-**	**1.617 (+0.017)**	**3.790 (+0.040)**	**6.210 (-0.760)**	**1.567**	**7.47**	**1.42**
B 1750-1900	Gund	GSS1	1.600 (0.000)	2.140 (-1.610)	5.850 (-1.120)	1.42	6.61	1.50
	Aragam	BSS2	1.640 (+0.040)	3.480 (-0.270)	5.600 (-1.370)	1.05	5.81	0.90
	Aloosa	BSS9	1.560 (-0.040)	2.730 (-1.020)	8.700 (+1.730)	7.50	6.99	0.75
	Shiltra	KSS7	1.500 (-0.100)	4.330 (+ 0.580)	10.00 (+3.030)	1.84	7.51	1.58
	Kalamabad	KSS4	1.510 (-0.090)	3.500 (-0.250)	5.900 (-1.070)	2.15	7.00	1.73
	Average	**-**	**1.562 (-0.038)**	**3.236 (-0.513)**	**7.210 (+0.240)**	**2.79**	**6.78**	**1.29**
C 1900-2050	Khanan	GSS4	1.600 (0.000)	2.20 (-1.550)	4.45 (-2.520)	1.39	6.82	1.65
	Arin	BSS5	1.650 (+0.050)	3.23 (-0.520)	4.23 (-2.740)	1.66	7.11	0.75
	Sumlar	BSS4	1.810 (+0.210)	3.460 (-0.290)	3.200 (-3.770)	3.60	7.32	1.58
	Haril	KSS6	1.560 (-0.040)	2.130 (-1.620)	5.450 (1.520)	1.04	6.41	1.88
	Mawar	KSS11	1.480 (-0.120)	3.160 (-0.590)	4.450 (-2.520)	1.65	7.29	1.50

	Indedeji	KSS12	1.490 (-0.110)	3.200 (-0.550)	5.570 (-1.400)	2.20	6.83	1.58
	Potwari	KSS13	1.480 (-0.120)	2.950 (-0.800)	4.000 (-2.970)	3.50	6.54	1.05
	Wanpur	BLSS3	1.640 +0.040)	2.370 (-1.380)	6.770 (-0.200)	5.98	7.13	1.13
	Average	**8**	**1.588 (-0.012)**	**2.837 (-0.913)**	**4.765 (-2.205)**	**2.63**	**6.93**	
D 2050-2200	Chitibandi	BSS3	1.600 (0.000)	3.020 (-0.730)	4.940 (-2.030)	1.48	7.04	1.73
	Malangam	BSS8	1.520 (-0.080)	3.020 (-0.730)	2.950 (-4.020)	12.60	7.46	0.75
	Waderbala	KSS5	1.570 (-0.030)	2.500 (-1.250)	4.600 (-2.370)	1.15	7.94	1.50
	Monbal	KSS9	1.510 (-0.090)	2.300 (-1.450)	4.770 (-2.200)	1.08	6.62	1.50
	Lache	KSS3	1.600 (0.000)	3.000 (-0.750)	3.300 (- 3.670)	1.50	6.20	1.88
	Potus	BLSS1	1.480 (-0.120)	2.600 (-1.150)	7.170 (+0.200)	3.65	6.91	1.50
	Average	**6**	**1.546 (-0.054)**	**2.740 (-1.010)**	**4.621 (-2.349)**			
E 2200-2350	Wangat	GSS5	1.780 (+0.180)	1.310 (-2.440)	5.500 (-1.470)	1.00	6.36	1.65
	Waniarm	GSS6	1.710 (+0.110)	2.090 (-1.660)	3.600 (-3.370)	1.18	6.39	1.50
	Mukam	BSS7	1.450 (-0.060)	2.380 (-1.370)	5.400 (-1.570)	1.34	6.82	1.73
	Rishwari	KSS10	1.500 (-0.100)	2.950 (-0.800)	5.500 (-1.470)	1.40	6.77	0.90
	Kandi	KSS1	1.540 (-0.060)	3.000 (-0.750)	9.000 (+2.030)	3.24	7.46	1.73
	Walthur	BLSS2	1.660 (+0.060)	0.550 (-3.200)	6.890 (-0.080)	2.12	6.89	0.75
	Average	**6**	**1.518 (-0.082)**	**3.554 (-0.196)**	**5.981 (-0.989)**			
F 2350-2500	Chont waliwar	GSS2	1.500 (-0.100)	0.100 (-3.650)	1.84 (-5.130)	2.30	7.85	1.65
	Kitsan	BSS6	1.550 (-0.050)	1.880 (-1.870)	4.80 (-2.170)	1.01	6.54	1.65
	Zachaldara	KSS8	1.520 (-0.080)	1.50 (-2.250)	4.900 (-2.070)	1.90	6.62	0.75
	Average	**3**	**1.524 (-0.076)**	**1.160 (-2.590)**	**3.847 (-3.123)**			
G 2500 & Above	Uninhabited							
Total	**32**	**32**	**1.570 (-0.030)**	**2.886 (-0.864)**	**5.439 (-1.354)**			

Control Soil Sample	Con. of I (mg/L)	Con. of Zn (ppm)	Con. of Cu. (ppm)	Ecms	pH	OC
GCSS	1.550	2.810	4.400	1.10	6.86	1.73
BCSS	1.680	3.640	3.370	0.80	7.62	0.90
KCSS	1.680	4.150	9.500	1.11	7.57	1.13
BLCSS	1.490	4.420	10.630	0.80	7.34	1.05
Average	**1.600**	**3.750**	**6.970**	**0.95**	**7.34**	**1.20**

Source: Data obtained through analysis of soil samples in RCRQA Lab, SKUAST-2014

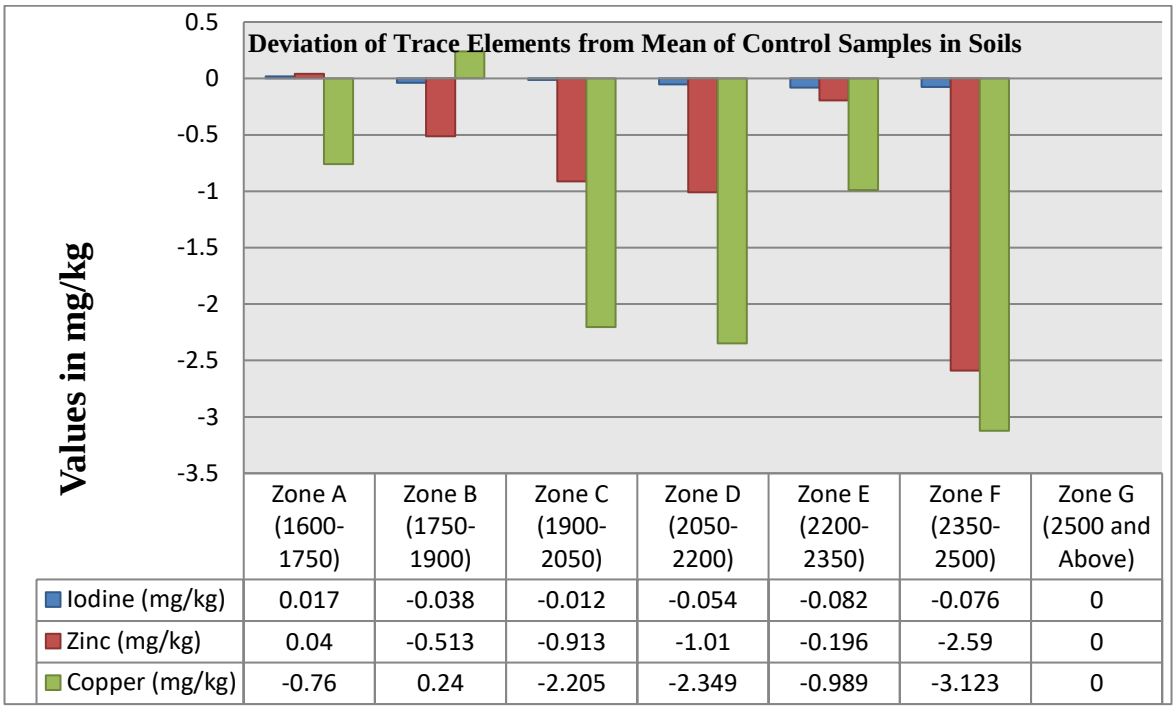

Figure 2: Concentration of Iodine, Copper and Zinc in Soils

The soils of the study area in all the altitudinal zones are deficient in Cu except zone B. The altitude zone B has high Cu content as compared to all other zones. The reason could be altitudinal variation of OM and Ph in the soils Since the soils at all the elevations are Zn deficit and the crops grown in these soils might be Cu deficient leading to its deficiency in human beings especially where people are more dependent on locally cultivated food items.

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