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Agriculture Science: Research and Review Volume IV

Editors

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PREFACE

We are delighted to publish our book entitled ***"Agricultural Science: Research and Reviews Volume IV".*** *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

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SEASONAL INCIDENCE OF SUCKING PESTS OF INDIAN BEAN, *LABLAB PURPUREUS* (LINN.) SWEET AND ITS NATURAL ENEMIES

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Introdution:

Indian bean, *Lablab purpureus* (Linn.) Sweet commonly known as hyacinth bean, Egyptian bean, dolichos bean or *sem* (Family: Fabaceae) is one of the most ancient crops among cultivated plants. It is presently grown throughout the tropical regions in Asia and Africa. It is a perennial herbaceous plant, occupies an important place among the fruit vegetable crops grown in the field as well as in kitchen gardens. It is primarily grown for green pods, while dry seeds are used in various vegetable food preparations. It is one of the major sources of proteins, minerals and dietary fiber. The green pods have a high nutritive value, comprising of protein 3.8 g, carbohydrate 6.7 g, vitamin-A 312 IU, mineral 0.9 g, fat 0.7 g and oxalic acid 1 mg in per 100 g. The foliage of the crop provides hay, silage and green manure. It is also grown for medicinal and ornamental purposes (Bose *et al.*, 1993).

It is well established fact that incidence of insect pests depends upon climatic conditions, crop growth stages, key weather parameters and natural enemies of a pest at a particular time. The interactions between pest activity with biotic and abiotic factors help in deriving predicative models that in turn forecast the pest incidence (Mrig and Singh, 1985; Dalwadi *et al.*, 2007 and Godwal, 2010). So, present study was in the direction to know the seasonal incidence of sucking pests and their natural enemies that occurred in Indian bean ecosystem and the most influential key abiotic factors that conditioned the pests under semi-arid conditions of Rajasthan

Material and Methods:

The materials used and methodologies adopted during the course of investigations on management of sucking pests of Indian bean, *Lablab purpureus* (Linn.) Sweet as envisaged in the plan of work has been described in detail here under**.**

General details

Location and site of experiments

The present investigations were conducted at Horticulture Farm, S.K.N. College of Agriculture, Jobner (Rajasthan) on Indian bean crop under field conditions during *kharif*,. Geographically, Jobner is situated at 75^0 28' East longitude, 26^0 06' North latitude and at an altitude (elevation) of 427 metres above Mean Sea Level (MSL). It falls under agro-climatic zone III A, the 'Semi-arid Eastern Plain' zone of Rajasthan.

Climate and weather conditions of location

The climate of this area is typically semi-arid, characterized by extremes of temperature both in summer and winter with low rainfall and moderate humidity. Maximum temperature in summer goes as high as 47 ^{0}C and minimum temperature in winter sometimes falls as low as 2-3^{0}C. The average annual rainfall of the locality varies from 400-500 mm occurring mostly from last week of June to September.

Preparation of land and manuring

The experimental field was thoroughly ploughed and cross ploughed with the help of mould board plough and cross-harrowing was done with tractor, followed by planking and leveling to bring the field to a good tilth. Fertilizers were applied at the rate of 20kg nitrogen ha^{-1} as starter dose and 40 kg P_2O_5 ha^{-1} in the soil before sowing the crop. The other cultural practices were done as and when required.

Seed and method of sowing

The seeds were sown @ 25 kg ha^{-1} in all experimental plots. Before sowing, seeds were treated with thiram @ 2.0 g kg^{-1} seed and also with rhizobium culture @ 20 g kg^{-1}. The seeds were sown by dibbling method keeping row to row and plant to plant distance of 60 and 30 cm, respectively.

Irrigation schedule and cultural practices

As rainy season crops need irrigation during long spell of drought for quick plant growth and formation of fruits, the Indian bean crop was irrigated at eight days interval. Gap filling was done during early stage of plant growth and row to row and plant to plant distance of 60 and 30 cm, respectively was maintained. The other recommended agronomical practices (weeding, hoeing etc.) were followed as and when needed.

Method of observations

The incidence of major sucking pests was recorded from appearance of pests till harvest of the crop. Observations on population of sucking pests were recorded on three leaves one each from top, middle and bottom canopy of the five plants selected randomly in each replications in

early hours (before 8.00 AM) at weekly intervals. The details regarding population counts of each pest has been described below:

Aphid, *Aphis craccivora* Koch

Aphid population was counted on the shoot of each of the five tagged plants in each plot. When the aphid population appeared, the observations were recorded early in the morning by visual counting method.

Jassid, *Empoasea fabae* (Ishida)

The population of jassids was recorded by counting both nymphs and adults as per method described by Rawat and Sahu (1973). In the initial stage of the crop, counting of jassids was done on whole plant and in later stage, on three leaves *i.e.* top, middle and bottom of each tagged plant.

Whitefly, *Bemisia tabaci* (Genn.)

The population of whitefly was counted visually on whole plant in the initial stage and in later stage, on three leaves from upper, middle and lower portion of each tagged plant. For counting the whitefly population, the leaf was held at the petiole by thumb and fore fingers and twisted until the entire under side of leaf became clearly visible (Butter and Vir, 1990).

Natural enemies

The estimation of natural enemies was recorded on five plants selected randomly and population per plant was calculated. Weekly data on different abiotic parameters were also recorded and were subjected to simple correlation studies.

Meteorological data

Meteorological data pertaining to minimum and maximum temperatures, relative humidity (RH) and rainfall were obtained from the meteorological section of Department of Agronomy, S.K.N. College of Agriculture, Jobner. The weekly mean meteorological observations have been presented in table 3.1 and fig. 3.1.

Harvesting

The green and tender fruits of marketable size were harvested manually at seven days interval.

Experimental details

To observe the response of different doses of nitrogen and phosphorus on the incidence of sucking pests of Indian bean, a field trial in Randomized Block Design was laid out at Horticulture Farm, S.K.N. College of Agriculture, Jobner. The variety, dolichus selection, most commonly grown in this area, was sown in the third week of July, 2013 in plots of 1.8 X 1.2 m^2

size keeping row to row and plant to plant distance of 60 cm and 30 cm, respectively. All the agronomical practices were done as and when required.

Result and Disussion:

A quantitative estimation of population build up of aphid, *Aphis craccivora* Koch, jassid, *Empoasea fabae* (Ishida), whitefly, *Bemisia tabaci* (Genn.) and its natural enemies were carried out in relation to abiotic factors, *viz.*, maximum and minimum temperatures, relative humidity and rainfall at jobner which is a true representative of the semi-arid region of Rajasthan to develop a management strategy in *L. purpureus* cropping system.

The data presented in the Table 1 showed that infestation of the aphids during *kharif* commenced in the first week of September (35th SMW), continued thereafter and reached to negligible level in the second week of November (45th SMW), before the crop matured. Initially, the population of aphids was recorded to be 1.33 aphids/plant which increased gradually and reached to peak 173.67 aphids/plant at 32.10 ^{0}C maximum, 21.80 ^{0}C minimum, 75.00 per cent relative humidity and 8.40 mm rainfall in the second week of October (41st SMW) after which a decline in the population was registered. The maximum and minimum temperatures, relative humidity and rainfall depicted a non-significant correlation with aphid population (r= -0.006, 0.114, 0.257 and 0.262, respectively). (Table 2).

The data presented in Table 1 revealed that the incidence of jassid commenced in the first week of September (35th SMW) and remained throughout the cropping season. Initially, the jassid population was 0.50/plant, gradually increased and reached to its peak (18.00/plant) at 34.20 ^{0}C maximum, 18.20 ^{0}C minimum and 55.00 per cent relative humidity in the third week of October (42nd SMW). Thereafter, jassids population declined from fourth week of October (43rd SMW) to second week of November (45thSMW) and ranged from 9.33 to 2.00 jassids/plant. The weather factors *viz.*, maximum and minimum temperatures, relative humidity and rainfall showed a non-significant correlation with jassid population (r= -0.004, 0.055, 0.177and 0.204, respectively). (Table 2).

Similarly the incidence of whitefly, *B. tabac*i commenced in the first week of September (35th SMW) and remained throughout the crop period. The population was 0.60/plant, which gradually increased and touched peak (19.50/plant) in second week of October (41st SMW) at 32.1 ^{0}C maximum, 21.8 ^{0}C minimum, 75.00 per cent relative humidity and 8.40 mm. Thereafter, it gradually decreased from third week of October (42nd SMW) to second week of November (45thSMW). The population declined in second week of November to negligible level *i.e.* 1.50/plant. The maximum and minimum temperatures and rainfall had non-significant

correlation with white flies population (r= -0.060, 0.430 and 0.437, respectively) whereas relative humidity showed a positively significant correlation (r= 0.605). (Table 2).

Beside the pest species, the incidence of predatory lady bird beetle, *Menochilus sexmaculatus* Fab. was also recorded during the cropping season. The incidence commenced in the third week of September (37thSMW) with a population of 0.60/plant. The population of the predator gradually increased and reached to peak (5.00/plant) at 32.1 ^{0}C maximum, 21.8 ^{0}C minimum, 75.00 per cent relative humidity and 8.40 mm rainfall in the second week of October (41st SMW), thereafter, their population started to decline from third week of October (42nd SMW) to first week of November (44thSMW) and ranged from 3.33 to 1.33/plant. The correlation matrix indicated a non-significant correlation with maximum and minimum temperatures (-0.013 and 0.220, respectively), relative humidity (0.374) and rainfall (0.301). However, its population showed a significant effect on the pest species (Table 2).

Table 1: Seasonal incidence of sucking pests and its natural enemies on *Lablab purpureus*

SMW*	Mean populations/plant**				Temperature (0c) (Max-min)		Average relative humidity (%)	Total rainfall (mm)
	Aphid	Jassid	White fly	*Menochilus sexmeculatus*	Max.	Min.		
35	1.33	0.50	0.60	0.00	32.90 -	22.80	70.00	0.00
36	5.00	1.00	1.60	0.00	33.90-	20.20	61.00	0.00
37	23.67	2.30	3.60	0.60	37.10-	22.70	55.00	0.00
38	43.50	5.50	7.33	1.33	33.50-	21.70	63.00	1.80
39	93.25	9.33	12.50	2.50	31.30-	23.70	79.00	19.00
40	125.33	12.67	17.00	3.67	31.50-	22.70	72.00	0.00
41	173.67	16.50	19.50	5.00	32.10-	21.80	75.00	8.40
42	138.50	18.00	9.50	3.33	34.20-	18.20	55.00	0.00
43	110.33	9.33	4.30	2.00	32.50-	14.20	51.00	0.00
44	45.67	6.50	3.67	1.33	31.30-	12.00	50.00	0.00
45	8.00	2.00	1.50	0.00	27.20-	13.30	61.00	5.20

* Standard Meteorological Week; ****** Mean of five plants

Table: 2 Correlation coefficient between the pest population, predator and weather parameters on *Lablab purpureus*

Particulars	Correlation coefficient (r)			
	Aphid	**Jassids**	**White fly**	***Menochilus sexmeculatus***
Maximum temperature	-0.006	-0.004	-0.060	-0.013
Minimum temperature	0.114	0.055	0.430	0.220
Relative humidity	0.257	0.177	0.605*	0.374
Rainfall	0.262	0.204	0.437	0.301
Menochilus sexmeculatus	0.976**	0.949**	0.939**	-

* Significant at 5% level ; ** Significant at 1% level

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RECENT ADVANCES IN PESTICIDE FORMULATION FOR ECO-FRIENDLY AND SUSTAINABLE CROP PEST MANAGEMENT

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Introduction:

In India, use of pesticides started from 1944 when 1st time DDT was imported and used for malaria control between 1948 to 1952 (Gupta, 2004)**.** Currently, there are 293 pesticides registered for use in India. Importance of continuous development in pesticide science is more in India because of predominant agricultural sector with about 75% of the population depending upon agriculture and allied fields and more people living in rural areas (Hazra and Purkait, 2019). Development and identification of new molecules pesticide has reached to almost at saturation point due to high demand.

Pesticide formulation is a process by which the active ingredient is combined with adjuvants to form a stable compound which can be easily stored, transported and applied by prescribed practical method in order to gain convenient, safe and effective method of pest control. There is an estimate that more than 65% of traditional pesticides with conventional formulations are not efficient due to repetitive use at a higher dose in achieving high bio-efficiency thus leading to environmental problems through some process including runoff, leaching and volatilization (Hazra and Purkait, 2019).

Some of the conventional formulated pesticides that are usually in wettable powder or emulsifiable concentrate forms reside in soil or groundwater for many years. The prolong degradation period has resulted in its accumulation in the food chain and hazardous effects on human, animal and environment. These pesticide formulations have been banned for any type of use through strict pesticide regulations in developed countries, but it is still dominantly used in many developing countries including India also (Lucas, 2019). Hence, water-based pesticide formulations using colloidal systems can be a good alternative in replacing the existing conventional formulation (Feng *et al.*, 2018).

Pesticides are toxic chemicals and using improper form of formulation makes it more dangerous to users. In India most of the users are often illiterate, untrained and lacking appropriate protective devices so risk get magnified here. The Poison Information Centre of National Institute of Occupational Health in Ahmadabad reported that organophosphorus pesticides with conventional formulation were responsible for the maximum number of poisonings (73%) among all agricultural pesticides (Dewan and Sayed, 1998).All the recent pesticide formulations have a view of reduction of the risks for the environment and the user of the pesticides (Meijis, 2008).

Following are the aspects of any pesticide which involves hazard assessment and determine the eco-friendliness of chemical - Physical-chemical properties of pesticide and active substance, Efficacy, Phytotoxicity, Toxicity, Residues, Fate and behaviour, Eco-toxicity (Meijis, 2008). Recent pesticide formulations are formulated with keeping all the above factors in mind. Hence recent formulations are more eco-friendly, less phytotoxic and having high application efficiency.

What is pesticide formulations?

A pesticide formulation is a mixture of chemicals which effectively control a pest. Alone active ingredient is unable to spray, penetrate and control the pest. Formulating a pesticide involves processing mixture of chemicals to improve its storage, handling, safety, application and effectiveness. Pesticide formulations play an important role in delivering pesticide to target sites and increasing their efficacy (Shao *et al.*, 2018).

Types of formulations:

Water based dispersion technology

- Microemulsion (ME)
- Suspension Concentrate (SC)
- Emulsion in Water (EW)
- Suspo-Emulsion (SE)
- Oil Dispersion (OD)

New dry product technology

- Water Dispersible Granules (WDG)
- Floating tablets

Control release technology

- Microencapsulation

Nanotechnology based formulations

Water based dispersion technology:

1. Microemulsion (ME):

Microemulsion is an optically isotropic and thermodynamically stable liquid solution with an ultramicroscopic droplet size and being completely water-dilutable (Shao *et al.,* 2018).The system is made up of oil, water, and surfactant(s) having optical transparency. In 1959, Schulman discovered new chemical formulation and proposed with name 'microemulsion'. The prefix "micro" was used in the sense of "very-small" without any direct link to the actual length scale (Tartaro *et al.,* 2020).They have a very fine droplet size of less than 0.10 microns (100 nanometres) (Hazra *et al.,* 2017).Small droplet size and lowered surface tension of the whole system produces higher pesticide application efficiency.

Microemulsions have relatively less active ingredient concentration, but the high surfactant content and solubilisation of the active ingredient give rise to increased biological activity (Feng *et al.,* 2005).The total concentration of surfactants for ME can be as high as 10–40% compared with about 3-5% for a typical oil in water emulsion(Hazra *et al.,* 2017), (Hazra, 2018). These high amount of surfactant makes it transparent. The preparation of microemulsions involves addition of two main different types of surfactants i.e. One water soluble (anionic having high HLB) and other oil soluble (ionic having low HLB) also one co-surfactant like hexanol with low HLB value (Hydrophile Lipophile Balance) is required (Pant *et al.,* 2016). New technology comprised of two non ionic surfactants i.e. alkyl polyglycoside and glycerol ester which can microemulsify a wide variety of oil-phase chemistries under a wide range of conditions (Greena and Beestman, 2007).

Commercial products:

Neemazal 30 ME, Pyrithiobac Na 5.4 + Quizalofop-P-Ethyl 10.6 ME etc.

2. Suspension concentrate or wet flowable (SC):

Suspension concentrate are the formulation in which the active ingredient is in the form of a stable dispersion of fine particles i.e. simply suspended particles in water. Hydrolytically stable and friable crystal compounds having low solubility in water or organic solvent are formulated in this form (Kumara and Sarkarb, 2018). This formulation consist of insoluble solid active ingredients dispersed (normally at high concentration) in water. The anti-settling agent is added to increase viscosity which prevents settling of ingredients and build up a three dimensional network which helps during long term storage. The antisettling agent normally used is swelling clay such as bentonite (sodium montmorillonite) (Knowles, 2008).

Long term storage stability, no settlement, easily pourable, easy dispersible in water these are some qualities of SC. In recent years, considerable attention has been given to the production of aqueous suspension concentrates by a high energy wet grinding processes such as bead milling (Knowles, 2008). Bead mills are one of the most popular and very effective methods for processing/grinding fine particles into sub-micron and nanomicron. In WP, dry grinding is done through a mechanical grinder such as a hammer type mill or by air milling.

Advantages:

No dust, no problem of toxicity or flammability due to solvents, low packaging volume, good efficiency due to the smaller particle size.

Example: Fipronil 5 SC, Carbendazim 50 SC, Sulphur 52 SC, Hexaconazole 10 SC etc.

3. Concentrated Aqueous Emulsions / Emulsion in Water (EW):

Emulsion in water (EW) consist of a dispersion of oil droplets in a continuous aqueous medium (Mulqueen, 2003).Volatile organic compounds (VOCs) are not safe for handling so Concentrated Aqueous Emulsions/ Emulsion in Water are now receiving considerable attention (Hiromoto, 2007). They can be considered as a safer and more environmental friendly alternative to EC. In EW the continuous phase is water instead of organic solvent used in EC.

In SC solid technical ingredient is dissolved in water but in EW liquid technical ingredient in dissolved in water. Emulsion is formed in EW at the time of mechanical processing, as in case of EC emulsion is formed upon addition to water at the time of tank mixing. EW are more compatible with SC for blending during application.EW is a precursor for suspo–emulsions (SE), which consist of a mixture of an oil-in-water and a suspension concentrate (SC) (Mulqueen, 2003). Emulsion in water (EW) creates smaller particle size than EC formulations, resulting in less drift.

Advantages:

Reduce phytotoxicity-ecotoxicity-dermal toxicity, higher flash point than EC, Good chemical stability, Ease to handling (Gasic *et al.,* 2012).

Example: Tricontanol 0.1 EW, Butachlor 50 EW, Cfluthrin 5 EW etc.

4. Suspo-emulsions (SE):

Mixed combination formulation is becoming more popular nowadays because of it's convenience, it ensures that the farmer applies correct amount of each component pesticide. Suspo-emulsions is mixtures of suspension concentrates and oil-in-water emulsions with added surfactants to prevent flocculation and thickeners to prevent separation of the dispersed phases

(Tadros, 1995). This formulation is popular to combine several types of solid-liquid, solid-solid or liquid-liquid technical compound into a single formulation (Mulqueen, 2003).

SE formulations are commonly used to combine two active ingredients having very different physical properties or solubility profiles into one formulation. This eliminates the disadvantage of tank-mixing incompatibility. SE is actually two different formulation combined into one single bottle.

Suspo-emulsion formulation consists of three phases-1) solid dispersed particles, 2) liquid oil droplets, 3) continuous phase, usually water (Knowles, 2008).

Advantages:

Active ingredients of different chemical and physical properties can be combined in one solution, Eliminate incompatibility during tank mixing, it provide a broad spectrum of pest control.

Example: Lambda Cyhalothrin-25.0 CS + Chloropyriphos-10.0 EW etc.

5. Oil Dispersion formulations (OD):

This is one of the latest formulation types. This technology allows very efficient and environmental friendly pesticide formulations. In OD, the solid active ingredient is dispersed in the oil phase. This formulation especially suitable for water sensitive or non-soluble active ingredients (Llacer and Jacas, 2012).

When OD comes in contact with water it creates milky emulsion or rarely suspo-emulsion. The oil-phase can comprise different oils such as vegetable oils, mineral oils or esters of vegetable oils (Hazra *et al.*, 2017). OD is also used for many other active ingredient due to good spreading and foliar uptake as the oil acts as an good adjuvant.

Advantages:

No need of water as solvent, suitable for those active ingredient which are unstable in water, penetration and retention period is high in oil.

Example: Cyantraniliprole 10.26OD.

New dry product technology:

1. **Water Dispersible Granules (WDG) or Dry Flowables (DF):**

Water dispersible granules are safer and more commercially attractive alternatives for wettable powders and suspension concentrates (Kim *et al.*, 2003). When user dissolves WDG in water it forms suspension solution. They are more attracting users because of their convenience in packaging, ease in use, being non-dusty, free-flowing granules which dissolve quickly when

added to water in the spray tank at the time of application. They represent a technological improvement over wettable powders (Hazra *et al.*, 2017).

The dispersion time of granules in water is a critical important property. To ensure that no problem occur in the spray tank , it is necessary for all the granules to dissolve completely within two minutes in varying range of degrees of water temperature and hardness (Hazra *et al.*, 2017).Wetting agent has a key role in this formulation which helps in disintegration and prevent nozzle blockage.

Water dispersible granular formulations are like WP formulations, except the active ingredient is formulated as granules. Water dispersible granules must be mixed with water to be applied. It requires continuous agitation to keep it suspended in water. (Kumara and Sarkarb, 2018).

Advantages:

Non-dusty so reduce inhalation hazard, High amount of active ingredient, Easy for handling as in solid phase.

Example: Captan 83 WG, Mancozeb 75 WG, Captan 83 WG, Cypermethrin 40 WG, Thiomethaxam 25 WG, Deltamethrin 25 WG etc

2. Floating Tablets:

The floating tablets are slow releasing pesticide tablets which after application in water bodies floats on the surface of water due to specific inert ingredients and low specific gravity. It continuously releases the active ingredient into the water. The floating tablets give a simple and practical remedy to overcome increased surface-residence time for the dosage form and sustained active ingredient's release. It can minimize the frequency of dosing required for mosquito's larva control and reduce variation in larvicidal concentration. Preparing the larvicides in a floating tablets can control the extent of bioavailability for many poor water-soluble active ingredients (Hazra, 2018).

Controlled release technologies:

1. **Microencapsulation/ Capsule Suspension (CS):**

Microencapsulation is a process in which minute droplets or particles (here active ingredients) of solid or liquid material are surrounded or coated with a continuous film of polymeric material (Pandya, 2012). In this method solids, liquids even gases can be enclosed forming a thin coating. Capsulated solid or liquid technical grade creates suspension with water as SC.The rate of release of the active ingredient can be controlled by adjusting the droplet size,

the thickness of the polymer membrane and porosity of the polymer (Fernandez-Perez, 2007). Higher concentration formulation is possible due to this technique. Larger volume of pesticide into a smaller volume of water is aim of microencapsulation.

Certain pesticidal combinations are antagonistic resulting in loss of efficacy for one, other or both the compounds. To overcome these effects the compounds must be applied separately or the rate of release adjusted to provide full efficiency. Controlled release is a more useful tool for this problem. Encapsulating one of the antagonistic material allow full biological efficiency when combined with the unencapsulated antagonized partner (Krause *et al.,* 2002).

Controlled release is now possible in liquid pesticides, molten lower melting point pesticides, and solid higher melting point pesticide crystals in the size range and volume demanded by pesticide products. (Beestman, 2003).

Advantages:

Prevent losses of active ingredient as a consequence of processes such as biodegradation, chemical degradation, photolysis, evaporation, surface runoff, percolating ground water also prevent inhalation or absorption through the skin (Fernandez- perez, 2007).

Example: Lambda-Cyhalothrin 25 CS, Lambda-Cyhalothrin 10 CS etc.

Nanotechnology-based pesticide formulations:

1. **Nano-emulsion:-**

Nano-emulsions have a particle size of less than 200 nm, which makes the systems inherently transparent and kinetically stable (Nair *et al.,* 2010).Nano-emulsion are colloidal dispersion system that are thermodynamically stable and composed of more than one liquid solvent with surfactant and co-surfactant (Gurpreet & Singh, 2018).As the particle size is very small, This formulation produces greater toxicity against insect pest than any other similar carrier formulation (Omar *et al.,* 2016).The nano-emulsion also can act as a coating layer for pesticides which provide protection against photodegradation and makes it stable(Nguyen *et al.,* 2013).

Pesticides formulated with nano-emulsions have low surfactant concentration than micro-emulsions (Kuzma & Verhage, 2010).This formulation systems brought many improvements in the agricultural sector for better efficacy towards antifungal activities and pesticide delivery systems (Mustafa & Hussein, 2020).Their attractive physicochemical properties such as nanosize particles have resulted in a larger surface area, thus enabling the release, accumulation and uptake of the active ingredients more effectively compared to other conventional formulations (Hazra, 2017).

Advantages:

This formulation shows better kinetic stability , enhances the solubility and dissolution of poorly water-soluble pesticides, low surface tension and good wettability which promotes to highly improved foliage adhesion with which the pesticide stay longer on leaves or other essential parts of the plant (Gin *et al.*, 2019).

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IN-VITRO APPROACHES FOR VEGETABLE CROPS IMPROVEMENT

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Introduction:

In an appropriate culture media and under regulated conditions, an entire plant can be regenerated from a small plant organ, tissue, or plant cells. Tissue-culture grown plants are the plantlets produced in this manner. Plant tissue culture is defined as "the technique of growing plant cells, tissues, and organisms in an artificially prepared nutrient medium, either static or liquid, under aseptic conditions." Tissue culture plants have disease-free growth, a more fibrous, healthier root system, a bushier branching habit, and a higher survival rate.

Tissue culture has a long history with vegetable crops, reaching back to the classic work of White (1934) and Caplin and Steward (1949). Techniques for micro propagation of most major vegetable species have been created as a consequence of the efforts of many researchers in this field. The majority of these procedures rely on vegetative meristematic tissue, however methods for regenerating plants from callus and protoplasts have also been established in various crops. Plant breeding programmes as well as basic genetic investigations rely heavily on haploids produced through anther/pollen culture. Haploid and doubled haploid lines generated by an effective anther culture procedure have now opened up new opportunities for genotype fixation and genetic research. Furthermore, it can be a valuable tool for molecular analysis and quantitative trait localization. Anther culture is the most practical, viable, and extensively used approach for haploid generation of the different ways available. Many species have benefited from anther culture, and it is well understood that the generation of haploid lines aids in the subsequent development of F1 hybrids (Arnison *et al*., 1990; Rotino *et al.* 1992). However, usefulness of this approach is limited in solanaceous species because some respond very poorly to anther culture (Yadav *et al.*, 1989).

When compared to current plant production technologies, using plants produced from tissue culture for field production of an edible product is not a viable alternative for most vegetables. This is partly owing to the fact that most vegetable crops are propagated from seeds. Seeds of these vegetables are typically abundant, making them comparatively affordable to produce when compared to the prices of micropropagation plants; also, seeds are robust, compact, self-sufficient, readily handled, and genetically stable. Micropropagation is not an economical solution for sick crops when asexual propagation is the established form of propagation (e.g. potato, sweet potato) due to the ease of obtaining a large number of plants by bedding or seed bits. Micropropagation of vegetable crops may currently hold the most potential as an addition to existing seed technology (e.g., in the creation of certain parental genotypes for hybrid seed production) and in specific cases when standard propagation methods for a given species provide difficulties.

History of plant tissue culture

- The aseptic cultivation of cells, tissues, organs, and their components in vitro under defined physical and chemical conditions is known as plant tissue culture. Gottlieb Haberlandt proposed the theoretical underpinning for plant tissue culture in his address to the German Academy of Science in 1902, based on his single-cell culture experiments. Gottlieb Haberlandt, a German botanist, introduced the concept of artificially culturing isolated *Tradescantia* cells in 1902. Despite the fact that his experiment failed to cause the cells to divide. The cell lived for 3 to 4 weeks. Haberlandt is known as the "Father of Tissue Culture" as a result of his efforts. Above all, he proposed the concept of totipotency.
- Henri-Louis Duhamel du Monceau (1756) was the first to experiment with wound healing in plants by forming a spontaneous callus (an unorganised mass of cells) on the decorticated region of elm trees.
- Vochting (1878) the presence of polarity as a fundamental element guiding the development of plant fragments has been hypothesised. He saw that the upper section of a stem always generated buds, while the lower portion always produced callus or roots.
- Organ culturing was attempted between 1902 and 1930. Hannig (1904) isolated crucifer embryos that developed well in mineral salts and sugar solutions.
- Simon (1908) grew a bulky callus, buds, and roots from a poplar tree on the surface of a medium containing IAA, which promoted cell division.

- The identification of auxin as a natural growth regulator and the awareness of the relevance of B-vitamins in plant growth were two key discoveries discovered in the mid-1930s that gave a big push to the development of plant tissue culture technology.
- Gautheret cultured cambium cells from a variety of tree species (*Salix capraea*, *Populus nigra*) in Knop's solution containing glucose and cysteine hydrochloride in 1934 and found that they multiplied for a few months.
- Gautheret obtained the first real plant tissue cultures from Acer pseudplatanus cambial tissue. Using an agar-solidified medium of Knop's solution, glucose, and cysteine hydrochloride, he also had success with *Ulmuscampestre*, *Robiniapseudoacacia*, and *Salix capraea* explants.
- Gautheret established the first continuously developing tissue cultures from carrot root cambium in 1939.
- White (1939a) described the production of comparable cultures from tumour tissue of the hybrid *N. glauca* × *N. langsdorffii*.
- Then the possibility for cultivation of plant tissues for unlimited period was announced simultaneously by P.R. White (1939) and R.J. Gautheret (1939).
- During the 1930s and 1940s, Gautheret and White were crucial for inventing the media composition we use today. Raghavan and Torrey (1963), Norstog (1965), and others continued to work on developing synthetic media for the culture of younger embryos.
- Suitable nutritional media for the cultivation of plant cells, tissue, protoplasts, anthers, root tips, and embryos were produced between 1940 and 1970. Plants were always successfully morphogenesis (regeneration of a whole plant from cultivated tissue) in vitro.
- In 1957, Skoog and Miller put forth the concept of hormonal control of organ formation.
- Murashige was crucial in elevating in vitro culture techniques to the position of a viable practical method for propagating horticultural plants. He worked hard to popularise the approach by inventing standard methods for in vitro multiplication of a variety of species, including ferns, leaves, flowering plants, and fruit trees.
- The discovery of kinetin by F. Skoog, C.O. Miller, and co-workers in 1959, as well as the proof of induction of shoot regeneration in tobacco callus, paved the door for plant multiplication via tissue culture.
- In 1960s, E. Cooking for the first time developed a method for isolation of protoplasts in large quantities using the fungal enzyme obtained from *Myrothecieum sp.*

Indian history

- In India, Panchanan Maheshwari, the father of embryology in India, began work on tissue culture in the mid-1950s at the Department of Botany (University of Delhi).
- Jones et al. developed a microculture method for cultivating single cells in conditioned medium in hanging drops in 1960.
- The first plant from a matured plant cell was regenerated by Braun in 1959.
- During the 1960s, the Botany School at the University of Delhi, led by P. Maheshwari, began involved in the in vitro production of flowering plant reproductive organs.
- Kanta, 1960 developed the technique 'intraovarian pollination' and 'test-tube fertilization'.
- For morphogenic research involving ovary, embryo, endosperm, ovules, and other tissues, several tissue culture approaches were used.
- Sipra Guha Mukherjee and S.C. Maheshwari (1964-67) produced the haploid for the first time at the University of Delhi using anther and pollen cultures.

Advantages of tissue culture

There are several advantages to using the tissue culture process. These are as follows -

- The new plantlets can be grown in a short amount of time.
- Only a small amount of initial plant tissue is required.
- The new plantlets and plants are more likely to be free of viruses and diseases.
- The process is not dependant on the seasons and can be done throughout the year.
- You need only a relatively small space to perform the process (ten times the plants in one-tenth of the space).
- On a bigger scale, the tissue culture technique aids in the delivery of novel subspecies and variety to the consumer market.
- People who want to grow difficult plants, such as specific orchid varieties, find that tissue culture works better than regular soil.

Disadvantages of tissue culture

- Tissue Culture can require more labour and more money.
- There is a chance that the propagated plants will be less resilient to diseases due to the type of environment they are grown in.
- It is critical that the material be tested before being cultured; failing to do so could result in the young plants becoming sick.

- While the success rate is high when the proper protocols are followed, tissue culture success is not guaranteed. There's still a danger that the process will cause a secondary metabolic chemical response, stunting or even killing the new explants or cells.

Application of plant tissue culture

Plant tissue culture work is frequently done for a specific purpose because it requires expensive apparatus, technical expertise, and a large capital cost (especially for commercial setup), which is described as follows:

Crop Improvement / Plant Breeding

- **Haploid production:** For producing homozygous plants, reduction of time involved in conventional breeding. (e.g., Anther / ovule / pollen culture)
- **Triploid production:** For production of seedless fruits (e.g. Endosperm culture)
- **In vitro pollination and fertilization:** In the creation of distant hybrids, to overcome the barrier of sexual incompatibility between distinct species or genera of plants.
- **Zygotic embryo culture:** To rescue the embryo after fertilization from abortion
- **Somatic hybridization and cybridization:** To develop inter-generic hybrids, or to develop hybrids between plants that are not crossable with each other
- **In vitro mutagenesis:** Mutagen is applied at cell-level usually with an objective to develop new varieties of crops, with novel traits, through mutagenesis.
- **Somaclonal and Gametoclonal variant selection:** At the cell or tissue level, selection pressure is applied, and tolerant/resistant cell types are chosen for regeneration. The regenerated plants should be tolerant of / resistant to the imposed selection pressure. (e.g., disease/toxin resistance, increased amino acid content, etc.)
- **Genetic Transformation:** Genes from other organism are used for development of transgenic plants using this technique.

Horticulture / Forestry

- Disease-free plant production: For disease-free plant/tree production, such as virus-free plant production (Meristem culture).

Micropropagation

- To propagate a vast number of plants on a large scale in a short period of time. Seasonal production is not required and can take place at any time of year.

General

- Cryo-preservation: Long and medium term storage of germ plans
- Secondary metabolite production: In vitro procedures are used to produce very useful secondary metabolites.

General technique of plant tissue culture

Plant cell, tissue, and organ culture techniques are nearly identical, with minor variations for different plant materials. For the regeneration of a whole plant from an explant cultivated on nutrient medium, there are a few basic processes to follow.

These basic steps for in-vitro culturing of plants are:

(a) Selection and Sterilisation of Explant:

Suitable explant is selected and is then excised from the donor plant. Explant is then sterilized using disinfectants.

(b) Preparation and Sterilisation of Culture Medium:

A suitable culture media is created, paying particular attention to the culture objectives and the type of explant to be cultured. The sterilised culture medium is placed into sterilised jars and autoclaved.

(c) Inoculation:

Sterilized explant is inoculated (transferred) on the culture medium under aseptic conditions.

(d) Incubation:

The cultures are subsequently incubated in the culture room, which has the proper lighting, temperature, and humidity for effective culturing.

(e) Sub culturing:

Cultured cells are transferred to a fresh nutrient medium to obtain the plantlets.

(f) Transfer of Plantlets:

Plantlets are transported to green houses or pots after the hardening process (i.e., acclimation of plantlets to the environment).

New approaches for crop improvement

Plant material can be cultured in a variety of ways. These approaches differ in terms of the explants employed and the products that result. Some of the most popular and advantageous methods in plant tissue culture are discussed below**:**

1. Cell Culture:

The technique of creating clones of a single cell is known as cell culture. Cell clones are cells that have been produced from a single cell by mitosis and are identical to each other and to the original cell. Haberlandt made the first experiments at cell culture in 1902. He was unable to culture a single cell, but his efforts inspired other researchers to pursue this path.

Cell culture is superior to other ways of culturing because it is the most effective way to study and comprehend cell metabolism and the impact of various chemical compounds on cellular responses. Through the application of genetic engineering techniques to higher plants, single cell culture is a huge assistance in crop development programmes.

The method of cell culture is done by following three main steps:

(a) Isolation of single cell from the intact plant by using some enzymatic or mechanical methods.

(b) In-vitro culturing of the single cell utilizing micro chamber technique, or micro drop method or Bergmann cell plating technique.

(c) Testing of cell viability done with the phase contrast microscopy or certain special dyes.

It's worth noting that cell cultures require a nutrient medium that's appropriately enriched, and that they should be done in the dark because light can harm the cell culture. Large-scale plant cell culture under in-vitro conditions is an effective method for producing a wide range of commercially significant phytochemicals.

2. Suspension Culture:

Suspension culture is a type of culture that comprises of cells or cell aggregates that are formed by placing callus tissues in an agitated liquid media. During a suspension culture, appropriate equipment called a shaker is used to continuously agitate the liquid medium, the most popular of which is the platform/orbital shaker.

Shaker agitation is vital because it breaks down cell aggregates into single cells or smaller groups of cells, allowing single cells and groups of cells to be distributed uniformly in the liquid medium. A excellent suspension is one in which there are more single cells than groupings of cells. Changes in the nutritional makeup of the medium could also be used to break up bigger cell clumps.

Batch and continuous cultures are the two types of cultures used in suspension culture. A batch culture is a suspension culture in which cells grow in a restricted amount of culture medium, gradually depleting the medium. A continuous suspension culture, on the other hand, is one that is continuously fed with nutrients through the input of fresh medium while maintaining a consistent culture volume.

3. Root Culture:

During the 1920s, Robbins and Kotte made pioneering experiments in root cultivation. Many workers attempted to create viable root cultures later on. White was the first to effectively nurture continuously developing tomato root tips in 1934.

Following that, root cultivation of a variety of angiosperm and gymnosperm plant species has been effective. Root cultures aren't always useful for producing whole plants, but they do have their own value. They provide useful information about nutritional requirements, physiological activity, nodulations, infections by various pathogenic bacteria or other microorganisms, and so on.

4. Shoot Culture:

Shoot cultures offer a wide range of applications in horticulture, agriculture, and forestry. Morel and Martin (1952) proposed the practical application of this technology after successfully recovering the entire Dahalia plant from shoot-tip cultures. Morel later realised that the technique of shoot culture could be a useful tool for quick plant multiplication (i.e. Micro propagation). The shoot apical meristem is cultivated on a suitable nutritional medium in this method. Meristem Culture is another name for this.

The region of a shoot's apical meristem that lies beyond the youngest leaf *primordium* is known as the apical meristem. Tissue culture techniques can also be used to recover pathogen-free plants, particularly virus-free plants, using meristem tip culture. The commencement of culture, shot multiplication, rooting of shoots, and finally the transfer of plantlets to pots or fields are all stages in this culture process.

5. Protoplast Culture:

A protoplast is a plasma membrane bound vesicle that comprises of a bare cell produced after the cell wall has been removed. Mechanical or enzymatic procedures can be used to remove the cell wall. In-vitro protoplast culture has a wide range of applications in plant biotechnology. It can be used not only for genetic alteration but also for biochemical and metabolic research in plants. Protoplast cultivation begins with the isolation of protoplasts from plants via a chemical or enzymatic technique.

Currently, a number of enzymes are available that allow protoplasts to be isolated from practically any plant tissue. After protoplasts have been isolated, they are purified and viability assessed. Finally, the live protoplasts are cultivated in vitro using an appropriate nutrient medium, which is commonly a liquid or semisolid agar media.

6. Haploid Production:

Haploid plants have half as many chromosomes as diploid ones (denoted by n). In investigations involving experimental embryogenesis, cytogenetics, and plant breeding, haploids can be beneficial. Haploids are extremely important in plant breeding and genetics. They are particularly valuable as a source of homozygous lines.

Furthermore, in-vitro haploid generation contributes in the development of genetic variabilities, disease resistance, salt tolerance, insect resistance, and other traits. At the moment, the focus is on increasing the frequency of haploid creation in order to better utilise them for economic plant improvement.

There are two approaches for in-vitro haploid production:

(a) Androgenesis:

Androgenesis refers to the process of producing haploids from anther or microspore culture. It is the method of choice for producing haploids on a big scale using tissue culture. The androgenesis approach for haploid generation is based on the in vitro development of a plant's male gametophyte, or microspore, which results in the production of a full plant. Another culture or microspore (pollen) culture is used to achieve this.

For practical reasons, the technique of another culture is a faster and more efficient form of haploid generation. Plantlets can, however, come from several other sections of the anther during another culture (along with from the pollens). Microspore cultivation, on the other hand, is free of any uncontrollable anther wall or other tissue effects. Microspore culture is an excellent tool for researching mutagenesis and transformation patterns.

(b) Gynogenesis:

It's a different way to make haploid cells in the lab. It is the process of creating a haploid plant from ovary or ovule culture. Gynogenesis for haploid generation has only been successful in a few plants so far, and as a result, it is not a particularly popular method for in-vitro haploid production. As a result, androgenesis takes precedence over gynogenesis.

7. Embryo Culture:

Embryo culture is a procedure that includes isolating and growing an embryo in vitro to produce a fully functional plant. Hannig achieved the first embryo culture success in 1904 when he separated and cultivated embryos from two crucifers, *Cochleria* and *Raphanus*. For the generation of hybrid plants, embryo culture is widely utilised in agriculture, horticulture, and forestry.

This technique enables for in-depth research on the nutritional needs of embryos at various stages of development. It also aids in determining the ability of embryos to regenerate. Embryo cultivation is useful for in-vitro plant micropropagation, seed dormancy removal, and the creation of beneficial haploid plants.

8. Endosperm Culture (Triploid Production):

Endosperm tissue is triploid, plantlets derived from endosperm culture are also triploid. The endosperm tissues are present in the majority of flowering plant families (with the exception of Orchidaceae, Podostemaceae and Trapaceae which do not have endosperm). After the double fertilisation of one male nucleus with two polar nuclei, endosperm is created. In culture, immature endosperm has a greater potential for growth, particularly among grains.

Endosperm culture has provided a fresh approach for producing triploid plantlets in plant breeding and horticulture. It's a simple way for making a big number of triploids in a single step. Furthermore, it is far more convenient than traditional procedures for triploid induction, such as chromosome doubling by crossing tetraploids with diploids. Plant species such as *Populus, Oryzasativa, Emblica officinalis, Pyrus malus, Prunus,* and others have produced full triploid endosperm plants.

Triploid plants are usually seedless, this technique is ideal for boosting the commercial value of fruits such as apple, mango, grapes, watermelon, and other similar fruits. Endosperm cultivation is also useful for investigating the production and metabolism of some natural compounds, in addition to the applications listed above.

Conclusion:

Tissue culturing is a critical component of applied biotechnology. The world's population will continue to grow in the future decades, requiring additional lodging space and agricultural lands. Global climate change is also a factor to consider. Keeping this in mind, we must secure a peaceful, healthy, and hunger-free environment for our children and grandchildren. Plant tissue culture is the only option for accomplishing this.

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STATUS OF ARBUSCULAR MYCORRHIZAL FUNGAL SPORULATION IN RHIZOSPHERIC SOIL OF *TOMATO PLANT* (*SOLANUM LYCOPERSICUM* L.)

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Abstract:

The present study was performed to isolate, identify and classify the AM fungal spores associated with rhizospheric soil of *Solanum lycopersicum* L. at five different locations in Tiruchirappalli district of Tamil Nadu, India. Spore and sporocarp were obtained from rhizospheric soil samples using wet-sieving method. Rhizosperic soil sample from five different locations were subjected to the recovery of AM fungal spores. The mean total average of spore density ranged from 214 to 511. To identify the spores at their species level by their morphological characters. We identified 13 species representing 8 genera belonging to 4 families. 5 genus from the family Glomeraceae i.e. *Glomus*, *Funneliformis*, *Septoglomus*, *Rhizoglomus*, *Dominikia*. 2 species of each from *Glomus* & *Septoglomus*. They are *Glomus microcarpum*, *Glomus sinuosum* and *Septoglomus constrictum*, *S. viscosum.* 4 species from *Rhizoglomus*, *Rhizoglomus aggregatum*, *Rhizoglomus fasciculatum*, *Rhizoglomus irregulare*, *Rhizoglomus*, *microaggregatum.* 1 species of each from *Funneliformis & Dominikia* i.e. *Funneliformis mosseae* and *Dominikia minuta. Claroideoglomus etunicatum* from Claroideoglomeraceae, *Acaulospora rehmii*, from Acaulosporaceae. *Gigaspora decipiens* from Gigasporaceae. The results revealed that the genus *Rhizoglomus* was seemed to be dominated among the other 7 genera. This could due to high sporolation capability and more viability of the species. Therefore the environmental conditions prevails in Tiruchirappalli is suitable for the sporulation of the genus *Rhizoglomus* which can be influence the yield of *Solanum lycopersicum L.*

Keywords: Arbuscular mycorrhizal fungi, *Solanum lycopersicum*, Species identification, Spore isolation, Spore density.

Introduction:

Mycorrhiza could be a symbiotic association between plant and fungus. They are very important in the uptake of nutrients such as P, N, K, Cu, Zn and Ca by plants especially in soils low in these nutrients. The filaments or hyphae of mycorrhizal fungi enhancing the water holding capacity of soil and promote drought resistance of the plant partner. Mycorrhiza will increase the speed of chemical action and thence improves plant growth, productivity and yield. In the spores of mycorrhizal fungi which become dormant during the unfavorable conditions and are germinated at the favourable conditions returns. Thus, they're higher equipped for combating the unfavorable conditions and have longer shelf lives as compared to the microorganism primarily based product.

There are many types of mycorrhizal fungi among them Arbuscular Mycorrhizal Fungi (AMF) are ubiquitous in their distribution and occur abundantly (Harley and Smith, 1983). They are found in rhizosphere of several vascular plants and have vital roles on sustainable agriculture also as agricultural ecosystem's management. Almost 90 % of crop plants are mycorrhizal mostly of arbuscular type, that help to increase the crop yield and quality with additional use of nutrient inputs, reduced need for pesticides (Hindumathi *et al*., 2011). The beneficial effect of indigenous AM fungi on the nutrition of agricultural crops depends on both the abundance and sort of fungi present within the soil (Abbott and Robson, 1982).

Different functionally diverse AM fungi can simultaneously colonize a single root segment. To understand the ecology of plant fungus interaction in natural ecosystems it is important to identify Arbuscular mycorrhizal fungi that specifically colonize roots of plant species. Therefore, field study is very important to grasp abundance and kind of native AM fungi present in the rhizosphere of the crop. In this view the present study was undertaken to isolate, identify and classify the AM fungal spores associated with rhizospheric soil of *Solanum lycopersicum* L. at five different locations in Tiruchirappalli district of Tamil Nadu, India.

Materials and Methods:

Collection of rhizospheric soil and root samples

Five different locations of Tiruchirappalli district, where the agricultural activity is comparatively more were selected for the present study. From the five study locations, initially four rhizospheric soil of *Solanum lycopersicum* L. from each location were randomly selected. A good amount of rhizosphere soil and root samples were collected with the help of a widger by lifting up gently a block of rhizosphere soil and placed in sterile polythene bags and transported to the laboratory. Root samples were separated from adhering soil, washed gently under tap

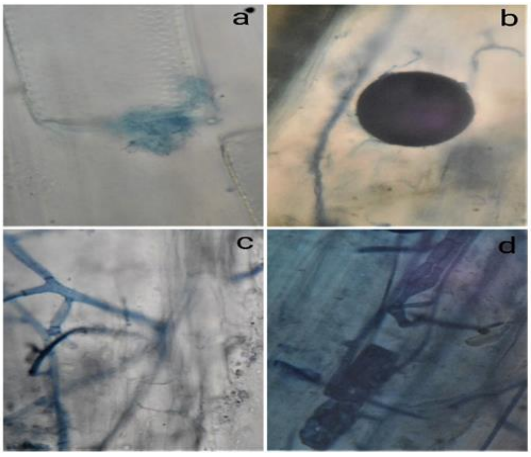
water and stained within 24 h or preserved in formalin-acetic acid-alcohol (FAA) before staining for the estimation of AM fungal colonization.

Analysis of soil samples

Soil pH was measured in the soil: water (1: 2) suspension using a digital pH meter (L1 model, Elico, India). Electrical conductivity (EC) was measured at room temperature in a 1: 5 suspension employing a conductivity meter (CM-180, Elico, India). Soil nitrogen and soil phosphorus were determined according to Jackson (1971), while available phosphorus was determined by Oslen's method (Oslen *et al*., 1954). Potassium was estimated after extraction in ammonium acetate solution (Jackson, 1971).

Evaluation of AM Fungal colonization

The root samples were washed with tap water cut into 1cm segments, cleared in 10 % (w/v) KOH, acidified with 1 % HCl, stained with 0.05 % trypan blue in lactophenol (wt/vol) (Phillips *et al*., 1970), mounted in glycerin and examined under a compound microscope (Olympus, Model CH-20i) at different magnifications (100× and 400×). A root segment was considered as mycorrhization when it showed the presence of any of the AM fungal structures *viz.* hyphae, hyphal coil, vesicle and arbuscule (Fig. 1).



a- arbuscules b- vesicle c- hyphae d- hyphal coil

Figure 1: AM fungal colonization structures

Spore extraction from rhizospheric soil

AM fungal spores were extracted from rhizosphere soil samples through wet sieving and decanting method proposed by Gerdemann and Nicolson (1963). 100 g of soil sample was dispersed in 1 L of water and the swirling soil suspension was decanted through two mesh sieves, 700 µm and 37 µm. Up to 40 percent of the spores are often left within the sediment from the primary sieving, that the sediments was re-suspended in swirling water and re-sieved.

The residues within the sieves were washed into the beakers and filtered through Whatman no. 1 filter paper. Each filter paper was then spread on a Petri dish from which spores were extracted under a dissecting microscope at ×40 magnifications. Calculate the spore population by count the spore number from 4 replicates per samples were mean averaged and the results was expressed as spore density per 100g of soil.

Spore propagation

AM fungi were propagated by using the pot culture method (Brundrett, 1995). The trap cultures were established to propagate the AM fungal spores present in the rhizosphere soils in order to identify. Rhizosphere soils collected from the five fields (served as inoculum) were mixed with sterilized coarse sand in 1:1 ratio (w/w), *Sorghum halepense* (L.) Pers, was used as catch plant. The culture were watered everyday and fertilized with Hoagland's solution (Hoagland and Arnon, 1938) devoid of phosphorus every fortnight. Since the catch plant complete its life cycle within ninety days, newly produced spores were harvested and examined.

Isolation and Identification of AM fungal spores

AM fungal spores used for identification were obtained from pot culture substrate by wet sieving method. The spores were mounted in PVLG (polyvinyl alcohol-lacto glycerol) (Koske and Tessier, 1983). The slides were examined under an Olympus CH-20i compound microscope at 100× and 400× magnifications. Identification of spores based on their morphology and sub-cellular characters (Schenck and Perez, 1990). For identification of spores, International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi website by Joe Morton: http://invam.caf.wvu.edu and other suitable references (Morton and Benny, 1990; Almeida and Schenck, 1990; Bentivenga and Morton, 1995; Walker and Vestberg, 1998) were used. The most recent compartmentalisation classification of AMF (Oehl *et al*., 2011) was followed to position the known species below the right genera.

Results and Discussion:

Physicochemical characteristics of soil samples are given in the Table 1. The results revealed that the rhizosphere soils of four study locations, Site-A, Site-C, Site-D and Site-E were sandy soil and Site-B alluvial soil. The soils were slightly alkaline in all the study locations except Site-B where it was near neutral. The Electrical Conductivity (EC) of rhizosphere soil samples ranged from 0.16 dSm^{-1} to 0.33 dSm^{-1}. It indicates that there is no salinity problem affecting the plant growth. Nitrogen (N) content in majority of the study locations appeared to be low to moderate. The total soil N ranged between 56 kg ac^{-1} to 110.6 kg ac^{-1}. The lowest N content was recorded in the study site-C and the maximum level of N content was recorded in the site-B. The highest

content of available P was recorded from the rhizosphere soil sample of location C and the lowest content of available P was recorded in site A. The Potassium (K) content was significantly higher in all the five locations. The K content was very low in the rhizosphere soil of site-A. The K content was very high in rhizosphere soil of site-E.

Table 1: Physicochemical characteristics of soil samples with the AM fungal spore density

Locations	Soil type	pH	EC	N	P	K	AM spore density
Site A	Sandy	8.03	0.16	95.2	2	116	303±31.9
Site B	Alluvial	7.61	0.30	110.6	4	250	511±63.0
Site C	Sandy	8.32	0.21	56	6	161	214±35.8
Site D	Sandy	8.23	0.23	91	5	382	322±41.7
Site E	Sandy	8.09	0.33	77	3	500	462±37.4

EC- Electrical conductivity; N- Nitrogen; P- Phosphorus; K- Potassium; EC ($dSm^{-1)}$ 0-0.12 very low level, 0.12-0.35 suitable range, 0.35-0.65 desirable range Nitrogen, Phosphorus and Potassium kg ac^{-1} .

Site A- Olaiyur; Site B- Kulumani; Site C- Mannachanallur; Site D- Valadi; Site E- Manapparai.

Rhizosperic soil sample from five different locations were subjected to the recovery of AM fungal spores. The spores are identified at their species level by their morphological characters shown in Table 2. Consistent with the classification of Oehl et al. 2011a; Oehl et al. 2011b we identified 13 species representing 8 genera belonging to 4 families from all rhizosphere soil samples of *Solanum lycopersicum* L. The identified species are, *Glomus microcarpum, Glomus sinuosum, Funneliformis mosseae, Septoglomus constrictum, Septoglomus viscosum, Rhizoglomus aggregatum, Rhizoglomus fasciculatum, Rhizoglomus irregulare, Rhizoglomus microaggregatum, Dominikia minuta, Claroideoglomus etunicatum, Acaulospora rehmii, Gigaspora decipiens* shown in Fig. 2 & 3.

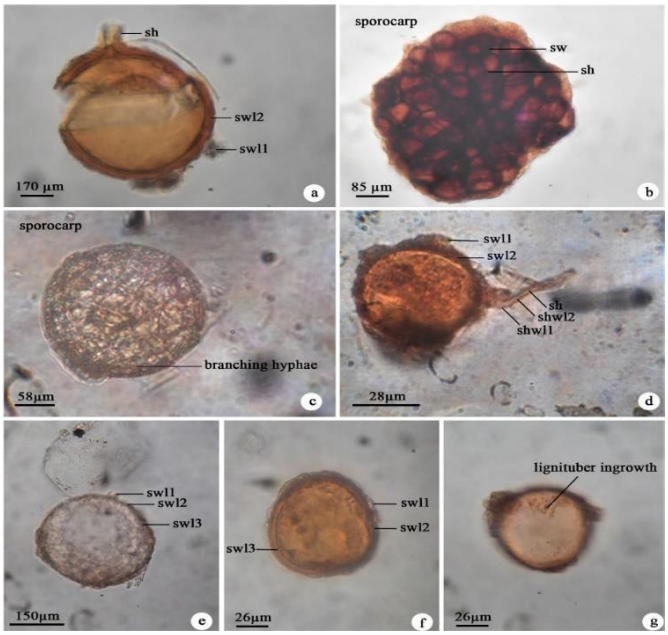


Fig. 2. AMF spores in *Solanum lycopersicum* L. (a- *Gl.microcarpum*, b- *Gl.sinuosum*, c- *F.mosseae*, d- *S.constrictum*, e- *S.viscosum*, f&g- *R.aggregatum*)

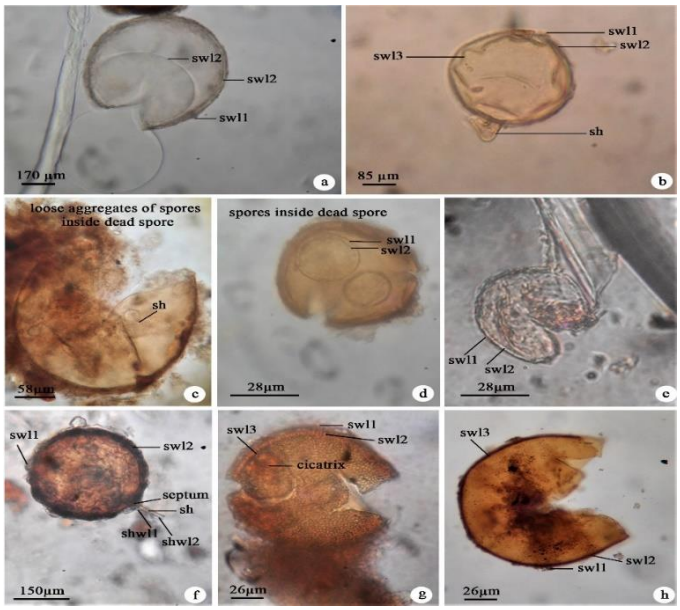


Fig. 3. AMF spores in *Solanum lycopersicum* L. (a- *R. fasciculatum*, b- *R.irregulare*, c&d- *R.microaggregatum*, e- *D.minuta*, f- *C.etunicatum*, g- *Ac.rehmii*, h- *G.decipiens*)

Spore wall layer 1(swl1), spore wall layer 2(swl2), spore wall layer 3(swl3), subtending hyphae(sh), subtending hyphal wall layer 1(shwl1), subtending hyphal wall layer 2(shwl2)

Table 2: Morphological characteristics of AMF spores in rhizospheric soil of *Solanum lycopersicum L.*

Species	Spore type	Shape	Diameter (µm)	Colour	Number of wall layers
Glomus microcarpum	Sporocarps (without peridium) rarely single	Globose – sub globose	22-55	pale yellow - golden yellow	Two layers
Glomus sinuosum	Sporocarps	Elliptical - clavate	58-88	yellow - reddish yellow	single layer
Funneliformis mosseae	Sporocarp (with peridium)	Globose – sub globose	100-260	pale orange-brown	Three layers
Septoglomus constrictum	Spore single	Globose – sub globose	100-220	brownish orange - dark brown	Two layers
Septoglomus viscosum	Spore single	Globose – sub globose	50-120	light brown	Three layers
Rhizoglomus aggregatum	Sporocarps loose clusters (without peridium)	Globose – sub globose	40-120	pale yellow	Three layers
Rhizoglomus fasciculatum	Spore single or in aggregates of 2-20 spores	Globose – sub globose	60-110	pale yellow-brown	Three layers
Rhizoglomus irregulare	Single rarely aggregate	Globose, ovoid, or irregular	70-165	yellow brown	Three layers
Rhizoglomus microaggregatum	loose aggregates, inside the dead spores	Globose – sub globose	11-35	yellow	Two layers
Dominikia minuta	Spores single or loose aggregates	Globose-subglobose or ovoid	20–65	Red brown	Two layers
Claroideoglomus etunicatum	Spore single	Globose - subglobose	60-160	orange - red brown,	Two layers
Acaulospora rehmii	Spores formed next to a‘‘sporiferous saccule’’	Globose - subglobose	90-160	pale yellow - light brown	Three layers
Gigaspora decipiens	Spore single	Globose - subglobose	280-440	white - cream	Three layers

The mean total average of AM fungal spore density ranged from 214 to 511. The wide variation in mean total fungal AM spore density among the species studied may be due to many reasons, namely differential host susceptibility to AM fungal proliferation (Mehrotra , 1998), different types of AM fungal species in the rhizosphere soils of individual host species, host efficiency in capturing and using soil resources (Koide, 1991; Clark and Zeto, 2000), soil types and quality (Raman and Gopinathan, 1992), root morphology of host species (Hetrick, 1991), mycorrhizal dependence on different host plants, and other edaphoclimatic factors (Fontenla *et al*., 1998; Abbott and Robson, 1991).In addition, seasonal sporulation of AM fungi, seasonal variation in host plant development (Sutton and Barron, 1972), acclimatization of a particular AM fungus to a particular site (Brundrett, 1991), and soil nutrient availability (Louis and Lim, 1987) could cause variation in AM fungal sporulation.

The frequency of these 13 AM fungal species of *Solanum lycopersicum* L. in five different locations of Tiruchirappalli district shown in Table 3. The results revealed that *Glomus microcarpum* and *Rhizoglomus aggregatum* present in higher frequency ie. Present in all loctions. *Acaulospora rehmii* and *Gigaspora decipiens* occured in lower frequency.

In general, the presence of more AM fungal species is sort of common within the perennial rhizosphere (Thaper and Khan, 1985). The occurrence of several AM fungi in soil or within the roots suggests the chance of an intra- specific competition between different species. Therefore, this study is very important to identify the native AM fungi present in the rhizosphere of the *Solanum lycopersicum L*. The above results revealed that the genus *Rhizoglomus* was perceived to be dominated among the other genera. Similar result was discovered within the case of chilli (Bagyaraj and Manjunath, 1980; Bagyaraj and Sreeramulu, 1982) *Solanum melongena* and okra. Formerly, *Rhizoglomus* conjointly known as *Glomus.* They have similar sporogenous characteristics that is the production of more spores rather small size within a short span of time compared with the massive spores of other genera (Hepper, 1984; Bever *et al*., 1996). AM fungi, particularly *Glomus* are able to live in alkaline pH and can reduce acidic stress in plant growth regions Hayman and Stovold (1979). Thus they're a lot of accommodative in adjustment of sporulation pattern in various environmental conditions (Stutz and Morton, 1996). Other genera was less common in the present investigation. There have been only a single species present in Gigasporaceae. It produce large spores and these need an extended time to develop the tiny spored species (Hepper, 1984). So that the species from Gigasporales are poor rival in colonizing plant roots. Therefore the host plants favours fungi from the Glomerales (Sykorova *et al*., 2007).

Table 3: Frequency of AM fungal spore distribution in *Solanum lycopersicum* L. of Tiruchirappalli district

S. No.	AM fungal species	Site A	Site B	Site C	Site D	Site E	Frequency of
1.	*Glomus microcarpum* (Tul. & C. Tul.)	+	+	+	+	+	100
2.	*Glomus sinuosum* (Gerd. & B.K. Bakshi)	-	+	-	+	+	60
3.	*Funneliformis mosseae* (T.H. Nicolson & Gerd.)	+	-	+	+	+	80
4.	*Septoglomus constrictum* (Trappe) Sieverd., G.A. Silva	-	+	-	-	+	40
5.	*Septoglomus viscosum* (T.H. Nicolson)	-	+	-	+	+	60
6.	*Rhizoglomus aggregatum* (N.C. Schenck &G.S. Sm.)	+	+	+	+	+	100
7.	*Rhizoglomus fasciculatum* (Thaxt.) Sieverd., G.A. Silva	+	-	+	+	+	80
8.	*Rhizoglomus irregulare* (Błaszk., Wubet, Renker & Olexia) Sieverd., G.A. Silva & Oehl,	+	+	-	+	+	80
9.	*Rhizoglomus microaggregatum* (Koske, Gemma & P.D.	+	+	-	+	-	60
10.	*Dominikia minuta* (Błaszk., Tadych & Madej)	+	-	+	-	-	40
11.	*Claroideoglomus etunicatum* (W.N. Becker & Gerd.) C.	-	+	-	+	-	40
12.	*Acaulospora rehmii* (Sieverd. & S. Toro)	-	+	-	-	-	20
13.	*Gigaspora decipiens* (I.R. Hall & L.K. Abbott)	+	-	-	-	-	20
	Total number of species	8	9	5	9	8	

Site A- Olaiyur; Site B- Kulumani; Site C- Mannachanallur; Site D- Valadi; Site E- Manapparai

Conclusion:

There are 13 species representing 8 genera identified in rhizospheric soil of *Solanum lycopersicum L*. among them the genus *Rhizoglomus* was dominated. This could due to high sporolation capability and more viability of the species, while, others were intermediate owing to the adverse edaphic conditions, longer reproductive times and short viability. Therefore the environmental conditions prevails in Tiruchirappalli is suitable for the sporulation of the genus *Rhizoglomus* which can be influence the yield of *Solanum lycopersicum L.*

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JOURNEY OF MICROBIAL SECONDARY METABOLITES IN PEST MANAGEMENT

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Abstract:

Microbial pesticides offer an eco-friendly method for the management of various pests which are difficult to manage with conventional pesticides. Microbial pesticides comprise of microorganisms such as bacteria, fungi, *actinomycetes* and cyanobacteria, they produce low molecular weight natural substances called as secondary metabolites. These metabolites unlike the primary metabolites have no any role in growth and reproduction of an organism. The milbemectins, avermectins and spinosads obtained from actinomycetes are the major insecticides of microbial origin. The strobilurins, kasugamycin, validamycin and blasticidin are the microbial fungicides effective against a wide range of pathogens. The bactericides comprise of oxytetracycline and streptomycin, while vulgamycin and bialaphos are commercially available bioherbicides isolated from actinomycetes. These microbial products being eco-friendly and less toxic to non-target pest emerge as a potential alternative for the management of the pest and hence can be exploited in the future for the synthesis of new products.

Introduction:

During the last few decades, pesticides have played a key role in protecting our crop from devastating pests. However, the continuous and indiscriminate use of these pesticides has led to serious problems such as pest resistance, pest resurgence and harmful effect on the environment due to their residual action. Therefore, microbial secondary metabolites offer a better alternative to conquer the pollution and resistance caused by the synthetic chemicals.

The secondary metabolites are low molecular weight organic compounds, varied structure and produced by certain microbial species. They do not play any role in the growth and development of organisms unlike the primary metabolites. The primary metabolites like enzymes, organic acids, lipids, carbohydrates, amino acids and nucleopeptides are essential for the growth while secondary metabolites play role in antagonism, competition and defense of an organism. These secondary metabolites are very diverse in structure and each unique metabolite is produced by a specific species. These secondary metabolites are derived from the common biosynthetic pathways which branch off the primary metabolic pathways. A characteristic feature

of these metabolites is that they are not produced during the phase of rapid growth (trophophase), but are produced during later stages of growth (idiophase). The SM are synthesized when growth become limited by the exhaustion of major nutrients like carbon, phosphorus and nitrogen. These metabolites showed potent control efficacy against various pests which are rather difficult to manage with conventional pesticides.

The microbial metabolites are produced by various microorganisms like fungi, bacteria and *actinomycetes*. The secondary metabolites produced by various microbes and their actions are dealt in details for proper insight of these metabolites and their further exploration in the future.

1. Fungal Secondary Metabolites

There are various fungi like *Metarhizium anisopliae, Beauveria bassiana, Trichoderma* spp., *Alternaria* spp., endophytic fungi, *Tolypocladium* spp., *Paecilomyces fumosoroseus, Verticillium lecanii* which produce tremendous number of secondary metabolites. Among these, the *Trichoderma* is the most extensively researched fungal biocontrol agent and is successfully used as biopesticide and biofertilizer in glasshouse as well as in field trials (Harman *et al*. 2004). It produces tremendous number of bioactive compounds used as antifungal, antibacterial and antitrichomonal agent (Table 1). Endophytic fungi proliferate within their host without causing any apparent disease symptoms (Petrini, 1991; Wilson, 1995). They are the novel and potential sources of bioactive compounds that can serve for the discovery and development of newer pharmaceutical product (Dompeipen *et al*., 2011). They have led to production of tremendous novel antidiabetic, antibiotics, anticancer, antimycotics and immunosuppressants and more of the unexplored products are on the way (Table 2).

The fungal secondary metabolites are exploited as herbicides, insecticides, nematicides and fungicides are summarized below:

1.1 Herbicides

a) **Maculosin:** It is cyclic dipeptide, isolated from the funus *Alternaria alternata* and effective against the weed spotted knapweed (*Cantaeurea maculosa*).
b) **Bipolaroxin:** It is obtained from *Bipolaris cyanodontis* and effective against the weed *Cynodon dactylon* (Bermuda grass). At higher concentration it can manage the weeds like wild sugarcane, oats and maize.
c) **Cornexistin:** It is a phytotoxin from *Paecilomyces variotii* and it can effectively manage both monocots and dicots weeds except maize (US Patent, 1991).
d) **LT-toxin**: It is a phytotoxin from *Lasiodiplodia theobromae* MTCC3068 and exhibit broad spectral herbicidal activity against duckweeds, carrot grass, prickly sida, jimsonweed and *Euphorbia hirta* (US patent, 2009).

e) **Cinnacidin:** It is a phytotoxin from *Nectria* sp. DA60047. It produces the chlorosis and stunting symptoms which progresses throughout the leaves (Irvine *et al*.,

f) **Mevalocidin:** It is a phytotoxin from *Roselliana* DA092917 and *Fusarium* DA056446. It exhibits herbicidal activity against monocot and dicot weeds. It is a xylem as well as phloem mobile herbicide (Gerwick *et al*., 2013).

g)

Table 1: Secondary metabolites of *Trichoderma* spp. and their biological potential

Structural class	Compound	Producer strain	Biological effect	Target pest	References
Peptaibols	Trichorzins TV B I, II, IV	*Trichoderma virens*	Antifungal	*Botrytis cinerea*	Rebuffat *et al*. (1996)
	Trichohorzianum TA Trichorzianum TB	*T. virens*	Antifungal	*B. cinerea*	Rebuffat *et al*. (1996)
Polyketides	Koninginin A, B, C, D and E	*T. harzianum* *T. koningii*	Antifungal, Plant growth regulator	*Gaeumannomyces graminis var. tritici*	Auvin-Guette *et al*. (1993)
	6-pentyl–α-pyrone	*T. harzianum* *T. koningii* *Trichoderma spp.* *T. viride*	Antifungal, Antimicrobial, Plant growth regulator	*Rhizoctonia solani*	Almassi *et al*. (1991)
	Massoilactone δ-decenolactone	*Trichoderma* spp.	Antifungal	Soil borne fungi	Almassi *et al*. (1991)
	Trichodermin	*T. virens* *T. polysporum* *T. reesei*	Antifungal	-	Sivasithamparam *et al*. (1998)
	Harzianum A	*T. harzianum*	Antifungal	-	Sivasithamparam *et al*. (1998)

Terpenes	Viridin	*T. viride* *T. virens* *T. koningii*	Antibiotic, Antisporulant	-	Sivasithamparam *et al*. (1998)
	Ergokonin A, B	*T. viride* *T. koningii* *T. longibrachiatum*	Antifungal	*Aspergillus sp.* *Candida sp.*	Vicente *et al*. (2001)
	Lignoren	*T. lignorum*	Antifungal, Antibacterial	*Sporobolomyces salmonicolo, Rhodotorula rubra, Bacillus subtilis, Pseudomonas aeruginosa*	Berg *et al*. (2004)
Other metabolites	Ferulic acid	*T. virens*	Antiviral, Bactericide, Antifungal	-	Dickinson *et al*. (1995)
	Gliotoxin	*T. virens* *T. lignorum* *T. hamatus*	Antibiotic	-	Wiest *et al*. (2002) Lorito *et al*. (1996)

Table 2: Secondary metabolites of endophytic fungi and their biological potential

Compound	Producer strain	Biological activity	Target pest	References
Cryptocin	*Cryptosporiopsis quercina* (endopyte in stem of *Tripterigeum wilfordii*)	Antifungal	*Pyricularia oryzae*	Li *et al*. (2000)
Cryptocandin	*Cryptosporiopsis quercina* (endopyte in stem of *Tripterigeum wilfordii*)	Antifungal	-	Strobel *et al*. (1999)

Cytochalins H, N	*Phomopsis* sp. (endophyte in *Gossypium hirsutum*)	Antifungal	*Sclerotinia sclerotium. Fusarium oxysporum, Botrytis cineria, Bipolaris sorokiniana, Rhizoctonia cerealis*	Fu *et al*. (2011)
Colletotric acid	*Colletotrichum gloesporoides* (endophyte in *Artemissia mangolica*)	Antifungal	*Helminthosporium sativum*.	Zou *et al*. (2000)
Volatile compound (alcohols, acids, esters and monoterpenes)	*Nodulisporium* sp. (endophyte in *Lagerstroemia loudoni*)	Antifungal	Green and blue mold decay caused by *Penicillium digitatum* and *P. expansum*	Suwannarach *et al*. (2013)
Nodulisporic acid	*Nodulisporium* sp. (endopyte in *Bontia daphnoides*)	Insecticidal	Fleas	Ondeyka *et al*. (1997)

h) **Macrocidins** (Macrocidin A and macrocidin B)**:** It is isolated from the *Phoma macrostom*a and potent herbicide against the *Cirsium arvense* L. (*Canada thistle*) (US Patent, 2010). It induces the chlorotic and bleaching symptoms on the broad leaved weeds by inhibiting the carotenoid synthesis in plants (Graupner *et al*., 2006).

i) **Phyllostictine A:** It is a phytotoxin produced by *Phyllosticta cirsii* and is potent mycoherbicide against *Cirsium arvense* (Zonno *et al*., 2008).

j) **Zinniol:** It is isolated from *Alternaria cirsinoxia* and it is used to control *Cirsium arvense* (Berestetskii *et al*., 2010).

1.2 Inseticides

a) **Destruxin:** Destruxin A and B from *Metarhizium anisopliae* were the first entomopathogenic metabolites discovered. Later many isomers of destruxin were

discovered and Destruxins A1, A4, A5 and homodestruxin B is obtained from the fungus *Aschersonia* spp (Strasser *et al*., 2000)

b) **Efrapeptins:** These are isolated from *Tolypocladium* spp. They exhibit miticidal and insecticidal activities against pests such as diamondback moth, spidermites and potato beetles (Krasnoff *et al*., 1991). Efrapeptins shows antifeedant activity and causes inhibition of insect growth (Bandani and Butt, 1999)

c) **Oosporein:** This secondary metabolite is produced by species of *Beauveria* on sterilized barley kernels in submerged cultures (Strasser *et al*., 2000)

d) **Beauvericin:** This hexadepsipeptide is isolated from *Beauveria bassiana* and *Paecilomyces* spp. It has two forms i.e. Beauvericin A and Beauvericin B (Lane *et al*., 2000; Miller *et al*., 2008)

e) **Nodulisporic acid:** This metabolite is produced by endophytic fungus *Nodulisporium* sp. which inhabits Hawaiian plant *Bontia daphnoides*. It possess the insecticidal activity against fleas (Ondeyka *et al*., 1997)

f) **Rugulosin:** It is isolated from the fungus *Phialocephala scopiformis* which is an endophyte in white spruce needles. It is effective against spruce budworm (*Choristoneurea fumifera*) by its anti-feedant activity (Miller *et al*., 2008).

g) **Citrantifidiene:** It is produced by the soil dwelling isolate of *Trichoderma citrinoviridae*. It shows antifeedent activity against *Schizaphis graminum*.

h) **4-(*N*-methyl-*N*-phenylamine)-butan-2-one:** This metabolite is produced by *Aspergillus gorakhpurensis* and shows larvicidal activity against *Spodoptera litura* (Busi *et al*., 2009).

1.3 Nematicides

a) **Omphalotin A:** This cyclic dodecapeptide is produced by *Omphalotus olearicus*. It exhibit higher efficacy than the well known nematicide ivermectin (Mayer *et al*., 1999).

b) **Caryospomycin A, B and C:** This is isolated from the *Caryospora callicarpa* and shows nematicidal activity activity against *Bursaphelenchus xylophilus* (pinewood nematode) (Dong *et al*., 2007)

c) **Dicarboxylic acid:** This novel nematicide is produced by *Paecilomyces* sp. It is effective against the nematodes *Meloidogyne incognita*, *Bursaphelenchus xylophilus* and *Panagrellus redivivus* (Liu *et al*., 2009)

1.4 Fungicides

a) **Cryptocin:** This metabolite is isolated from the endophytic fungus *Cryptosporiopsis quercina* inhabiting the stem of *Tripterigeum wilfordii* and effectively manage the blast fungus *Pyricularia oryzae* of rice (Li *et al*., 2000).

b) **Cytochalsins**: These are isolated from the fungus *Phomopsis* sp. which is an endophyte in *Gossypium hirsutum*. It is effective against *Botrytis cineria, Rhizoctonia cerealis, Sclerotinia sclerotium, Bipolaris sorokiniana* and *Fusarium oxysporum* (Fu *et al*., 2011).

c) **Colletotric acid:** This phenolic compound is isolated from the fungus *Colletotrichum gloesporoides* existing as an endophyte in *Artemisia mongolica.* It can effectively manage the pathogen *Helminthosporium sativum* (Zou *et al*., 2000).

d) **Rufuslactone:** It is obtained from the fruiting bodies of a basidiomycetes fungus *Lactarius rufus*. It inhibits the growth of pathogens such as *Botrytis cineria, Fusarium graminearum, Alternaria alternata* and *Alternaria brassicae* (Luo *et al*., 2005).

e) **Strobilurins**: It is isolated from the fungus *Oudemansiella mucida* and *Strobilurus tenecellus.* These strobilurins were too photosensitive that they were not commercially used. The synthetic analogues of this strobilurins have been developed namely azoxystrobin, kreso-oxim methyl, trefloxystrobin and picoxystrobin, which are photostable and are used to manage many diseases

2. Bacterial Secondary Metabolites

The bacteria producing the bioactive compounds can be categorized into four groups namely obligate pathogens (such as *Bacillus popilliae*); crystalliferous spore formers (such as *Bacillus thuringiensis*); facultative pathogens (such as *Pseudomonas aeruginosa*) and potential pathogens (such as *Serratia marcesens*). Among these, the spore formers are commercially employed at field level due to their effectiveness and safety (Roh *et al*., 2007). The *Bacillus thuringiensis* and *B. sphaericus* are most commonly used bacteria. The insecticidal property of these bacteria is due to crystalline proteins encoded by the cry genes. The Bacilli are rod-shaped, gram positive, aerobic bacteria and motile by peritrichous flagella. The Secondary metabolites of bacilli can be broadly classified as bacteriocins, lantibiotics and miscellaneous antibiotics based on their structure and shows remarkable antiviral, antitumor, antimicrobial, immunosuppressant and antifungal properties (Table 3).

Pseudomonads are gram-negative, aerobic, motile, straight or slightly curved rods belonging to gamma - Proteobacteia (Galli *et al*., 1992). Secondary metabolites produced by fluorescent Pseudomonads exhibit a wide range of antimicrobial activity (Thomashow *et al*., 1990), this makes fluorescent Pseudomonads as promising plant growth promoting rhizobacteria. *Pseudomonas fluorescens* produces secondary metabolites such as phenazines (Sunish Kumar *et al*., 2005); phenolics (Vincent *et al*., 1991); polyketides (Kraus and Loper, 1995); pyrrole-type compounds (Pfender *et al*., 1993); peptides (Sorensen *et al*., 2001); 2, 4-diacetylphloroglucinol (DAPG) (Rosales *et al*., 1995); cyclic lipodepsipeptides (Nielsen *et al*., 2002) which exhibit various antibacterial, antifungal and antihelmenthic activities (Table 4).

The various compounds of bacterial origin exhibiting herbicidal, insecticidal and fungicidal properties are listed below.

2.1 Herbicides

a) **Tabtoxin:** This wildfire toxin is isolated from the bacteria *Pseudomonas syringae* var. *tabaci* causing wildfire disease of tobacco. It acts by inhibiting the activity of the enzyme glutamine synthetase.

Table 3: Secondary metabolites of *Bacillus* spp. and their biological potential

Structural Class	Compound	Producer strain	Biological effect	References
Bacteriocins	Thuricin	*B. thuringiensis* HD2	Bacteriolytic	Favret and Yousten (1989)
	Entomocin	*B. thuringiensis*	Bactericidal entomocidus HD9	Cherif *et al*. (2003)
	Coagulin	*B. coagulans* Le	Bactericidal, bacteriolytic	Marrec *et al*. (2000)
	Kurstakin 18	*B. thuringiensis* BMG1.7	Fungicidal	Hathout *et al*. (2000)
Lantibiotics	Subtilin	*B. subtilis* ATCC6633	Antibacterial	Stein *et al*. (2002)
	Subtilosin A	*B. subtilis* 168, ATCC6633	Antibacterial	Stein (2005)
	Ericin	*B. subtilis* A1/3	Antibacterial	Stein (2005)
Cyclic lipoheptapeptide	Surfactin 5	*B. subtilis*	Hemolytic, cytotoxic	Carrillo *et al*. (2003)
	Iturin 7	*B. amyloliquefaciens* B94, FZB42	Antifingal, haemolytic	Aranda *et al*. (2005) Yu *et al*. (2002)
Polyketides macrolactone	Difficidin 10	*B. amyloliquefaciens* FZB42, GA1	Antibacterial	Arguelles-Arias *et al*. (2009)
Aminopolyol Antibiotic	Zwittermicin 14	*B. thuringiensis, B. cereus*	Antifungal	Silo-Suh *et al*. (1998)

b) **Phaseolotoxin:** This phytotoxin is produced by *Pseudomonas syringae* pv. *phaseolicola*, the causal agent of Halo blight of bean. Its shows broad range of activity by inhibiting the ornithine carbamoyl transferase (OCT), which regulates the synthesis of arginine.

c) **Coronatine:** It is produced by *Pseudomonas coronafacience*. It produces chlorotic symptoms on the leaves by inhibiting the jasmonate controlled pathways in the host (Block *et al*., 2005).

2.2 Insecticides

a) **Bt-Toxins**: These widely exploited bacterial endotoxins are produced by *Bacillus thuringensis* and related species. Transgenic plants expressing the genes responsible for production of bacterial toxin can potentially control Lepidopteran (butterfly and moths), Coleopteran (beetles) and Dipteran (flies) insects.

b) **Diabroticin A:** This toxin is produced by *Bacillus subtilis* and *Bacillus cereus,* which can effectively manage the Southern corn rootworm (*Diabrotica undecimpunctata*) (Stierle *et al*., 1990).

2.3 Fungicides

a) **Macrolactin A:** Macrolactin A and iturin A isolated from the *Bacillus* sp. *sunhua.* It can inhibit the pathogens *Streptomyces scabies* (Scab of potato) and *Fusarium oxysporum* (Dry rot disease) (Han *et al*., 2005).

Table 4: Secondary metabolites of fluorescent pseudomonads and their biological potential

Metabolites	**Producer strain**	**Biological effect**	**Target Pest**	**References**
Pyrrolnitrin	*P. chlororaphis* *P. cepacia* *P. fluorescens*	Antifungal	*F. graminearum, R. solani, Pythium ultimum* and *Aphanomyces cochliodes*	León *et al*. (2009); Park *et al*. (2011)
2,4-DAPG	*P. fluorescens* *P. aeruginosa*	Antifungal, Antibacterial, Antihelmenthic,Herbicidal	*F. oxysporum, G. graminis* var. *tritici, C. michiganensis* subsp. *Michiganensis* and *Xanthomonas oryzae* pv. *oryzae*	Lagzian *et al*. (2013); Lanteigne *et al*. (2012)
HCN	*Pseudomonas* sp. P76 *Pseudomonas* sp.	Antifungal	*Sclerotium rolfsii, Clavibacter michiganensis* subsp. *michiganensis*	Priyanka *et al*. (2017); Lanteigne *et al*. (2012)
Phenazine-1-carboxylic acid	*P. fluorescens* 2-79 *P. aureofaciens* 30-84	Antifungal, Antibacterial	*Gaeumannomyces graminis* var. *tritici, F. oxysporum* f.sp. *ciceris* and *F. udum*	Pathma *et al*. (2010)
Pyoverdine	*P. fluorescens* 3551 *P. fluorescens* CHAO	Antifungal	Competitive inhibition of phytopathogens	Loper (2008)
Cyclic lipopeptides	*P. fluorescens*	Antifungal	*R. solani, P. ultimum* and *Phytophthora infestans*	Tran *et al*. (2007)

b) **Syringomycin E:** It is produced by *Pseudomonas syringae* and can potentially manage the *Penicillium digitatum* causing the citrus green mold (Bull *et al*., 1998).

3. Actinomycetes Secondary Metabolites

Among the soil microbes, the actinomycetes occupy the most important place. They are source for the production of many novel biologically active substances which have been commercialized as pharmaceuticals and agrochemicals. Among the actinomycetes, *Streptomycetes* are best known for their ability to control pests as it produces tremendous number of bioactive compounds which have the potential to be used as fungicide, herbicide, insecticides, acaricides and bactericides. *Streptomyces* are gram positive soil dwelling microbe with high G+C content and comprise largest genus of actinobacteria. They are the versatile producers of secondary metabolites which exhibit potential antiproliferative, antihelmintic, antimicrobial, etc. (Table 5).

The numerous secondary metabolites of *actinomycete*s exhibiting herbicidal, insecticidal, bactericidal and fungicidal properties are listed below.

Table 5: **Secondary metabolites of *Streptomyces* spp. and their biological potential**

Structural Class	Compound	Producer strain	Biological effect	References
Lipopeptide	Daptomycin	*S. roseosporus*	Antibiotic	Woodworth *et al*. (1992)
Tripeptide	Bialaphos	*S. hygroscopicus*, *S. viridochromogenes*	Herbicidal	Kondo *et al*. (1973)
Aminoglycoside	Streptomycin	*S. griseus*	Bactericidal	Singh and Mitchison (1954)
	Neomycin	*S. fradiae*	Antibacterial	Waksman and Lechevalier (1949)
	Istamycins A and B	*S. tenjimariensis*	Antibiotic	Okami *et al*. (1979)
Macrocyclic lactones	Avermectin	*S. avermitilis*	Anthelmintic, Insecticidal	Burg *et al*. (1979)
Quinones	Marinone	*Streptomyces* sp.	Antibiotic	Pathirana *et al*. (1992)
	Komodoquinone A	*Streptomyces* sp. KS3	Neutritogenic	Itoh *et al*. (2003)
Cyclic Peptides	Cyclomarins	*S. arenicola*	Anti-inflammatory	Renner *et al*. (1999)

3.1 Herbicides

a) **Bialaphos**: This tripeptide is isolated from *Streptomyces hygroscopicus* and *Streptomyces viridiochromogenes* in 1978 (Mase *et al*., 1984). This is a post-emergence herbicide used in apple, brassicas, vines or on the uncultivated land (Copping and Duke, 2007). The bialaphos is converted into active toxic compound phosphinothricin inside the plant (Tachibana, 2003). This herbicide is produced by Meiji Seika through fermentation and available in market as Herbiace. This herbicide is also available with the other common names such as phosphinothricyl-alanyl-alanine, bilanafos and phosphinothricinalanyl-alanine. The glufosinate ammonium is the chemically synthesized analogues of phosphinothricin and introduced by Hoechst (now Bayer Crop Science) as herbicide and available in the market with trade names Liberty, Basta and Ignite.

b) **Herbimycin:** This herbicide is isolated from *Streptomyces hygroscopicus*. It is broad spectrum herbicide effective against both monocot and dicot weeds. It is pre- as well as post-emergence herbicide (Hahn *et al*., 2009).

c) **Pyrizadocidine:** This is produced by the *Streptomyces* and induces symptoms like chlorosis and necrosis in the weeds (Gerwick *et al*., 1997).

d) **Albucidin:** This metabolite is isolated from *Streptomyces albus* subsp. *chlorines*. This is broad spectrum herbicide and produces chlorosis and bleaching symptoms (Hahn *et al*., 2009).

3.2 Insecticides

a) **Abamectin:** It is obtained from the fermentation broth of *Streptomyces avermitilis*. It is effective against the motile forms of broad range of suckers, beetles, mites, leafminers and other insects which attack on potatoes, vegetables, cotton, citrus, ornamental plants, *etc*. It acts by inhibiting the gamma-aminobutyric acid (GABA) receptor in insects. It is an insecticide and acaricide and effective against lepidopteran insects and nematode *Bursaphelenchus xylophilus*. It is sold in market with different names as Denim, Agri-Mek, Clinch, Proclaim, Arise, Avid, Affirm and other names (Dunbar *et al*., 1998).

b) **Spinosad:** This secondary metabolite is isolated from *Saccharopolyspora spinosa* (Mertz and Yao, 1990; Thompson and Hutchins, 1999). It is a mixture of spinosyn A and spinosyn D and can effectively control wide range of thrips, caterpillar, foliage feeding beetles and leaf miners

c) **Milbemycin:** This metabolite is isolated from fermented broth of *S. hygroscopicus* subsp. *aureolacrimosus* (Mishima *et al*., 1983). It is used to manage leaf miners, citrus red mite, kanzawa spider mite, etc. by inhibiting the neurotransmission signals in the insects.

3.3 Fungicides

a) **Blasticidin-S:** It is product obtained from actinomycete *Streptomyces griseochromogenes* and has the potential to control *Pyricularia oryzae* (Blast of rice). It is a contact fungicide having both protective and curative action (Fukunaga, 1955). It is available in market with trade name of Bla-S as emulsifiable concentrate (EC), dustable powder (DP) and wettable powder (WP).

b) **Kasugamycin**: This another fungicide produced by *Streptomyces kasugaensis* is also used to effectively control *Pyricularia oryzae*, scab in apple and pear (*Venturia inaequalis*) and leaf spot in celery and sugar beet (*Cercospora* spp.) (Umezawa *et al*., 1965). It is sold in market as wettable powder, dustable powder, soluble concentrates and granules with the tradename of Kasumin and Kasugamin.

3.4 Antibiotics

a) **Oxytetracycline:** This antibiotic is a product of soil actinomycete *Streptomyces rimosus* and can effectively manage the diseases caused by *Xanthomonas* and *Pseudomonas* species (Finlay *et al*., 1950).

b) Streptomycin: This metabolite is produced by soil actinomycete *Streptomyces griseus*. It is a potential antibiotic against *Xanthomonas citri*, *X. oryzae* and *Pseudomonas tabaci* and widely used in fruits, vegetables and ornamentals. It is also used to control bacterial canker, bacterial rots and other bacterial diseases.

Conclusion:

The secondary metabolites from different antagonists such as bacteria, fungi and actinomycetes have served as a novel source of microbial pesticides. Many of these microbial pesticides are in commercial use in agriculture and pharmaceutical, but more research need to be done in this direction, as being sensitive to UV light, heat and desiccation their efficacy is not up to the mark. Their performance in the field is also inconsistent and unpredictable. The short shelf life of these microbial products is another concern; research should be focused to make special formulation to increase their shelf life. The metagenomics can serve as a new scientific tool to explore various silent and unculturable microbial consortia to produce novel compounds which in turn can serve in the field of agriculture. Bioantagonists and their metabolites enable us to do sustainable farming without depleting the environment.

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PRECISION AGRICULTURE: A WAY TO DO SMART FARMING FOR SUSTAINABILITY

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Abstract:

Precision agriculture, also known as precision farming, is a type of farming in which in-field variability is taken into consideration and planting, fertiliser management, and plant protection measures are implemented based on the local conditions of a given field. As a result, resources/inputs will be used more efficiently, resulting in agricultural sustainability. Meeting food demands with the planet's finite resources is a difficult task. In order to meet this challenge, a number of cutting-edge technologies are being implemented in the agricultural sector. Precision agriculture (PA) is a collection of sensors and near- and far-field sensing systems that help monitor crop conditions at various stages of growth. PA entails gathering and analysing a vast amount of information about crop health. PA allows a farmer to know exactly what parameters are required for a healthy crop, where they are required, and in what quantity at any given time. Initially, it become used primarily as a mechanism to adjust fertilizer charges surrounding high, medium, or low producing zones of a subject. Precision farming provides great opportunity for accuracy and efficiency in every agricultural operation right from sowing, fertilizing, irrigation, protection till harvesting with precision tools like GPS, GIS, VRT, Remote Sensing etc. PF shows immense potential environmental prospects like reduction in nitrate leaching, emission of greenhouse gases and agrochemicals like fertilizer, herbicide, pesticides etc. which may help in achieving sustainable agriculture of future

Keywords: crop health, efficiently, greenhouse gases, remote sensing and sustainable

Introduction:

Precision agriculture is a relatively recent technology-driven approach to agriculture. It is a philosophy or management approach to agricultural production that takes into account regional

and temporal variation in soils, rainfall, and management practises to minimise costs, improve input efficiency, and reduce pollution. Precision agriculture, also known as precision farming, is a type of farming in which in-field variability is taken into consideration and planting, fertiliser management, and plant protection measures are implemented based on the local conditions of a given field. As a result, resources/inputs will be used more efficiently, resulting in agricultural sustainability.

Human survival on Earth has become more difficult due to the continually rising human population. Meeting food demands with the planet's finite resources is a difficult task. In order to meet this challenge, a number of cutting-edge technologies are being implemented in the agricultural sector. Precision agriculture (PA) is a collection of sensors and near- and far-field sensing systems that help monitor crop conditions at various stages of growth. PA entails gathering and analysing a vast amount of information about crop health. PA allows a farmer to know exactly what parameters are required for a healthy crop, where they are required, and in what quantity at any given time. This necessitates gathering a large amount of data from various sources and parts of the field, such as soil nutrients, the presence of pests and weeds, plant chlorophyll content, and some weather conditions. (Mondal and Basu, 2009)

To generate agronomic recommendations, all collected data must be analysed. Plants' amount of greenness (chlorophyll content), for example, reflects the nutrients required based on their growth stage. This data is coupled with the soil parameters of the plant's location, as well as the weather forecast. All of the data gathered is then utilised to calculate how much fertiliser should be applied to that plant the next day.

Although PA is defined and referred to in a variety of ways, the core principle remains the same. Precision farming, information-intensive agriculture, prescription farming, target farming, site-specific crop management, variable rate management, variable rate technology (VRT), farming by soil, grid soil sampling agriculture, grid farming, Global Positioning Systems (GPS) agriculture, farming by the inch, farming by the foot, and so on are some of the terms used to describe this type of farming.

Definitions:

- ***The United States Department of Agriculture (USDA)*** define it as ‘a **management system** that is information and technology based, is site specific and uses one or more of the following sources of data: soils, crops, nutrients, pests, moisture or yield, for optimum profitability, sustainability and protection of the environment.

- Precision agriculture (PA) is conceptualized by a system approach to re-organize the total system of agriculture towards a low-input, high-efficiency, sustainable agriculture (Shibusawa, 1998).
- Precision agriculture (PA) is an approach to farm management that uses information technology (IT) to ensure that crops and soil receive exactly what they need for optimum health and productivity. The goal of PA is to ensure profitability, sustainability and protection of the environment. PA is also known as satellite agriculture, as-needed farming and site-specific crop management (SSCM).
- Precision farming is the only solution to identify the causes of variability within the field and to carefully tailor soil and crop management to fit in each cultivated field (Gautam and Sharma, 2002).
- Precision farming can be defined as farming system, which enables profit to be maximized and where inputs (tillage operations, seed, fertilizer and chemicals) are varied according to the yield potential of individual parts of a field. It facilitates the optimal use of inputs, resulting in increased gross margins with reduced impact on the environment. It is sometime known as Variable Rate Technology (VRT) and site-specific agriculture (Sahoo *et al*., 2002).
- Precision agriculture is defined as the management of inputs to small plots as a fact on of diversity of the physical medium and the environment (Basso *et al*., 2001).

Concept:

- The management of variability lies at the heart of concept of precision farming.
- It is a modern agriculture practice involving the **use of technology** in agriculture like remote sensing, GPS and Geographical Information System (GIS) for improving productivity and profitability.
- PF is based on the philosophy of heterogeneity within homogeneity and requires precise information on the degree of variability to optimize input resources.
- **More efficient utilization** of inputs to improve resource use efficiency without affecting the productivity is one of the **key concept** of Precision Farming. Hence Precision Agriculture is based on the concept of applying inputs:

(i) At right time **(ii) In right amount**

(iii)At right place **(iv) In right manner**

Evolving Farming Technology

Today	**Tomorrow**
Mechanical	Information
Hardware oriented	Software oriented
Manual and on-site control	Automatic and remote control
Simple machines	Networked smart machines
Unpredictable quality and quantity	Predictable in quality and quantity

Figure 1: Evolving Farming Technology in future

Objectives:

- To increase the accuracy and efficiency of agricultural input applications.
- To conduct lab and field experiments and researches on the economic and environmental negative impacts of agricultural chemical misapplications.
- To conduct researches on the economic and environmental feasibility of the precision farming technology that would promote this technology to be accepted and adopted by farmers and environmentalists in India.
- To localize this technology through collaborative research efforts.
- To deliver this technology to a wide segment of local researchers, environmentalists, farmers and agro-economists.

Need for Precision Farming

- To increase agriculture productivity
- Prevents soil deterioration
- Reduction of chemical use in crop product

- Effective use of water resources
- Dissemination of current farm practices to ameliorate quality, amount and reduced cost of yield
- Developing favourable attitudes
- Precision cultivating changing the socio-economic status of agronomists
- Depletion & Degradation of Water resources

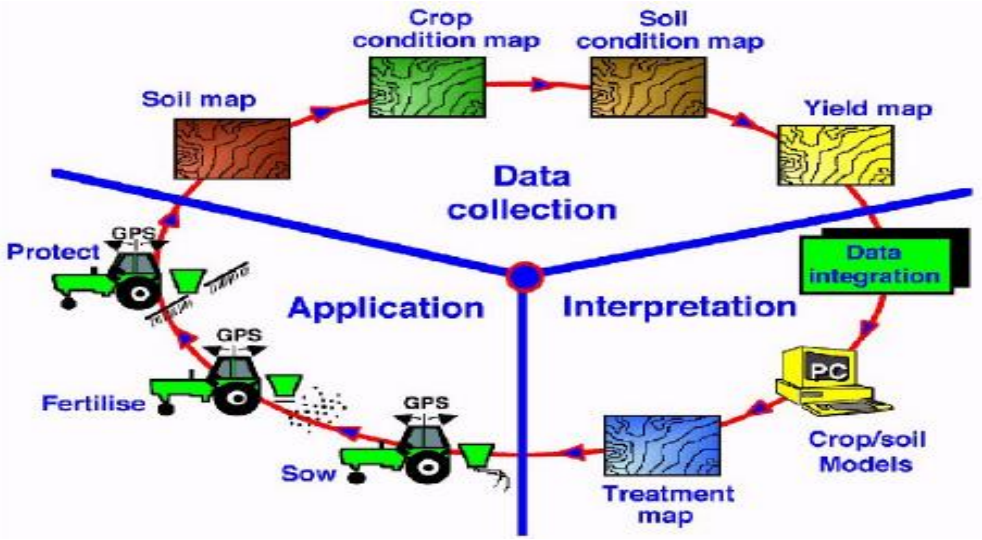

Figure 2: The Precision Agriculture Cycle

Components or tools of Precision Agriculture:

1. Global positioning system (GPS)

GPS is a global radio navigation system made up of 24 satellites and ground stations that gives geographic accuracy to farm practises by allowing farmers to identify each field site. GPS precision has improved to roughly 20 metres when the US government decided to switch off selective availability (an artificial defect generated into satellite data to lower positional accuracy to 100 metres) on May 1, 2000. However, differential GPS (a GPS receiver combined with a ground reference station) is required for yield mapping, crop scouting, and variable input applications to achieve precision of 1-3 m. Field border and topographical maps can also be created using GPS. (Usery *et al.,* 1995)

Crop rotations in a region can be tracked year to year using a set of maps with each field labelled with the crop planted in each season. GPS auto-guidance (automated steering for application equipment) has recently gained popularity in the United States, Australia, Brazil, and South Africa as a means of reducing labour costs.

2. Geographic information system

GIS is a computerised data storage, retrieval, and transformation software tool that is used to manage and analyse spatial data linking agricultural productivity and agronomic parameters. Data for diverse fields can be derived from a variety of sources, including existing digital maps, data digitised from maps and images, topographic surveys, soil or crop sampling, and sensor data with GPS location information. GIS can present analysed data in maps, allowing for (1) a better understanding of interactions between yield, fertility, pests, weeds, and other elements, as well as (2) decision-making based on these spatial linkages.

Land ownership, crop cover, soil type, topography, N. P., K., and other nutrient levels, soil moisture, pH, and other base maps are all included in a precision agriculture GIS. Rotations, tillage, nutrient and pesticide applications, yields, and other information are also maintained. Fertility, weed, and pest intensity maps, as well as prescription maps that depict appropriate application rates of farm chemicals at various field areas, are all created using GIS.

3. Remote sensing

Low-flying aircraft and satellites have come to be a prime source of spatial records because of discount in each value and time of photograph acquisition and transport. Although the use of remote sensing in agriculture is numerous decades old, improvements in spatial, spectral, and temporal resolutions of recent satellites (e.g., IKONOS) and systems consisting of airborne videography, and latest traits in new hyper-spectral sensors are more and more turning into useful to determine size, region, and purpose of variant. Imagery can show all fields in a location and spot anomalies associated with crop power and bio mass a good deal earlier than ground inspections, thereby improving the performance of crop scouting in massive fields and allowing spark off remedial remedies. Temporal changes in vigor, as decided from NDVI [Normalized Difference Vegetation Index] evaluation of snap shots received at critical times, may be used to characterize spatial and temporal dynamics of crop overall performance and are expecting crop yields. Upon integration of imagery with other records layers in a GIS, maps can be organized. GPS receivers can then be used to discover susceptible spots and practice corrective measures. Likewise, pictures of various vegetation planted in rotation can display awesome crop vigour responses because of agronomic techniques followed in previous crops. Imagery also can be used to reveal land use changes at local degree. In numerous tropical countries, cloud cover persists for most a part of the cropping season and in such cases acquisition of radar imagery may be beneficial.

4. Yield Monitoring

If the "crop is the excellent sensor of its own environment" (Legg and Stafford, 1998) then sensing structures which can faucet into what the crop is "announcing" might also offer information on crop condition important to direct spatially variable inputs. Many studies and traits, specifically based on spectral mirrored image traits of the crop, have potential to allow inputs to be numerous to optimize use and decrease environmental harm. Yield mapping, the primary crop mapping device to be commercialized, offers valuable information to the crop supervisor to apply within the following season.

Others are:

LCC

- It provides reading of Leaf color intensity and leaf N status and saves nearly 26% fertilizer N. It also helps to synchronize N supply and crop demand.

SPAD Meter

- It measures the chlorophyll concentration in the leaf sample.
- Meter emits specific wavelengths in the red and infrared ranges. Chlorophyll transmission is characteristically high in the near infrared range and very low in the red range.
- The detector analyses the ratio of the two wavelengths to determine chlorophyll concentration index (CCI).

Green Seeker

- It uses active light source to measure NDVI to determine N rate by comparing it with a N rich strip within the field.

Laser Land Levelling

- Increase in water application efficiency up to 50 per cent.
- Saves 20-30% water hence saving in energy (diesel/electricity) also
- Better crop stand
- Increase in 8 to 10 % area under the crop
- Increases resource (N and water) use efficiency
- Increase weed control efficiency
- Enhances productivity
- Reduced labour requirement
- Improvement in crop yield by 10 to 15 per cent
- Improves uniformity of crop maturity (Jat *et al*., 2004)

Advantages:

Initially, it become used primarily as a mechanism to adjust fertilizer charges surrounding high, medium, or low producing zones of a subject. As technology and agronomic studies has improved, we've got begun to see not only improved use instances for precision farming gear, however even more potent outcomes and results related to stacking numerous technologies and practices collectively.

1. **Reduced costs** Being able to accurately lower fertilizer, herbicide or seed quotes in regions where it'll now not be reasonably-priced to utilize is one of the key benefits of precision agriculture.
2. **Increased Profitability** Increasing yields because of making use of agronomic ideas at an excessive decision, whilst decreasing expenses increases common profitability. Farmers Edge gives one of the lowest-priced, excessive-cost applications inside the industry via our precise software of era.
3. **Enhanced Sustainability** Ensuring that crop enter products carried out truly get into the plant and now not someplace else affecting the environment delivers now not simplest a superior bottom line however also helps a more secure surroundings, and inside the destiny, may even come up with get right of entry to to new markets on your crops. Using our precision services, we were able to quantify that, on common, a Farmers Edge Variable Rate (VR) purchaser decreased their carbon footprint by means of over 10% whilst growing output.
4. **Better Harvestability** One of the maximum widespread blessings of precision agriculture is the capacity to recognize the farm nutrient levels and soil types throughout the farm. We know that fields and geographies aren't created equal, and this will affect the quantity of nitrogen mineralization's, water keeping capacity, and plenty extra. When we apprehend these variances, we can make sure we do no longer over practice nitrogen, that can cause accommodations, or we will boom vitamins like potassium that help with standability in regions wherein it is low. To pinnacle it off, we will do VR desiccation, meaning we can have a decrease quantity of desiccant on hilltops or sandy areas and higher prices in low spots to ensure your crop may be harvested with no trouble.
5. **Increased Land Values** We recognize precision is an outstanding practice from an agronomic attitude, however it could also force the fee of land fees, as mentioned here.

   "The evidence that precision ag makes land greater efficient and profitable truely interprets into higher lease value and market cost for farmland to aggressive customers of precision ag."

6. **Higher Resolutions Understanding of Your Farm** Farmers realize their land higher than each person. Precision agriculture gives you the capacity to apprehend why certain areas of your farm beneath produce, or are generating higher, supplying you with the foundation to make decisions that always enhance the farm.
7. **Better In season Yield Understanding** Using precision imagery, or precision weather offerings can help to not handiest benefit an information of regions of your farm which might be seeing challenges or want greater attention however through this combination of records concerning the farms and fields, we will offer accurate yield prediction in-season, empowering higher decisions agronomically in addition to for advertising and marketing or asset shopping functions.
8. **Precise nutrient applications** can give crucial environmental and financial blessings. The purpose is to use handiest the nutrients that the vegetation requires and might use. Moreover, there can be a demand to manipulate utility in regions that are environmentally touchy. Rates of software will fluctuate inside the field based on the form of soils, levels of fertility, and sensitivity to the surroundings. There are a few types of soils in a discipline that does not have the capacity to validate maximum fees of nutrient software. On the opposite hand, there might be regions that need to be reduced prices because of sensitivity to the environment. (Bana *et al*., 2020)
9. **Precise pesticide applications** can provide each economic and environmental advantages. One of the cheapest and fastest environmental payoffs for packages of pesticides is the use of light bar steerage systems. These affordable light bar steering systems offer an smooth approach to steer system across an area to save you overlapping while pesticides are being sprayed

Challenges and limitations:

PA has been used since the previous couple of decades to decorate plants' yield with reduced costs and human effort, even though the adoption of these novel strategies by means of farmers remains very restrained attributable to the subsequent reasons or demanding situations.

1. **Hardware Cost PA** is predicated totally on hardware inclusive of sensors, wi-fi nodes, drones, spectral imaging sensors, etc., which can be used to assess more than one parameter in real time. These sensors have a couple of boundaries which include high development, renovation and deployment value. Some structures in PA are cost powerful and are appropriate for small arable land, i.e., clever irrigation systems that require low-value hardware additives and sensors. However, drone-based systems for plants' health tracking are viable for huge arable land because of high set up fee.

2. **Weather Variations** Environmental version is one of the fundamental challenges that affects the accuracy of records gathered through sensors. Sensor nodes deployed in the subject are sensitive to environmental versions, i.e., rain, fluctuation in temperature, wind pace, sun mild, and so on. Communication between wi-fi nodes and the cloud may be interrupted due interference caused in wireless communique channels by atmospheric disturbance. The satellite tv for pc, air borne and drone systems are also sensitive to weather variations. Imagery received by using those systems is affected by contamination of clouds and other natural aerosols. The development of superior techniques for atmospheric correction, cloud detection and noise interpolation is a modern open task, which requires difficult efforts from the studies community.
3. **Data Management** The sensors in PA constantly generate facts. To make sure the integrity of statistics, a few facts security measures wish to be in place, as a way to in flip enhance the value of the system. The readings from the sensors ought to be accurate so as to take appropriate actions exactly whilst and wherein required. An intruder can corrupt the readings, and fake readings will adversely lessen the effectiveness of the gadget. PA structures generate monstrous quantities of records, which require enough assets to perform data analysis. Real-time facts collected from sensors deployed across the fields after a couple of minutes and spectral imagery received from excessive-altitude or low-altitude structures produce the bulk of the facts, which growth the storage and processing necessities. New software structures and centers for scalable control of Big Data resources are demanded. In this regard, the generation of software program-as-a-provider solutions is focused on merging statistics control and IoT thorough cloud computing platforms.
4. **Literacy Rate** Literacy is an important component that influences the adoption ratio in PA. In growing international locations where the illiteracy rate is high, farmers grow vegetation primarily based on their experience. They do not utilize the modern-day technology in agriculture, which results in lack of manufacturing. Farmers want to be knowledgeable for you to recognize the era or they need to accept as true with a third birthday celebration for technical help. Therefore, in underdeveloped areas wherein the literacy rate isn't always high, PA isn't always very not unusual because of the constraints of sources and training.
5. **Connectivity** Next-generation 5G networks can be one hundred-times quicker than 4G ones, making verbal exchange among gadgets and servers much quicker. 5G can also convey much greater information than different networks, which makes it a really perfect technology for transmitting records from remote sensors and drones, key gear that are

being examined in PA environments. The adoption of latest conversation networks based totally on 5G is a must in modern-day packages where steady and rapid records transfer enables actual-time records management and help for choice making.

6. **Interoperability** One of the biggest problems PA faces is the interoperability of device because of distinctive digital standards. This lack of interoperability isn't always best obstructing the adoption of new IoT technologies and slowing down their boom, but it additionally inhibits the gain of manufacturing efficiency through smart agriculture applications. New techniques and protocols to combine one of a kind machine communique standard to unlock the potential of efficient system-to-system verbal exchange and data sharing between machines and management records systems are required in the modern-day state of affairs of PA.

A few of possible solutions may be

- ✓ Identifying ways and means of reducing the cost of RS, GIS and precision farming technologies and time gap in collection, interpretation and dissemination of data to enable their usage on a large scale.
- ✓ Providing convincing evidence to prove the utility and economic viability of these technologies so as to mobilize support for R & D work
- ✓ Human resource development to hasten the process of large-scale use of unexplained and cutting-edge technologies that have tremendous scope and potential
- ✓ Improving the internet connectivity and speed in rural areas
- ✓ Use of Artificial intelligence for handling and analysing big data
- ✓ Farmer’s co-operatives and Farmer Producer Organizations
- ✓ Pilot projects.
- ✓ Combined effort of Researchers and Government
- ✓ Entry of Private players in this sector

Conclusion:

- ➢ Precision farming technology looks promising as a future farming tool, however its effective use in Indian agriculture is yet to be realized and research on Precision Farming is at infancy stage in our country.
- ➢ Precision land levelling, precision planting with VRT, real time N application using LCC, SPAD (chlorophyll meter), Green Seeker, Nutrient Expert and other sensor-based technology have demonstrated potentialities for improving crop yields and increasing resource-use efficiency in real farming situation.

- Precision farming provides great opportunity for accuracy and efficiency in every agricultural operation right from sowing, fertilizing, irrigation, protection till harvesting with precision tools like GPS, GIS, VRT, Remote Sensing etc.
- PF shows immense potential environmental prospects like reduction in nitrate leaching, emission of greenhouse gases and agrochemicals like fertilizer, herbicide, pesticides etc. which may help in achieving sustainable agriculture of future.
- Precision Farming may help farmers to harvest fruits of frontier technologies by achieving more profitability with increase in productivity, availability of cost-effective technology and decrease in cost of cultivation with better management of inputs.
- The PF may trigger a techno-green revolution in India which is the need of the hour.

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HONEYBEE MANAGEMENT FOR POLLINATION

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Abstract:

Honeybees are social insects which live in colonies, pollinate many crops and provide their valuable products like honey, bees wax, royal jelly, bee venom, pollen and bee gum (propolis). Though, honeybees have been known since immemorial time for their honey but their role in pollination of crops was realized only later on. There are at least six species of honeybees in India including the one imported European. *Apis dorsata*, a huge rock bee, *Apis cerana*, a medium-sized oriental species, *Apis florea*, a small bee, and two kinds of stingless bees, Mellipona and Trigona, are among the native species. Few other honey bees reported are *Apis koschevnikovi* from South- East Asia. *Apis andreniformis* from Thailand, Malaysia and southom peninsular region of China. *Apis laboriosa* probably the largest honeybee is foundin the mountain region of Nepal. Out of these, *A. cerana* and *A. mellifera* are maintained for commercial purposes and are domesticated for both pollination and bee product collection. These two species construct parallel combs in dark and concealed places, have mild behaviour and are commercially viable.

Keywords: Honeybees, pollination, Products.

Introduction:

Bees are said to have evolved from hunting wasps that developed a taste for honey and decided to go vegetarian. Bees presumably appeared on the earth at the same time as flowering plants in the Cretaceous period, 146 to 74 million years ago, according to fossil evidence. *Trigona prisca*, the oldest known fossil bee, was discovered in the Upper Cretaceous of New Jersey, USA, and dated from 96 to 74 million years ago. Fossils of the authentic *Apis* type were first unearthed in Western Germany during the Lower Miocene (22 to 25 million years ago). Honey bees are eusocial flying insects belonging to the *Apis* genus of the bee clade, all of which are native to Eurasia. They're well-known for their construction of perennial colonial nests from wax.

It plays an important role as *natural pollinators* of crops and propagation of many plants growing in nature thereby maintaining the stability of ecosystem, environmental quality and biodiversity. Also beekeeping is crucially important for agriculture wellbeing. European honey bees (*Apis mellifera*) to fields and orchards for pollination services. It also helps rural populations to become selfreliant. It favors diversification to the local economy. So, beekeeping recognized as a low input and high output activity, suitable for rural, tribal and other weaker section of population.

According to the National Bee Board's most recent statistics, the country's total honey production reported in 2019 - 2020 was 1.05 lakh metric tonnes (MTs). With international demand for honey growing, India exports half of the world's honey, and during the past 12 years, shipments have grown by 207% as a result of rising demand worldwide. Punjab is the major state in beekeeping in the nation, with around 35,000 beekeepers delivering around 15,000 metric tonnes of honey. This is more than 39% of the nation's total honey production. Next to that, Karnataka has produced 1200 tonnes of honey.approximately as updated of 2019.

Habit and habitat of important honeybee species:

Table 1: Races of honeybees and their native habitat

Sr. No.	Common Name	Scientific Name	Native habitat
1.	Rock Bee	*Apis dorsata* Fab.	Asia
2.	Little Bee	*Apis florea* Fab.	Asia
3.	Largest Bee	*Apis laboriosa* Smith.	Asia
4.	Asiatic Hive Bee	*Apis cerana* Fab.	Asia
5.	Smallest Bee	*Apis andreniformis* Smith.	Asia
6.	European Bee	*Apis mellifera* Lin.*	Europe
7.	Enderlein Red Bee	*Apis koschevnikovi* Enderlein	Malaysia
8.	Malaysian Bee	*Apis nuluensis* Lin.	Malaysia
9.	Smith Black Bee	*Apis nigrocinta* Smith	Indonesia

1. Rock bee (*Apis dorsata* F.)

This species are found in Malaysia, China, Philippines, Sri Lanka, Malaysia, Pakistan, as well as Indonesia. It is found upto an elevation of about 2100 m a.s.l and builds single comb in open-air, generally under the shade attached to the branches of trees, rocks, ceilings etc. Combs of *A. dorsata* are 1.5 2.0 m from side to side and 0.6-1.2 m in depth. The length of the workers is 16–18 mm. This species is quite migratory and frequently shifts its location in quest of food. It is vicious and is triggered by even the smallest disruption. A large group of worker bees assault and

follow the victim for a considerable distance. The *Apis dorsata* is an effective crop pollinator and has a large honey reserve. 20–25 kg of honey can be produced by a single colony. It has not yet been domesticated in ancient hives for economic gain.

2. Little honeybee (*Apis florea* F.)

This species is widely distributed in India, Sri Lanka, Pakistan, Indo-China region, Malaysia, Philippines, Iran and Oman. It is found in plains and sub-mountain regions upto an elevation of 300m a sl. *A. florea* builds a solitary, little comb in the open, in the shade, dangling from the branches of small trees, bushes, hedges, etc. It gathers honey poorly and has a great propensity to swarm. 200g to 900g of honey can be obtained from a comb. This species helps pollinate a variety of crops.

3. Stingless bees (*Melipona sp.* & *Trigona sp.)*

Tropical and subtropical areas are inhabited to these species. Rather than being fully stingless, these have a weak sting. Members bite to defend themselves. Stingless bees prepare their nets in the ground, tree or bamboo hollows, on rocks, or in wall crevices. Wax and plant resins are used to make nest cells. In colonies, these bees may also be domesticated. During the reproductive season, a stingless bee colony consists of a queen, a few hundred workers, and a few drones. They have different parts for their honey and brood cells. These bees produce a relatively small amount of honey-just a few ounces. Crops are pollinated by workers, although there isn't much information on this topic.

4. Oriental honeybee (*Apis cerana* F.)

This species is native to the Indo-China region, the Philippines, China, USSR, Japan, and Indonesia in addition to India, Pakistan, Sri Lanka, Malaya, and Malaysia. *A. cerana* inhabits plains and hilly terrain up to an altitude of 2500m a.s.l. Under non-migratory conditions, a single colony produces 8 to 9 kg of honey per year on average. It has a moderate disposition and a strong propensity for swarming and absconding in addition to being more vulnerable to wax moth attack. In places that are dark, such as cracks in walls, mound-forming termite nests, tree trunk cavities, etc., it constructs parallel combs that have a count of 7 to 10. This species typically gathers very little propolis for repairing the joint or caulking the fissures.

5. European honeybee (*Apis mellifera* L.)

This originated in Europe and has been extensively domesticated in many nations. It is now practically universal in its incidence. Even more so than the Asian honeybee *A. cerana*, *A. mellifera* has an amiable disposition. In gloomy areas, it also builds parallel combs in wall crevices, tree hollows, etc. It is an effective pollinator and honey collector. Under non-migratory conditions but in optimum migratory conditions, a single colony may typically produce 15 to 20 kg. of honey each year. 35 to 40 kg of honey are very typical. Compared to the oriental

honeybee, this species has less of a tendency to scurry away and is less frequently attacked by wax moths, but it is more susceptible to mite infestations. Around the 1960s, the Dept. of Entomology at Punjab Agricultural University in Ludhiana successfully introduced this species to India from Italy, and it has since spread to practically all of the country's states.

Domestication of honeybees:

The management of a honeybee colony requires knowledge and expertise. Before starting a beekeeping business, one must make certain that there is either an abundance of bee flora nearby that is available throughout the year or that colonies can be moved to flower-rich locations during times of shortage. After confirming that the location is suitable for beekeeping, the necessary training can be received from the closest beekeeping organisation. Discussing all facets of beekeeping, its issues, and surrounding marketing resources with other beekeepers in the area is advised.

1. Suitable environment:

1.1 Climatic conditions

Except in extreme cases where temperatures typically remain below 15-16 °C or above 38 °C, relative humidity is above 75% with frequent rainfall and higher wind speeds, climatic conditions such as temperature, humidity, rainfall, etc. prevalent in different parts of India and Nepal do not pose significant obstacles. Since beekeeping relies on crops and forests, subtropical climates with plains and hilly forests within 100 kilometres are excellent. Pathogens, nutrient shortages, climate change, and deforestation are a few of the external factors that limit bees' ability to perform their pollination function. Threatening pollination services of crops and wild plants, pathogens including viruses and bacterial infections have a severe impact on bee health and longevity. Bee immune systems are weakened by viral infections, which lead to illness across entire colonies.

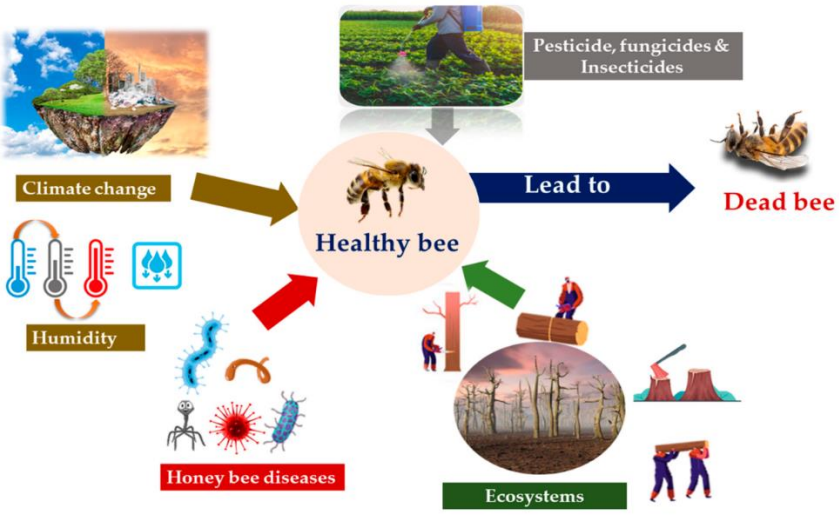

Figure 1: Challenges faced in bee pollination

1.2 Bee Flora

Successful beekeeping requires a sufficient supply of bee vegetation. Eucalytus, Neem, Jamun, tamarind, Australian acacia, drumstick, karanj, silk cotton, guava, lemon, litchi, rubber, apple, banana, pear, ber, shoesum (Dalbergia sisso) are import crops. Maize, pearlmillet, buck wheat, mustard, tonia, sunflower, and berseem seed crops are also significant. Bottlebrush and Plectranthus sp. (Shain) are shrubs. Calendula, roses, tick seeds, omamental poppies, creepers, Antigonon leptopus, and other flowers are highly beneficial. Planting day-neutral crops like sunflower close to the hive may increase the availability of pollen and nectar.

2. Equipments

For the European honeybee *A. mellifera*, a Langstroth hive or one similar to it is necessary, while a Newton, Jeolikote, or one similar to it is required for the oriental honeybee *A. cerana*. The following items are needed: a bee veil, gloves, smoker, hive tool, queen gate (typically needed for oriental honeybees), queen excluding screen, division board (dummy board), honey extractor, honey storage tanks, honey bottles, and a machine to seal bottle caps.

Bee gloves and veils prevent stings, while smokers are employed to control aggressive bees. In order to prevent the queen from laying eggs outside of the brood chamber, a queen-excluding screen is placed between the brood chamber and the super chamber (honey chamber). During periods of low population, dummy boards are used to separate and contain colonies, which aids in preventing infestations of natural enemies like wax moth.

3. Start of beekeeping:

The best months to start beekeeping are February and March. Any reputable beekeeper or the closest government beekeeping center can provide beehives, bees, and other accessories. When purchasing bees, it is important to make sure the queen is strong, healthy, and successfully laying eggs. The packaging and transportation of the bee hives are best done during the dusk or evening hours. The hive's gate can be secured with wire mesh during shipment, and the floor board can be fastened to the brood chamber using iron strips and nails.

4. Precautions for handing of bee hive:

The safety of the beekeeper depends heavily on handling beehives and their frames properly. When handling honeybees, the following safety measures can be taken:

- Avoid blocking the foraging bees by standing to one side of the hive:
- Hives should only be opened on sunny, clear days when bees are actively foraging. Hives should not be opened on gloomy or rainy days.
- Break the upper and lower frame joints by moving the super sideways.
- Use the hive tool to disassemble inter-frame joints.

- Wear gloves, a bee veil, and thick pants and shirts or a pron.
- Carefully remove supers and brood chambers to prevent physical harm to the bees.
- When handling bees, keep an eye out for any behavioural changes, and if necessary, smoke the bees with a smoker.
- Rub any green leaf on the injured area after it has been stung to alter the warning pheromone's characteristics and prevent further stings.

Seasonal management of apiary:

Bees can be maintained as follows to ensure effective apiary management and good honey production as well as to maintain healthy colonies for seasonal pollination:

1. Monsoon period (June to September):

- This period follows the end of honey flow season Leave sufficient honey in supers for feeding during flora deficient periods.
- Protect hives from rain, wind and natural enemies like wax moth, ants wasps, lizards and other reptiles.
- Clean floor-board regularly and remove uncovered frames to protect them from the attack of wax moth.
- Replace comb frames which are infested with the wax moth larva keep such frames in strong sunlight for about 30-60 minutes to kill the larvae.
- Removal replace fungus attacked combs.
- Inspect colonies on sunny days only.
- When honey is deficient in combs, feed bees with 50% sugar solution.

If, these steps are taken, bees may be managed effectively and absconding may be avoided particularly, in case of *A. cerana*.

2. Post-monsoon period (October - November):

- Continue to feed sugar solution, if required.
- Some healthy colonies may exhibit swarming signs. To lessen swarming drive in robust colonies and strengthen weak ones, combs carrying honey from such colonies should be moved.
- Frames drawn out earlier (done so) may be given to the colonies.
- Weak colonies may be united by removing the older queen.
- Capture natural swarms, if available.
- Maintain proper ventilation.
- Extract surplus honey.
- Prepare colours for the December–January period of flora shortage..

- Do queen rearing and remove old queen.
- The weekly provision of feed and brood combs from other colonies to a few selected colonies to encourage drone and queen reproduction.
- For comb raising, add combs or comb foundation sheets.

3. Winter management (December - January):

- Protect colonies from cold weather and enemies.
- For protection against severe cold, use grass, saw dust for packaging, without affecting proper ventilation.
- Migrate colonies to the plain areas where there is lesser cold.
- If required, feed colonies with 2:1 sugar, water solution.
- For better carry over of winter season, maintain new queen a supply good quality feed.

4. Post-Winter (February-May):

This includes peak activity period of spring season when greater amount of honey is collected. Observe following practices during this period:

- Add one or two sealed brood frames to the selected colonies to be used for queen rearing, strengthen and induce drone brood rearing.
- Unite weak colonies and remove old non-prolific queen.
- Undertake queen rearing/grafting work in the selected colonies.
- In colonies meant for queen rearing prepare mating nuclei, introduce, distribute and select fully developed queen cell to mating nuclei and watch till the queans are mated. Unite nucle where new queen remained either unmated or lost during mating Continue this for more queen production.
- In the colonies meant for harvesting bee’s products, add supers to the colonies, avoid congestion in brood or honey chambers and check drone brood rearing which may lead to swarming.
- Schedule final honey extraction by the end of April, leaving about 2-3 frame honey for feeding honeybees.
- Inspect colonies for supersedure and if there are queen cells, break them.
- Remove uncovered super frames at the end of nectar flow season, dry and preserve them in para-di chlorobenzene treated chamber.

6. Management of swarming:

Swarming is a natural occurrence that helps colonies grow. To maintain healthy colonies for a decent honey harvest, it is under supervision. In swarming, a new queen takes the place of the old one. The old queen departs the hive with nearly half of the colony's chambers and

establishes a new colony in a natural setting. Break old queen cells or eliminate the old queen when the future queen is well along in the pupal stage to regulate it.

7. Management of absconding:

Absconding happens because the hive is starved of nourishment, such as nectar and pollen, or because of human meddling, ant or wax moth attacks, etc. Provide a 50% sugar solution, replace any frames damaged by wax moths, apply ant-proofing measures, and utilise queen gates to stop absconding.

8. Management of natural enemies:

The most significant natural enemies include Vespa species, mites, wax moths, and infectious illnesses.

8.1. Wax moth:

The colonies of oriental honeybees are significantly harmed by wax moths. The following actions could be taken for its efficient management:

- Clean floor-board regularly.
- Replace the damaged frames with new ones and set the new ones in direct sunlight to destroy the moth larvae.

8.2. Mites:

The European bee, *Apis mellifera*, is particularly vulnerable to mite attack. When all the bees are inside the hive in the evening, seal the entrance and crown board holes for an hour while using illuminated Folbex strips (one strip every 10–12 frames). Up to six sessions of this therapy should be administered at weekly intervals. Before the beginning of the honey flow season, such treatments are typically used.

8.3. Diseases:

There are numerous diseases that have been linked to honeybees. Amoebic and viral illnesses are among them, and they have a significant economic impact on India and its neighbour’s areas.

8.3.1. Amoebic disease:

This is brought on by the amoeba *Nosema apis* and develops as a result of drinking tainted rainwater. Clean water needs to be available close to beehives to deter its attack. One @250 mg tetracycline capsule may be dissolved in @250 ml of sugar solution in the event of infection. A bottle with a large mouth and a thick cloth- and rubber-band-covered entrance is used to hold the solution. Over the hive's frames, the bottle is maintained upside down.

8.3.2. Viral diseases:

There have been numerous reports of viral infections in honeybees. Around ten years ago, India saw a Thai-sac brood disease epidemic that affected *Apis cerana*. The spread of viral

illnesses can be stopped by taking measures, but there is no effective pharmaceutical treatment for them.

- Check robbing and dritting in bees.
- Do not interchange contaminated equipment with other beekeepers.
- Infected colonies should be kept apart at least 500m and preferably more than 1,0km from the healthy ones.
- Dead bees and their immature stages should be buried deep in soil.
- Inform government agencies.
- The hive of bum bees. If the hive is not burned, properly clean it, sanitise it with a good disinfectant, and store it in a sunny location for more than 30 days.

9. Harvesting and storage of honey:

When super frames are sealed and filled with honey during the honey-flow time. One by one, the frames are removed. Jerk the frame diagonally over the hive lightly. The remaining bees are brushed off the frame before it is transported into a bee-proof area for extracting honey. With the use of a knife and heated water, cape cells are sliced. After that, frames are placed within the honey extractor, and when the extractor basket rotates, honey falls to the bottom of the extractor as a result of centrifugal force. The collected honey is filtered through muslin cloth and then kept, along with its container, in a hot water drum for around 30 minutes. It should be mentioned that the hot water shouldn't be heated above 60 °C and should instead be between 50 and 55 °C. Honey is heated before being filtered through two layers of muslin cloth. If there is a wax or pollen covering on the surface the next morning, it should be removed before honey is put into wide-mouth bottles and sealed.

10. Release of honeybees inside a cage:

During the nighttime, honeybee colonies are transported inside a cage. To condition the bees inside the cage, such colonies are initially given a 50–80 percent sugar solution. Honeybees frequently show signs of continued disturbance and attempt to flee the cage. This state continues for roughly two days. Around 10-15% of bees perish during such escape attempts, hence in the beginning, 10-15% more bees should be retained. It should be noted that the cage's length, width, and height must be adequate to allow for the bees' proper movement. For *Apis mellifera* and *A. cerana*, nylon nets with minimum sizes of 200 and 100 sq. m., respectively, are typically employed. Such nets should be 1.5–2.0 m above the crop canopy in height. One more hive gate may be provided opposite the first gate if a favoured crop is not nearby, and the hive may be positioned so that the second gate opens outside of the cage. Bee mortality is decreased as a result.

11. Protection of honeybees from pesticides:

Most fungicides, weedicides, organic pesticides, microbiological pesticides, and granular insecticides are generally harmless for honeybees. Phosalone, endosulfan, menazon, formothion, and permethrin are insecticides that are safe in their EC formulations. The majority of synthetic pyrethroids, including diazinon, parathion, dichlorvos, monocrotophos, malathion, dimethoate, and carbaryl, are extremely hazardous to honeybees. In light of this, it is clear that, with a few notable exceptions, the majority of the insecticides now in use are very harmful to honeybees. Therefore, the following precautions should be taken before using insecticides:

- Can use insecticides with less or no toxicity to honeybees while being effective against the target species.
- The economic injury level (EIL) of the pest should be taken into consideration when using insecticides.
- Right from sowing or planting of the crop till harvest, continuous vigil should be maintained and wherever feasible mechanical, physical, cultural and biological means of pest management measures should be adopted.
- It should be encouraged to apply dust formulations, and granular, EC, WP, and WDP formulations should all be used. Granules should be applied in the soil, though, as this may reduce the likelihood that they may replace pollen in the soil.

Aeroplane or large scale application of pesticides should be discouraged because it may annihilate large number of bees.

- It will be better if pests are managed before or after the bloom period.
- Use natural pesticides besides augmentation and conservation of natural enemies of the pests.
- Effective repellents may be mixed with the pesticides to repel honeybees.
- Proper dosage along with suitable appliances should be used.

Increases quantity and quality of crops by Bee pollination

Honeybee pollination services result in increased yields that are worth between 15 to 20 times more than the gross margin of all hive goods. Studies conducted of honeybee pollination in improving the yield and quality of some crops such as Yield increase in almond, kiwi, avocado, and soybean crops and increase in fruit weights in *Cucurbita* spp., fava bean, and sunflower. The yield increment was varied from crop to crop due to honeybee pollination.

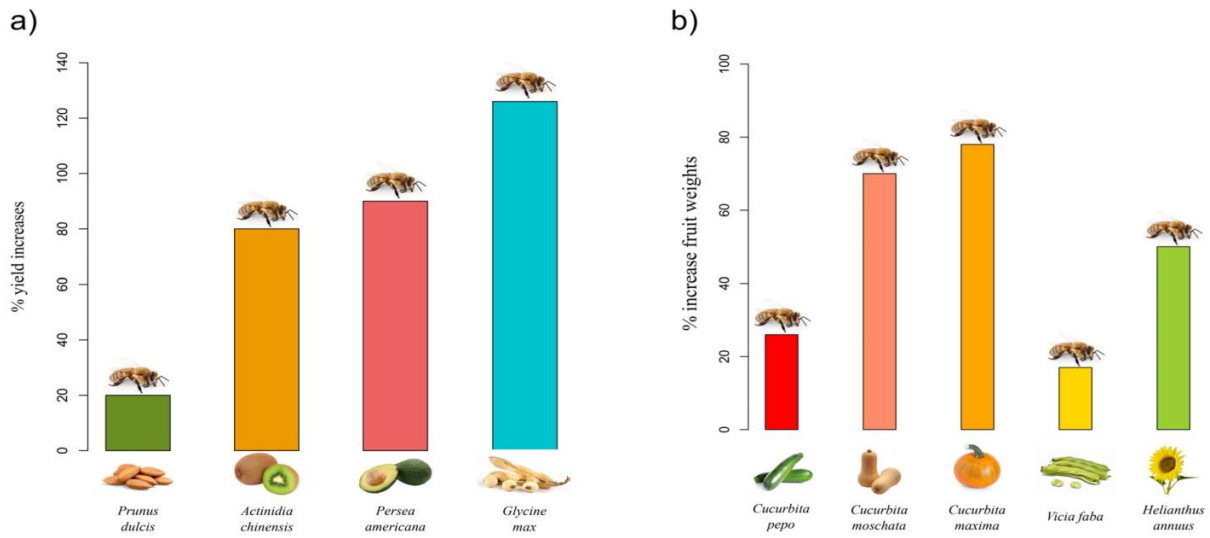

Figure 2. Benefits provided by *A. mellifera* in crop production.
(a) Yield increase in almond, kiwi, avocado, and soybean crops;
(b) increase in fruit weights in *Cucurbita spp.*, fava bean, and sunflower

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EL NIÑO AND LA NIÑA AND ITS EFFECTS ON INDIAN SUMMER MONSOON

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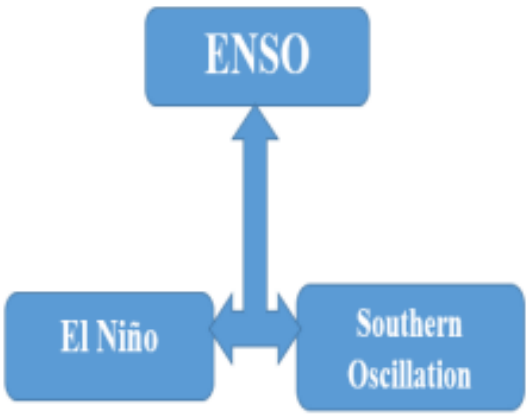
Abstract:

El Niño and La Niña represent opposite phases of the El Niño-Southern Oscillation (ENSO) cycle. These deviations from normal surface temperatures can have a significant effect on the global climate and weather conditions. Trade winds played an important role in pushing the warm ocean current. El Niño is often called the warm phase and La Nina is called the cold phase of ENSO. El Niño and La Nina typically occur every 4 to 5 years. El Niño is more frequent than La Nina. Typically, the duration of an episode is nine to twelve months. The main source of rainfall in India is the southwest monsoon which is normally sufficient for agriculture. ISM is intensified during La Niña but weakened during both the eastern and central Pacific El Niño events. The weakening of the ISM is particularly more pronounced during the eastern Pacific El Niño. El Niño results in heavy rainfall in Peru, depriving the Indian subcontinent of its normal monsoon rains. La Nina causes drought in Peru and Ecuador, severe flooding in Australia, high temperatures in the Western Pacific, Indian Ocean, and off the coast of Somalia, and abundant monsoon rains in India. The Indian monsoon actually benefits from a La Nina. The summer monsoon rainfall (SMR) and the tropical Indo-Pacific climate drivers affect Kharif crop production in different parts of India and hence the economy of the country.

Introduction:

El Niño is a phenomenon in the equatorial Pacific, in which sea-surface temperatures rise over a threshold of +0.5 degrees Celsius. There are a few other acronyms that one comes across while tracking El Niño. For instance, the Southern Oscillation Index, or SOI, gives an indication of the development and intensity of El Niño or La Nina. The SOI is calculated on the basis of the atmospheric pressure differences between Tahiti (South Pacific Ocean) and Darwin (Australia), separated by 8,569 km. Sustained positive SOI values are indicative of La Nina conditions while negative values suggest El Niño conditions. Another acronym is the ENSO comprises two words El Niño and Southern Oscillation (Fig 1) which refers to the oscillation between El Niño and La

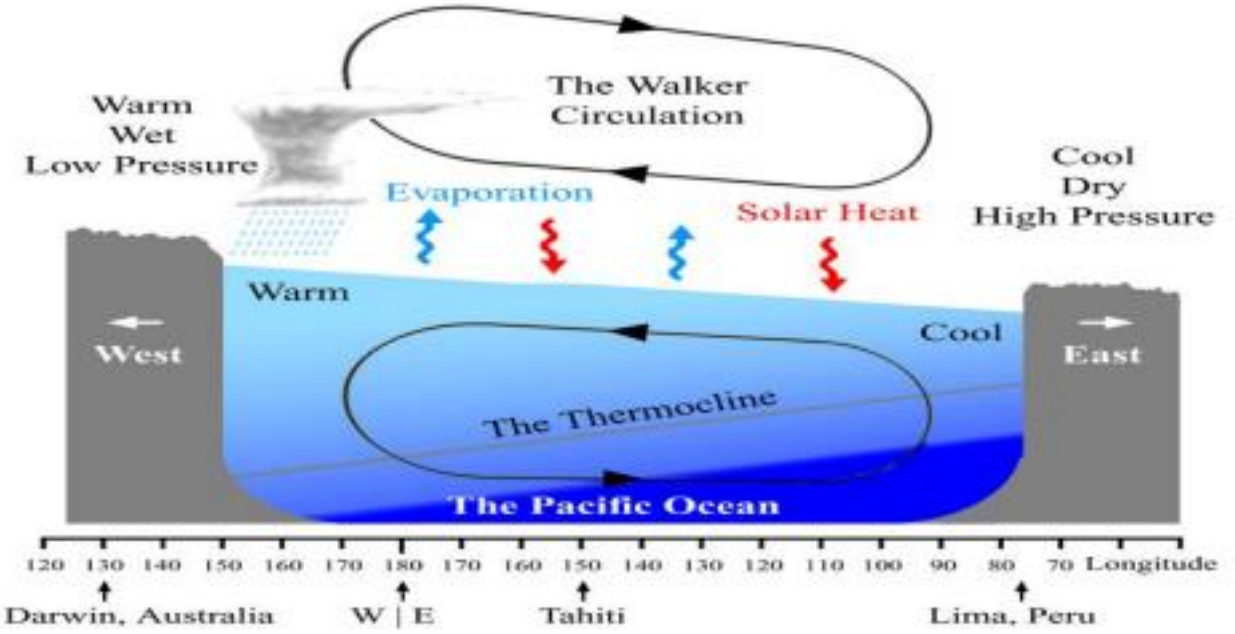
Nina. ENSO shifts irregularly back and forth between El Niño and La Niña every two to seven years.



It is the ocean temperature that oscillates back and forth from warm ocean temperature to cold ocean temperature and vice versa in the Pacific Ocean. The Pacific Ocean is a large pool of water that exists on the western side of the American continent and the Eastern side of the Asian and Australian continents.Each phase induces predictable fluctuations in temperature, precipitation, and winds that disrupt large-scale air circulation in the tropics, resulting in a chain reaction of global side effects. However, under "normal" conditions, the western basin of the tropical Pacific is warmer than its eastern basin. Additionally, the ocean's warmer region is a source of convection and is associated with cloudiness and precipitation. Warmth shifts to the Central and East Tropical Pacific (Niño 3.4 region) during El Niño years, bringing cloudiness and precipitation with it.

How does ENSO occur?

The majority of ENSO events take place in the southern hemisphere. As a result of its location on the equator, the Pacific Ocean experiences excruciatingly high temperatures. Equatorial regions receive maximum solar radiation throughout the year so the temperature is high so the trade wind (Tropical easterlies) pushes warm ocean currents towards the Asia side because they move from east to west making the western pacific ocean warm(the region around New Zealand, Australia and Indonesia). This warm ocean current has an effect on the surrounding atmosphere, causing an increase in both the temperature and the amount of moisture that is present.



Walker Circulation

After that, the process of convection causes warm air to rise high into the atmosphere, where it then causes clouds to form and ultimately results in precipitation. This warm air rises until it reaches the end of the troposphere, which is colder than the top of the troposphere. This warm air then moves eastward toward the eastern Pacific Ocean, which is close to Ecuador and Peru. When warm air collides with cold air, the moisture content of the air is evaporated, leaving behind dry air. This dry air moves towards the eastern side of the Pacific Ocean, and it eventually makes its way down the Peruvian coastal side, which causes the region to be cold. This pattern of rising air in the west and falling in the east continues and it is known as the Walker circulation (Fig 2). Here trade winds played an important role in pushing the warm ocean current towards the western Pacific Ocean.

El Niño:

El Niño is the large-scale ocean-atmosphere climate interaction associated with the periodic warming of sea surface temperatures in the central and east-central Equatorial Pacific. It is associated with the western Pacific's high pressure. December is one of the few months during the year when the trade winds are weak. As a result, the warm ocean current does not receive any push, and the warm pull of ocean water in the western Pacific moves slowly towards the central and eastern Pacific. In the central and eastern Pacific Ocean, the warm ocean current is gradually replacing the cold ocean current. When the warm ocean current moves, everything

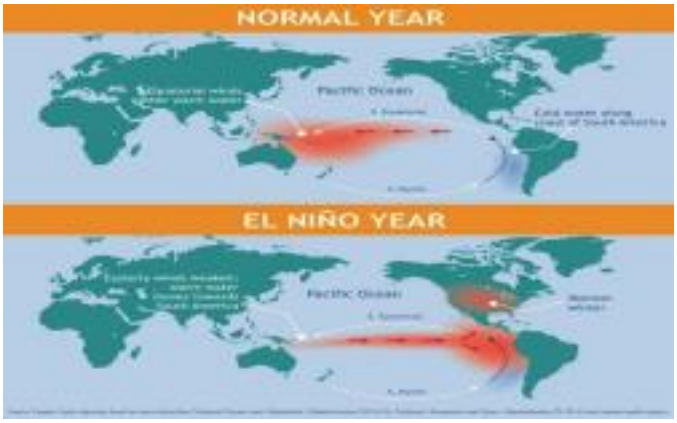
associated with it, such as the convection process and the formation of rain clouds, moves along with it. This walker circulation, which is a large looping pattern, is now splitting into two sections. As a result, the ocean near Australia is cold and there is no precipitation; consequently, the interior of Australia is experiencing a severe drought. On the other hand, the warm pool of ocean current near the Peruvian coasts brings heavy rain to the American continent. The events, i.e., warm waters in the Pacific Ocean, tended to occur in December, hence, the name was chosen.

Let us see how El Niño affects India

In a typical monsoon season (in the absence of El Niño), the pressure distribution is as follows:

1. The pressure along the Peruvian coast in South America is higher than the pressure near northern Australia and Southeast Asia.
2. The Indian Ocean has comparatively lower pressure as it is warmer than the surrounding oceans. Thus, winds laden with moisture moves from the western Pacific to the Indian Ocean.
3. India's landmass has a lower pressure than the Indian Ocean; consequently, the moisture-laden winds travel farther from the ocean to the land.

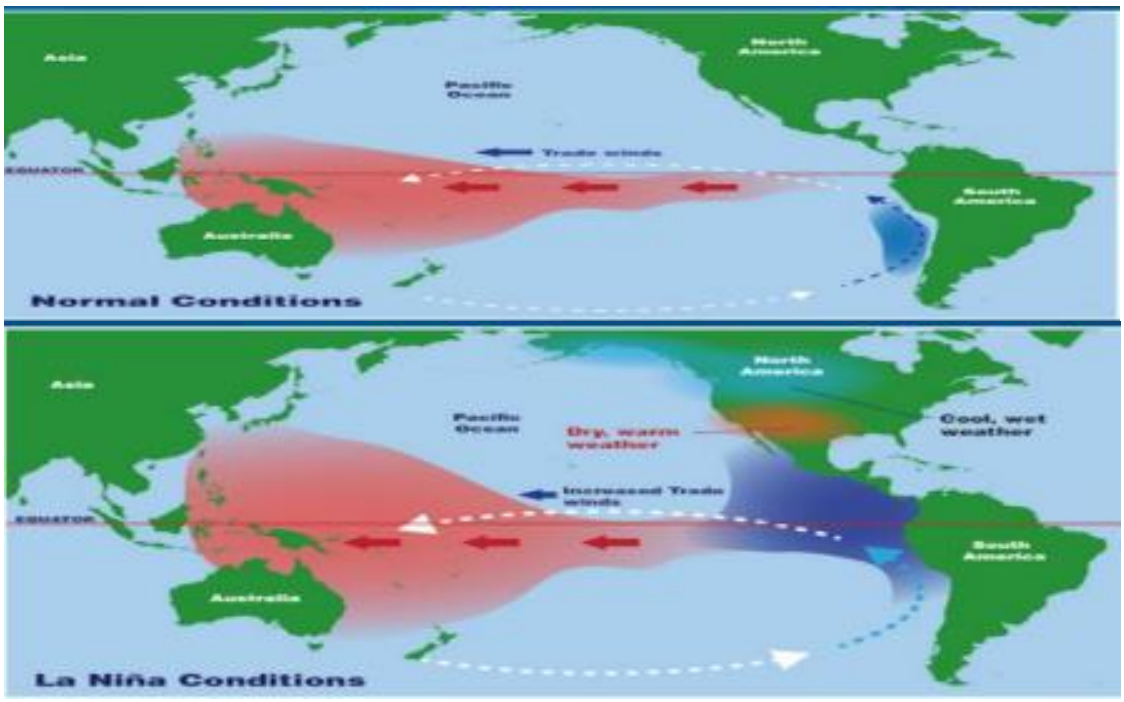
If for some reason this normal pressure distribution is altered, the monsoons will be affected. What effects does El Niño have?



El Niño causes the surface water off the Peruvian coast to warm. When the water is warm, normal trade winds dissipate or reverse course. Therefore, the flow of moisture-laden winds from the western Pacific is directed toward the coast of Peru (the region near northern Australia and South East Asia). During El Niño years, this results in heavy rainfall in Peru, depriving the Indian subcontinent of its normal monsoon rains (see fig 3). The larger the temperature and pressure difference, the larger the rainfall shortage in India.

La Nina

In Spanish, La Nina means "little girl" and is also known as El Viejo or "cold event." Here, the Eastern Pacific's water temperature drops below normal. When trade winds are strong, they blow from east to west and push the warm eastern Pacific Ocean current to the western Pacific Ocean (fig 4). As a result, cold water from the deep ocean rises to the surface, and the remaining process is identical to walker circulation. As a result, strong high pressure prevails over the eastern equatorial Pacific. Currently, there is low pressure in the Western Pacific and off the coast of Asia. La Nina causes drought in Peru and Ecuador, and severe flooding in Australia, high temperatures in the Western Pacific, Indian Ocean, and off the coast of Somalia, and abundant monsoon rains in India. The Indian monsoon actually benefits from a La Nina.



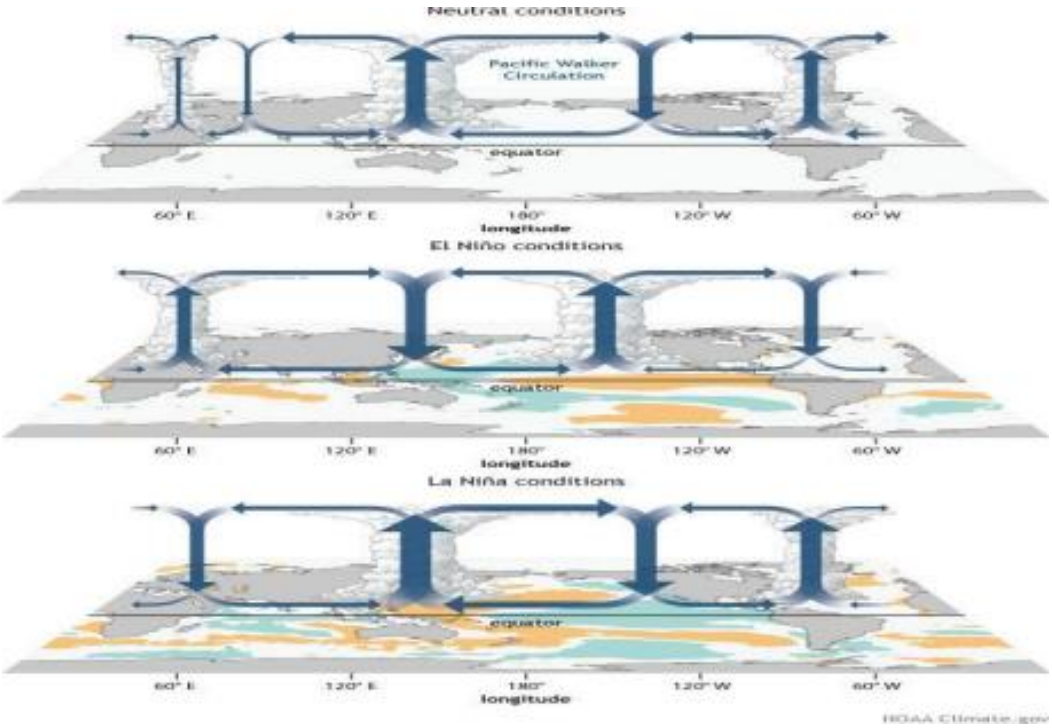
Difference etween normal conditions and La Nina conditions

How did it originate?

El Niño was first recognized in the late 1800s when South American fishermen observed the warming of coastal waters around Christmastime. It was called "El Niño" (Spanish for "the boy child") because it appeared around Christmas. British mathematician Sir Gilbert Walker discovered the Southern Oscillation (SO), or large-scale variations in sea level pressure across Indonesia and the tropical Pacific. However, he did not recognize the connection to Pacific Ocean changes or El Niño. Jacob Bjerknes, a Norwegian American meteorologist, and others did not discover the connection between ocean and atmospheric changes until the late 1960s. This is how the term "ENSO" originated.

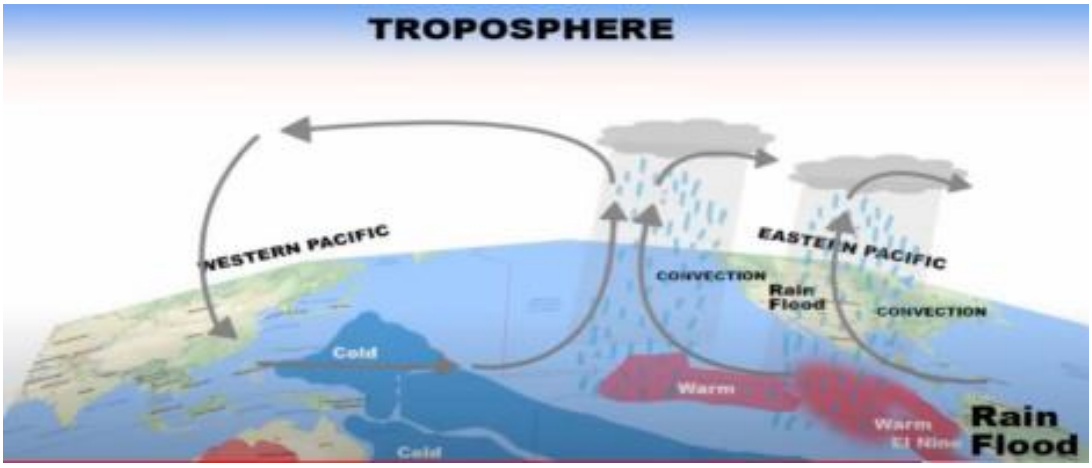
El Niño has been found to affect nearly half of the world, causing droughts in Australia, India, and southern Africa and floods in Peru, Ecuador, the United States, the Gulf of Mexico, and the Colorado River basin, as previously mentioned. If Sir Gilbert discovered in the 1920s that many global climate variations, including monsoon rains in India, were correlated with the Southern Oscillation, Bjerknes deserves credit for linking it with El Niño as a component of ENSO involving both the ocean and the atmosphere. However, it took until the 1980s or later for the terms 'La Nina' and 'neutral phase' (neither El Niño nor La Nina) to become popular.

Changes in the ENSO-Monsoon system



Different Phases of ENSO

Neutral Phase of ENSO



El Niño

The monsoon influences environmental conditions in much of Asia, thereby affecting the majority of our planet's most populous region. The seasonal reversal of monsoon winds is caused by the differential warming of the north Indian Ocean, the North West Pacific Ocean, and the Indian landmasses. During the summer, this wind blows from the oceans to India, bringing massive quantities of moisture to the surrounding land. This subsequent heavy rainfall can have devastating effects on human and animal life. In contrast, agriculture in India and Asia is dependent on monsoon rain, and the amount of nutrients in ocean surface waters is influenced by the monsoon and crucial to the success of fisheries. El Niño has a significant impact on the monsoon system.

The variable nature of India's summer monsoon rainfall has a demonstrable effect on the country's socio economic development. The Indian summer monsoon dominates the Indian subcontinent for four months, from June to September, with significant temporal and spatial variation. In an agricultural nation like India, the extreme deviation from normal seasonal rainfall has a significant impact on agricultural output and, by extension, the economy. It has been established that the Indian summer monsoon is a fully coupled land atmosphere-ocean system and that it is linked to ocean temperature variability. Interannual variability of the Indian summer monsoon rainfall is linked to the El Niño-Southern Oscillation phenomena directly.

El Niño has strengthened and its pattern has shifted. A 400-year-long seasonal record of El Niño created by Australian scientists has revealed that a new type of El Niño has become more prevalent in the central Pacific in the last three decades than at any time in the previous four centuries, and that El Niño events have become more intense and significant. El Niño has fluctuated in type over the last four centuries, as indicated by its historical trend. There has been an increase in events in the Central Pacific and a decrease in events in the Eastern Pacific. Since the late 20th century, there have been fewer but more intense Eastern Pacific El Niño events

over the last 30 years. The trend indicates that the intensity of the stronger Eastern Pacific El Niño events, such as those that occurred in 1997-1998 and 2015-2016, has been increasing.

Impacts of the El Niño-Southern Oscillation on the strength of the Indian summer monsoon

ISM is intensified during La Niña but weakened during both the eastern and central Pacific El Niño events. The weakening of the ISM is particularly more pronounced during the eastern Pacific El Niño.

During the eastern Pacific El Niño, cyclonic wind anomalies develop from north of the Arabian Sea to central India, which are accompanied by negative mean sea level pressure anomalies in these regions. During the central Pacific El Niño, a similar, but weaker pattern is observed.

These cyclonic wind anomalies are opposite to the climatological winds in this region. Thus, moist southwesterly winds of the ISM are weakened during the eastern and central Pacific El Niño events.

During La Niña, a similar pattern but with a reversed sign develops over the Indian subcontinent, leading to the strengthening of the moist southwesterly winds, and thus strengthening of the ISM.

Variability of summer monsoon rainfall over the Indo-Gangetic plains in relation to El-Niño/La-Nina

The Indo-Gangetic plains (IGPs) incorporate seven meteorological subdivisions, viz. Punjab, West Uttar Pradesh and East Uttar Pradesh, Bihar, Jharkhand, and Gangetic West Bengal. IGPs have fertile soil, a warm climate, and plenty of water. They are regarded as the "breadbasket" of the majority of South Asia. The main source of rainfall is the southwest monsoon which is normally sufficient for agriculture. Indian monsoon rainfall forecasting is an important consideration for India’s economy. El- Niño and La-Nina events are associated with a number of weather extremes, particularly droughts and floods. A better understanding of the El-Niño (La-Nina)-related factors influencing monsoon rainfall can improve the ability to forecast monsoon rainfall (in terms of temporal and spatial parameters), thereby facilitating the management of food production. Furthermore, the study reveals that

The most drought-producing subsidence is concentrated over India during El Niño events with the warmest sea surface temperature (SST) anomalies in the central equatorial Pacific, as opposed to events with the warmest SSTs in the eastern equatorial Pacific.

As shown by 142-year historical rainfall record data that severe droughts over the IGPs have generally been associated with El Niño events. However, El Niño events have not always been accompanied by severe droughts.

SMR (summer monsoon rainfall) variability is greater during the El-Niño years and is repressed during the La-Nina years, but the occurrence of El-Niño (La-Nina) does not guarantee droughts (floods) over the Gangetic plains.

Global warming and associated changes in the Indian summer monsoon circulations and the general atmospheric circulation are responsible for the spatiotemporal changes in rainfall activity.

Droughts of Indian summer monsoon associated with El Niño and Non-El Niño years

Variability in Indian summer monsoon rainfall (ISMR) frequently causes abnormal droughts and floods in South Asia, resulting in natural disasters and negatively affecting the Indian economy. Interannual variation is caused by a variety of external and internal forces, such as oceanic and atmospheric environments. Droughts are crucial for a variety of reasons, especially in the planning and agricultural sectors. Except for peninsular India and the eastern region, the majority of the Indian subcontinent experiences below-normal precipitation in drought years unrelated to El Niño. The majority of drought conditions of the Indian summer monsoon rainfall are related to El Niño (13 of 18 years), indicating that approximately 72 percent of drought years are related to the influence of the Pacific Ocean. North India and the majority of central India are experiencing below-average precipitation, with the severity of drought being particularly severe over west coast stations. Droughts associated with El Niño are more severe in the majority of the subcontinent, impacting the entire west coastal belt, monsoon zone, and eastern regions more severely than droughts associated with non-El Niño years. The spatial patterns of precipitation during flood years associated with La Nia and drought years associated with El Niño reveal the highly nonlinear spatial structure of precipitation. Over central India and the Western Ghats (WGs), the drought associated with El Niño gives clear indications of droughts beginning in early June; however, in the case of droughts unrelated to El Niño, indications of drought are not visible until the first week of July. The study suggests that El Niño-related droughts bring severe drought conditions to the WG region. However, there is no significant difference in total precipitation between the two types of drought over central India. Non-El Niño and El Niño droughts exhibit strikingly distinct intraseasonal precipitation characteristics. During El Niño droughts, the variance of rainfall in both the central Indian and WG regions is weaker than the droughts that are not associated with El Niño.

Indian Summer Monsoon and Tropical Indo-Pacific Climate Drivers for Kharif Crop Production

India's GDP depends primarily on agriculture. The agriculture sector generally relies on the Indian Summer Monsoon Rainfall (ISMR), which occurs from June to September (JJAS). India has two cropping seasons, Kharif and Rabi. Kharif season crops grow during the summer monsoon and are harvested in consecutive late fall/early winter months of October and November. Rabi crops are grown during October–March and harvested from April–May. ISMR is a significant contributor to both cropping seasons, supplying water directly for Kharif crops and providing soil moisture and irrigated water for Rabi crops. Kharif crops such as rice require more water than Rabi crops. However, several other issues are also important for crop production in India. El Nin o-Southern Oscillation (ENSO) is one of the most important drivers of Indian summer monsoon rainfall variability. Strong El Niño's cause a drier-than-normal summer monsoon in India.

Interestingly, since the late 1970s, we see an increasing frequency in the El Niño Modokis, also referred to as central Pacific El Niño as against the canonical El Niño. The El Niño Modokis are distinguished by a warmer than normal central tropical Pacific flanked on both sides by cooler than normal anomalies. While both types of El Niño's cause drier than normal rainfall conditions during summer monsoons.

The relationship between rainfall and crop yield is not a straight line, of course. For instance, the effect of too little rain on GDP and grain production is stronger than the effect of too much rain. Also, in La Nina years, crops produce more than in El Niño years. So the summer monsoon rainfall (SMR) and the tropical Indo-Pacific climate drivers affect crop production in different parts of India during the Kharif season. Also, because of recent changes in the evolution and frequency of the tropical Indo-Pacific drivers, especially the more frequent occurrence of the ENSO Modokis instead of the canonical ENSOs, a correlation analysis was done to estimate the effect of the different Indo-Pacific climate drivers on the rainfall of each Indian state from 1998 to 2013. Crop production data for the most productive Indian states, including West Bengal, Odisha, UAP (Undivided Andhra Pradesh), and Tamil Nadu, are used.

ISMR relevance for the Kharif crop production (KCP)

1. The results show that the KCP of the respective states is significantly correlated with the summer monsoon rainfall at the 95–99% confidence levels.
2. The NIÑO 3.4 and ENSO Modoki indices have a statistically significant correlation with the KCP of most of the Indian states, particularly in states such as UAP and Karnataka.

3. The KCP of the districts in UAP also responds strongly to all of the climate drivers, which can be used to predict local crop yield.
4. Variations in the central tropical Pacific SST can significantly impact the KCP over the majority of the Indian states under consideration.

What lies ahead?

El Niño is known to reduce monsoon precipitation in India, while La Nina increases it.

El Niño years tend to be drier than average, but one of the strongest El Niño of the century (1997-1998) caused India to experience an above-average monsoon season .

According to researchers, even the location of the warming in the Pacific may have an effect on the monsoon.

Anomalous warming in the Central and East Pacific (Niño 3.4 region) may have a greater negative impact on the monsoon than warming in the adjacent far east Pacific (Niño 3. region).

Several private forecasters and international organizations have already explored the possibility. According to scientists, additional factors may interact with the prevailing Pacific conditions to determine the fate of the monsoon. The gradual warming of the land during April, May, and June is one example. The Himalayan/Eurasian snow cover is another factor. Less snow cover results in a warmer subcontinent, which can aid in intensifying the monsoon circulation and bringing more precipitation. Last but not least is the 'dipole' effect closer to home, in which the Indian Ocean mimics El Niño-La Nina by causing the western and eastern basins to warm relative to one another every few years, thereby influencing the monsoon. The west Indian Ocean's warming enhances the monsoon, and vice versa.

Conclusion:

In conclusion, El Niño and La Nina are the dominant mode of tropical climate variability: the ENSO phenomenon. ENSO affects climate, ecosystems, and societies globally. It's vital to understand past and future ENSO variations. ENSO activity and characteristics depend on the tropical Pacific climate system, which will change in the 21st century due to radiative forcing (including increased greenhouse gases) and internal climate variability. El Nin o-Southern Oscillation (ENSO) is one of the most important drivers of Indian summer monsoon rainfall variability and it results in below-average precipitation in India. Summer monsoon rainfall (SMR) and the tropical Indo-Pacific climate drivers affect kharif crop production in different parts of India. As a result of the dependence of Indian agriculture on the monsoons,

less rainfall during the monsoons typically results in below-average crop yields. Since the phenomenon affects India and global weather conditions, it is a global issue. Yet we are fairly confident that ENSO variations will continue to occur and influence global climate in the coming decades and centuries. Changes in a continental climate, however, could alter the remote impacts of El Niño and La Nina.

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CROP RESIDUE: A TOOL FOR NUTRIENT RECYCLE AND IMPROVING SOIL HEALTH

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Introduction:

Every year a large quantity of agricultural waste is generated. The amount of crop residue produced in India was 501.73 million tonnes (Mt), of which 93.51 Mt of wheat, 105.24 Mt of rice, 22.26 Mt of maize, 16.03 Mt of millets (Jowar, Bajra, Ragi, and Small millet), 341.20 Mt of sugarcane, 7.79 Mt of fibre crops (Jute, Mesta, Cotton), 18.34 Mt of pulses, and 30.94 M (NPMCR, 2014). The crops that are most vulnerable to agricultural residue fire include sugarcane, wheat, and rice. Some of these residues are used as animal feed, industrial fuel, residential cooking fuel and thatching for rural homes. With the introduction of combine harvesters, more than 75% of the rice area is harvested mechanically. Most farmers remove wheat straws for animal feeding. However, because of its high silica concentration, rice straw is thought to be poor fodder for animals, making its management a significant difficulty. A swath of loose rice leftovers left by a combine harvester prevent the wheat seed drill from functioning properly. To avoid these problem farmers burns this crop residue 90-140 Mt annually. From the perspective of the farmers, burning may be viewed as the best way to get rid of residue. It is not only a cost-effective method but it acts as an effective weed and pest control procedure and prepares the fields for the next crop rapidly and inexpensively. Farmers gain certain advantages, such as time and cost savings, by burning residue in the field.

Burning agricultural residues pollutes the air and results in the loss of a significant quantity of biomass and plant nutrients, including all of the carbon (C), 80–90% of the nitrogen (N), 25% of the phosphorus (P), 20–% of the potassium (K), and 50% of the sulphur (S) that are contained in crop residues (Ponnamperuma, 1984) are lost in the form of various gaseous and particulate matters, resulting in atmospheric pollution and global warming, but also cause an adverse effect on soil properties as well as soil flora and fauna. Stubble burning also impacts human and animal health both medically and by traumatic road accidents due to restricted visibility.

So, there is a need to adopt ways and options to manage this valuable resource. The management of crop and other plant residues on a soil surface, as well as their usage as a raw material for the creation of other valuable goods, is referred to as crop residue management. Crop residues are an important source of plant nutrients and carbon (C), and their incorporation back into the soil can help prevent soil erosion, increase SOC, improve water conservation, and recycle nutrients. Returning crop residues back to the soil follows the principle of taking whatever you want and ploughing the rest back into the soil for sustainability. Growing mushrooms, composting, making biochar, creating non-woven composites, and in-situ mechanical intensification management employing crop management practises aimed at agricultural conservation are a few of the methods available for managing crop wastes.

Types of Residues

After crops have been harvested and threshed, parts of the plants are still present in the field as crop leftovers. These come in two types:

1. **Field residues:**

   When the crop has been harvested, items are left in a field or orchard. Stalks, stubble (stems), leaves, and seed pods are some of these leftovers.

2. **Process residues:**

   Are components that are still there after a crop is converted into a useful resource. Seeds, bagasse, roots, husks, and molasses are some of these remnants. They can be utilised in industry, as a soil amendment, as fertilisers, and as animal feed.

Adverse Effects of Crop Residue Burning

1. **Loss of nutrients:** In addition to organic carbon, it is predicted that burning one tonne of rice straw results in the loss of 5.5 kg of nitrogen, 2.3 kg of phosphorus, 25 kg of potassium, and 1.2 kilogramme of sulphur. The top soil's temperature rises when straw is burned, and the soil's carbon-nitrogen balance swiftly shifts. As a result, the soil loses a significant amount of NPK. Burning sugarcane waste results in the greatest loss of nutrients, followed by burning rice and wheat straw. Burning sugarcane waste resulted in the loss of 0.84 Mt of nutrients each year, 0.45 Mt of rice residue, and 0.14 Mt of wheat residue, of which 0.39 Mt were N, 0.014 Mt were K, and 0.30 Mt were P. The soil will be enhanced if these agricultural wastes are integrated or kept, especially with SOC and N (Meena *et al*., 2020).
2. **Impact on soil properties:** The temperature of the soil is raised by the heat from burning wastes, which kills beneficial soil organisms. Burning residue frequently causes a total eradication of the microbiological population. Death is just momentary, though, since the microbe regenerates after a few days. But frequent field burning permanently reduces the

microbial population. Burning raises the exchangeable NH_4^+-N and bicarbonate extractable P (Olsen-P) content right away, but the soil profile does not see any appreciable nutrient build-up. The 0–15 cm soil layer's total N and C content as well as possibly mineralizable N are decreased by prolonged fire.

3. **Emission of greenhouse and other gases:** Burning crop residues has the potential to release Green House Gases (GHGs) as well as other harmful gases and aerosols that are significant in terms of chemistry and radiation, such as CH_4, CO, N_2O, NO_X, and other hydrocarbons. The open burning of rice straw in fields results in the emission of GHGs, SO_2, NO_2, and CO_2 into the atmosphere. In the presence of sunshine, these gases mix with nitrogen dioxide to produce photochemical oxidants, which cause photochemical smog to occur.

Strategies for residues management as nutrient cycling and improving soil health tools:

Crop leftovers are processed for use in building projects. For instance, cement is sometimes mixed with rice husks. The paper industry makes use of sugarcane and banana peel waste. Scientists and agriculturists have proposed various alternatives to agricultural waste burning during the past ten years, but due to farmers' lack of understanding and social conscience, these alternatives have not been effectively adopted. Information on four such agricultural applications that have been either disregarded or omitted for a variety of reasons is offered in this section. These include mechanical intensification, in-situ management, composting, charcoal, surface retention and mulching integration, and residue. Each is discussed below:

1. **Residue incorporation:**

Figure 1: Residue incorporation under loose residue after harvesting of maize (left) and rice (right)

One of the finest methods for using most of the leftover residue in fields is straw inclusion. It raises the amount of organic matter, N, P, and K in the soil. Compared to the control treatment, crop output is increased by 15–35 percent by incorporating crop residue. The best way for incorporating residue is ploughing. Although the integration of crop residues into the soil is

advantageous for recycling nutrients, it also temporarily immobilises certain nutrients, such as nitrogen, and necessitates the use of additional nitrogenous fertiliser to balance the high C:N ratio at the time of residue incorporation. In addition to lowering air pollution, this will enhance the fertility and health of the soil. It would also reduce dependence on chemical fertilizers on one hand and prevent soil pollution from agro chemicals on the other.

2. Surface retention and mulching:

Figure 2: Crop residues are used as surface retention and mulching material

Mulch is a layer of plant leftovers or other materials that is spread over the soil's surface naturally or artificially in order to retain soil *via* low bulk density and low energy yield per unit weight basis. When straw remnants from a previous crop are drilled directly into surface-mulched residues, they are not incorporated into the soil. Surface residue retention aids in preventing wind and water erosion of the rich surface soil. The higher number of leftovers that are still on the surface sometimes causes equipment failures, which affects the planting of seeds for the subsequent crop. Therefore, when no-till or conservation tillage methods are common, farmers typically employ this technique. In many circumstances, surface retention of part or all of the residues may be the optimum choice. The top 5 to 15 cm of soil, where residues breakdown slowly, gains more organic carbon and total nitrogen while preventing soil erosion. In comparison to burning, retention of residues on the surface enhanced soil nitrate content by 46%, nitrogen absorption by 29%, and yield by 37%. However, retention enhances microbial activity, soil carbon and nitrogen content, lowers the need for fertiliser nitrogen in rice, and offers home for both dangerous and beneficial species. It also supplies a carbon substrate for heterotrophic nitrogen fixation. The faster decomposition and release of nitrogen to soil is possible if it is treated with urea and applied during field preparation.

3. Biochar:

Figure 3: Biochar prepared by different crop residues

A high carbon substance known as biochar is created by the gradual pyrolysis of biomass, such as wood, dung, or leaves, at relatively low temperatures. The agricultural research community has been continually exploring for strategies to efficiently increase natural rates of carbon sequestration in the soil as a metric for reducing GHG emissions. This has piqued the scientists' growing interest in using biochar, charcoal, and black carbon as soil amendments to stabilise the SOC concentration. These methods are seen to be a good choice for decreasing GHG emissions while significantly lowering the amount of agricultural waste. In order to reduce the amount of CO_2 or CH_4 released into the atmosphere, the process of carbon sequestration fundamentally calls for longer residence times and resistance to chemical oxidation of biomass to CO_2 or reduction to CH_4 (Srinivasarao *et al*., 2013). The partially burned by products, pyrogenic carbon/carbon black, undergo a very gradual chemical change to create a long-term carbon sink that is perfect for soil amendment (Izaurralde *et al*., 2001; McHenry, 2009). Biochar is a fine-grained, porous substance made by pyrolysis, a thermochemical reaction that takes place at low temperatures in an atmosphere devoid of oxygen (Amonette and Joseph, 2009). It is a mixture of ash, C, H, N, O, and S in various ratios (Masek, 2009). Biochar made from leaves, dung, agricultural waste, or wood. The very porous characteristic of biochar, when applied to the soil, aids in better water retention and increased soil surface area. According to Lehmann and Joseph (2009), it primarily interacts with the soil matrix, soil microorganisms, and plant roots, aids in nutrient retention, and activates a variety of biogeochemical processes. Numerous researches have noted that using it raises pH. (Tryon, 1948; Gaunt and Cowie, 2009). Particularly, biochar is utilised in many different fields including water treatment, building, food, cosmetics, metallurgy, wastewater treatment, and many more chemical applications. Currently, only a few small towns and villages in India are using biochar applications. The encouragement of biochar production and use appears to be a better idea in India given its broad application.

4. Crop Residue as compost:

Crop residue, like animal manure and food waste, is a good source material for compost because of its higher organic carbon content. The natural rotting or breakdown of organic waste by microorganisms under regulated circumstances is known as composting (Misra *et al*., 2003). Compost is a significant source of organic matter and helps to maintain soil fertility, which is essential for maintaining sustainable agricultural production. Composting the soil enhances its physical, chemical, and biological entities, and it can augment the use of agricultural chemicals like fertilisers while reducing the need for pesticides. The advantages of compost enriched soil include greater potential for improved yields and resilience to outside stresses including drought, disease, and toxicity (Shilev *et al*., 2006; Lei *et al*, 2010). Due to increased soil microbial activity, these methods also aid in greater nutrient absorption and active nutrient cycling. After composting, the leftovers from a one-hectare rice harvest produce around three tonnes of nutrient-rich manure (FYM). Using an indigenous supply of low-grade rock phosphate, rice straw compost may be reinforced with P to create value-added compost that contains 1.5 percent N, 2.3 percent P_2O_5, and 2.5 percent K_2O. It is the biological maturity in aerobic circumstances when the organic matter of plant or animal origin is broken down to components with shorter molecular chains and more stable, hygienic, humus-rich compost, useful for crops and eventually created (Sequi, 1996). Organic matter undergoes two processes during composting: degradation and maturation. Degradation's initial phase begins with the breakdown of organic compounds that are easily broken down, such as sugars, amino acids, and organic acids. Oxygen is used by the aerobic microbes, who then produce CO_2 and energy. The initial thermophilic phase lasts for many weeks to months and is characterised by high temperature, high pH, and humidity, all of which are necessary for activating the microorganisms (Aladjadjiyan, 1992). The substrate is also adequately chilled during this phase, along with a suitable amount of oxygen (Beck-Friis *et al*., 2000). A decline in the microbial population is followed by the breakdown of more complex organic compounds during the second phase, which lasts for a few weeks. When the temperature drops to about 40 and 45^oC, the phase transition from thermophilic to mesophilic occurs (Maynard, 2000; Wu *et al*., 2000). At the last step, the temperature drops even further.

5. Residue Recycling:

Crop leftovers may be recycled in two different ways. Using the Happy Seeder machine and zero seed drill for direct planting of next crops without land preparation, chopping up the previous crop residue with a mulcher so that it can decompose quickly, or integrating them into the soil with a mouldboard plough, harrowing/disking, or chiselling. These might also be recycled into fields using animal dung, as has been done in conventional agriculture, or utilised

as mulches and composts. According to several studies in soil science, plant science, and agronomy, recycling of leftovers offers a number of important and mostly irreplaceable environmental and soil health advantages.

6. Conservation tillage:

Figure 4: Conservation tillage (Residue retention and sowing)

In order to maximise certain economic and environmental benefits, a tillage and planting strategy that covers 30% or more of the soil surface with crop residue after planting reduces the frequency or intensity of tillage operations. Reduced usage of agricultural machinery and equipment, lower carbon dioxide and greenhouse gas emissions, and lower fuel and labour expenses are a few of these benefits. Additionally, it has been demonstrated that conservation tillage techniques enhance soil health, decrease runoff, and restrict the degree of erosion. A well-developed and well-integrated conservation tillage method may support the sustainability of an agricultural system and offer a variety of possible environmental and financial benefits.

Benefits of crop residues/ the detrimental effects of removing them:

Table 1: General benefits of crop residues to soil quality

Primary effect ⇒	**Secondary effect** ⇒	**Tertiary effect**
Contributes to soil organic matter	Improves chemical, physical and biological properties	Increases yield and yield sustainability
Provide physical buffer	Reduce raindrop impact and wind shear	Reduce soil erosion

1. Protecting Soils against Erosion and Improving Water Retention:

Farming that is sustainable is seriously threatened by excessive soil erosion. There is debate about how much erosion reduces agricultural production, but there is little doubt that poor soil quality brought on by soil erosion causes a large loss of plant nutrients (Troeh *et al*., 1991). The best way to prevent erosion is to retain residues; the amount of erosion control is directly

correlated with the amount of field covered by residues. Wind erosion may be reduced by more than 95% by increasing the bulk of wheat residue from 0.56 to 1.12 t ha^{-1} (Finkel, 1986). According to Shelton *et al*. (1991), soil erosion will be 50% lower when residues cover 20% of the soil surface, and a 90% cover will reduce water erosion by as much as 93% when compared to bare soil (Wischmeier and Smith, 1978). Crop yields are boosted as a result of less erosion and greater soil water storage. Residues control erosion primarily by two modes of action:

i. Reducing wind speeds below the threshold level for soil particle movement, and
ii. Intercepting falling raindrops, thereby preventing them from detaching soil particles.

Besides, the presence of residues reduces surface water runoff by way of increasing water infiltration rates and soil retention capacity.

2. Enhancing Soil Organic Matter:

A noticeable decrease in soil organic matter contents has been the universal result of converting grasslands to agriculture (SOM). Long-term data reveal a loss in soil carbon by up to 50% over periods of similar lengths of time, and a fall in soil N content of 25–70% during intervals of 30–90 years (Aref and Wander, 1998). A decrease in SOM is frequently accompanied by structural deterioration of the affected soils, which results in surface crusting; as a result, less water infiltration and less phytomass litter have led to a decrease in the presence of the soil microorganisms and invertebrates whose activity is crucial for the sustenance of highly productive soils (Madsen, 1995). Earthworms are extremely successful at causing favourable physical and chemical changes in soils; but, when agricultural leftovers are removed from the soil and burned in the field, their population rapidly decreases (Knight *et al*., 1989). Reusing agricultural residue can address all of the aforementioned issues. In soils where crop rotations include "green manure" (*i.e*., leguminous cover crops growing for brief periods and then ploughed under) or leguminous forages, recycling roots and stubble may be sufficient to sustain high levels of SOM. There is a maximum amount of SOC that can be stored in mineral soils with simply stubble ploughed in, and the pace of this growth mostly depends on variables regulating decomposition. After recycling all straw and stubble for just six years, wheat fields at Sanborn Field Experimental Plots in Missouri accumulated around 2.6 times this amount of SOC than the initial planting (Buyanovsky *et al*., 1997).

3. Recycling Nutrients:

Approximately one-third of the N, one-fifth to one-third of the P, and more than 100 percent of the K treated with inorganic fertilisers would come through recycling leftovers and their eventual mineralization. However, macronutrients in agricultural leftovers are not as easily accessible as nutrients from inorganic fertilizers. Crop leftovers can't degrade quickly because of

their high cellulose and lignin concentration, especially in colder locations. The high C: N ratios of crop residues are also significantly greater than those of fresh leafy phytomass (12–15 for grasses) or animal dung, with only those of leguminous leftovers falling below 40 (typically, 15-25). Biomass with a C: N ratio under 20 will release net nitrogen for plant development rather quickly. However, the breakdown of residues with a high C:N ratio will remove N from the soil, momentarily immobilising it during the early stages of decay and lowering the soil's short-term productivity. P immobilisation follows a similar trend as N. Of course, the immobilised nutrients ultimately become accessible, but they cannot be relied upon to increase growth, yields, or profitability in the short term. The rate at which the nutrients are released is determined by the microbial decomposers' activity, which is mostly temperature- and moisture-dependent. More than half of the residue left on the surface may not degrade after a year in colder and drier locations (Schomberg *et al*., 1994). In contrast, leftovers quickly disintegrate in warm, humid regions, making nutrients considerably more accessible but also making it harder to reduce soil erosion and water flow year-round. The breakdown of wastes can be sped up in cold or dry conditions by using the right agronomic techniques. Research using wheat and sorghum straw in Texas revealed that degradation rates rose linearly with the amount of applied water and that nitrogen in residues left on the top was immobilised three times longer than nitrogen in the buried phytomass (Schomberg *et al*., 1994). When used alone, rice straw was shown to be a poor source of nitrogen, but when combined with fertilizer (used as urea), its agronomic efficiency was only marginally greater than when Fertilizer-N was used alone. In comparison to other organic sources of nitrogen, rice straw had a stronger residual benefit (*i.e*., it delivered N over a longer period of time). It was also a superior source of organic carbon due to its high C:N ratio and was able to boost bacterial N fixation. Therefore, recycling rice straw may have a larger chance of lowering inorganic N application requirements than using green manure. Tropical lowland rice fields with good management might obtain up to 75 kg ha^{-1} yr^{-1} of nitrogen from straw. By applying fertiliser N at the ideal time, improving the way recycled straw is incorporated into the soil, and eventually using mechanical harvesters that leave the straw in the field (instead of hand harvesting, which involves removing all phytomass), efficient recycling of this N would be encouraged. To preserve the quality of the soil and increase crop yield, crop leftovers should be handled properly as a valuable renewable resource.

4. Crop residues operate as a physical buffer, shielding soil from the direct effects of wind, rain, and sunshine. This improves soil structure, lowers soil temperature and evaporation, increases infiltration, and decreases runoff and erosion.

5. Crop waste helps promote microbial and macroinvertebrate activity by increasing soil organic matter, nitrogen levels, and water retention. Generally speaking, these outcomes result in enhanced plant development, elevated soil productivity, and greater agricultural output.

Conclusion:

- Crop residues are of great economic value as livestock feed, fuel and industrial raw material, and in conservation agriculture for which it is a pre-requisite.
- Crop residue is a dynamic material that improves the physical, chemical and biological entities of the soil and ultimately leads to better soil health.
- Crop residues, either partly or entirely must be used for conservation agriculture for ensuring the country's food safety, generating sustainable agriculture and the soil resource base healthy.
- For the sustainability and resilience of Indian agriculture, it is imperative that all stakeholders, including farmers, service providers along the supply and value chains, researchers, extension agents, policymakers, and civil servants, work together to fully understand and utilise the potential of these priceless resources.
- We believe that the research, policy and development programs as outlined in this assignment and residue management policies in the future will serve a great deal in managing crop residues at local and regional scales.

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CROP WATER STRESS AND ITS ASSESSMENT USING REMOTE SENSING TECHNIQUES

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Abstract:

Currently, the world is facing high competition and market risks in improving yield, crop illness and crop water stress. In order to meet the demand for increased global food production under limited water resources, implementation of suitable irrigation scheduling technique is crucial, particularly in irrigated basins experiencing water stress. Optimizing water use in agriculture requires innovations in detection of plant water stress, at various stages of the growing season to minimize crop physiological damage, and yield loss. With increasingly advanced remote sensing systems, more accurate retrievals of crop water status are being made at the individual crop level as they are fully equipped to address these complex and technical issues in an easy and efficient way. They also provide simple and timely solutions for a diverse set of ecological zones and also help in accurate monitoring of those areas which are difficult to reach by man especially in large agriculture fields. Through an examination of previous literature, this study presents the assessment of different remote sensing techniques that are used in the analysis of crop water stress.

Introduction:

Drought, salt, severe temperatures, chemical toxicity, oxidative stress and other abiotic stresses that the biosphere is subjected to generate imbalances in the normal state of the environment. Every year, stressors on arable plants in various parts of the world interrupt agriculture and food supply, resulting in famine. Due to global climate change and the recent COVID-19 geopolitical issues that have been affecting food production and imports in many regions of the world, such a scenario is considerably more problematic. Water is the fundamental molecule in all physiological processes of plants since it is the principal channel for transporting metabolites and nutrients, accounting for 80-90 per cent of the biomass of non-woody plants. Drought is a condition in which plant water potential and turgor are reduced to the point where plants are unable to perform regular physiological processes. In water management, the availability of water for the roots of irrigated agricultural crops (IACs) is a limiting issue. As a

result, the principles utilised in water management are critical in determining irrigation efficiency in the agriculture sector, which is the world's greatest consumer of freshwater resources. When IACs are exposed to a lack of soil water held in the root zone, they show signs of particular water stress, which has a direct impact on the quality and yield of the crop. In this sense, efficient irrigation water use should be based on objective crop water stress data, which can improve actual irrigation practises and play a critical role in agricultural crop management in light of regional and local climate change trends.

An early warning system for water stress (i.e., before symptoms appear in the plant) would be a valuable tool for greenhouse managers looking to maximise resource efficiency. Furthermore, such an approach could serve as the foundation for breeding programmes aimed at selecting genotypes for biotic and abiotic stress adaptability as well as high yield in both stress and non-stress situations. Through the recording, measurement, and interpretation of pictures and digital representations of energy patterns, remote sensing allows for the detection of chemical or physical aspects of crops at any distance. Thanks to highly novel and complex data analysis methods, these technologies can be used to analyse entire fields over the course of entire crop seasons, allowing for accurate, early and reliable diagnosis of crop stress.

Water stress

When the water supply to the roots becomes insufficient or the transpiration rate becomes excessive, plants experience water stress. Water stress is produced by a lack of water, such as drought or high soil salinity. Water exists in the soil solution in cases of excessive soil salinity, as well as other situations such as flooding and low soil temperature, but plants are unable to absorb it, a scenario known as 'physiological drought.' Drought occurs every year in many regions of the world, and it is especially common among field-grown plants in arid and semi-arid climates. Water limiting settings can also be found in areas with adequate but non-uniform precipitation. Drought has been one of the key output limiting variables since the birth of agriculture. As a result, the ability of plants to tolerate such stress is extremely important economically.

Effect of water stress on plants

a) Photosynthesis

Water deprivation has a particularly negative impact on photosynthesis. The fall in relative water content (RWC) and leaf water potential reduces photosynthesis in higher plants. Lower photosynthetic rate is a common side consequence of water stress in plants and it's been linked to stomatal restriction and metabolic impairment. Although the relative impact of stomatal or metabolic inhibitions is uncertain, metabolic impairment is a more complex issue than

stomatal limitation. Under drought conditions, the photosynthetic rate of leaves in both C3 and C4 plants decreases.

b) Protein synthesis

LEA (late embryogenesis abundant), desiccation stress protein, proteins that respond to ABA (abscisic acid), dehydrins, cold regulation proteins, proteases etc are the most common proteins generated in response to water stress. Protein kinases and transcription factors, which are important in the regulation of signal transmission and gene expression, are produced as well. Dehydrin-like proteins, which increase during seed production and embryo maturation in many higher plants, as well as in water deprived seedlings, make up the majority of these stress response proteins. These proteins have a highly conserved region that is associated to hydrophobic interactions that are necessary for macromolecular stability. During various stresses, including water stress, heat-shock proteins (HSPs) and LEA type proteins are two primary types of stress-induced proteins. These proteins are widely known for protecting macromolecules including enzymes, lipids and mRNAs against dehydration. They also play a vital role in protein and membrane stabilisation, as well as helping protein refolding under stress. Under osmotic stress, both ABA-dependent and independent signalling mechanisms influence the expression of LEA-type genes.

c) Morphological, anatomical and cytological changes

Cutinization, hairiness, vein density, stomatal frequency and palisade layer and total leaf thickness are typically enhanced. This frequently results in the xeromorphic kind of foliage, which is thick, leathery and strongly cutinized. By shading tobacco to generate thin leaves for cigar wrappers, water stress is reduced to a minimum. Other morphological changes include reduced leaf size, fewer stomata, thickening of leaf cell walls and underdevelopment of the conductive system - increased number of large vessels, submersion of stomata in succulent plants and xerophytes, formation of tube leaves in cereals and induction of early senescence. Under water stress, the root-to-shoot ratio increases to aid water absorption and sustain osmotic pressure. The ABA content of roots and shoots has been linked to a higher root-to-shoot ratio under dry conditions. Although reduced leaf area saves water, it also reduces crop production by reducing photosynthesis. Plant biomass declines as a result of water deficits in crop plants, owing to reduced photosynthesis, plant development and leaf senescence under stress circumstances.

d) ABA accumulation

Under water deficiency conditions, the plant hormone ABA accumulates and plays a key role in dehydration response and tolerance. The function of ABA is known to be the closure of stomata and the stimulation of the expression of numerous genes involved in water shortage

defence. When water availability in the soil is restricted, the amount of ABAs in xylem saps increases significantly, resulting in an increase in ABA concentration in different compartments of the leaf. The water stream in the leaf apoplasm cannot permeate the plasma membrane and translocate ABA to the guard cell. High ABA concentrations around guard cells cause stomata to close, allowing water to be conserved.

e) Reduced ion uptake

Plants with reduced transpiration owing to water stress may experience a decrease in the rate of ion transport (long distance movement from root to shoot occurs in transpiration stream). Because of the slow flow of soil water and minerals, ions/mineral absorption may be reduced in soil with lower water content (soil water stress). It is further reduced in water stressed plants due to inadequate root extension and increased root suberization, both of which reduce permeability to water.

f) Reduced translocation

Leaf water shortage reduces the transfer of photosynthate and herbicides from leaves to other regions. Because the translocation system is resistant to dehydration, the amount of material that may be translocated is restricted by either a decrease in photosynthesis or a reduction in sink size due to cell growth. (In water stressed plants, reduced translocation is caused more by reduced source or sink activity than by direct effects on the conducting system's capacity to operate.)

g) Reduction in respiration

Although the rate of respiration reduces as water stress increases, the enzyme system involved is remarkably resistant to dehydration. Because photosynthesis is reduced more than respiration, carbohydrate stocks are likely to be depleted. Because of the hydrolysis of starch to sugar in response to water stress, a brief increase in respiration can occur with decreasing water content due to the availability of additional substrate for respiration.

Remote Sensing Methods

The term "remote sensing" refers to a group of methods for determining the chemical or physical characteristics of real things at any distance by capturing, measuring and analysing images and digital representations of energy patterns obtained through non-contact sensor systems. Precision farming and plant phenotyping for the goal of resistance breeding use it as a quick, non-destructive way to find both biotic and abiotic stressful circumstances. The processes at play primarily depend on how electromagnetic radiation affects plants. Healthy crop status could be deduced from changes observed in the plant-electromagnetic radiation relationship, provided on specific spectral domains, because any stressful condition can cause numerous and

complex physiological and biochemical reactions in plants (i.e., altered stomatal conductance, pigments concentration, and biochemistry). Agricultural sciences have been dominated for decades by sensors that measure reflectance (in the visible, VIS, 0.4-0.7 m, near-infrared, NIR, 0.7-1.3 m, and short wave-infrared, SWIR, 1.3-2.5 m areas), thermal (in the thermal infrared, TIR, 7.0-20.0 m region), and fluorescence (at 0.68 and 0.74 m wavelengths). In addition to its resolution in detecting signal fluctuations, each sensor is also characterised by its spatial resolution, which depends on the distance between it and the object being analysed. It is possible to classify sensors using both non-imaging [such as VIS and IR spectroscopy, fluorescence spectroscopy] and imaging [such as VIS, multispectral and hyperspectral imaging, thermal imaging, fluorescence imaging, and x-ray imaging]. Because they transmit data without spatial information, non-imaging sensors could generally be more beneficial for lab or leaf scale studies.

Table 1: Satellite that monitor global soil moisture content with major applications

Systems	**Application**
AMSR-2	Global observation of soil moisture (from the soil surface to a few cm depth), soil water-related parameter analysis
NISAR	Spatially based maps of global soil moisture in 6–12 days
Sentinel-1	Dynamics observation
SMAP	Analyze soil surface and vegetation status
Tandem-L	Global soil moisture

2.1. Fluorescence Spectroscopy

Fluorescent molecules absorb energy from a particular wavelength, modify their electronic shell and then return to their normal state after a brief period of time, emitting some of the energy they had previously absorbed in the form of an electromagnetic wave. Each molecule has its own unique absorption and emission wavelengths. For example, chlorophyll a fluorescence (ChlF) emits in the range of 650 to 800 nm naturally, with two maxima in the red (680 nm) and far-red (735 nm) wavelengths. Changes in leaf Chl concentration result in changes in the shape of the fluorescence spectra as well as the ratio between the two greatest emission peaks (F685/F735) (Buschmann, 2007; Pandey *et al*., 2015).

Sun induced fluorescence (SIF) is calculated using Earth's atmosphere absorption lines and faint absorption lines obtained from the solar irradiance range (Fraunhofer lines) between 650 and 800 nm (Meroni *et al.*, 2009). The conventional technique for extracting SIF uses a sub-nanometer spectral resolution between 760.5 and 687.5 nm (Moya *et al.,* 2004). SIF has been

demonstrated to be a reliable visual predictor of leaf and crop water stress in studies and its use in assessing leaf stress has been confirmed. However, it is not yet clear whether this relationship can be used at the canopy level (Frankenberg *et al.*, 2011, Frankenberg *et al.*, 2014). In order to monitor crop water stress, studies of SIF in the red (FR) and far red (FFR) bands may be useful (Guanter *et al.*, 2012). A thorough analysis of the SIF temporal variable is necessary to comprehend stress levels. To determine if they can be utilised to forecast agricultural water stress, SIF and the photosynthetic connection need to be further investigated.

2.2. VIS/NIR Spectroscopy

In a range of biotic and abiotic stressful situations, active and passive sensors have been employed to analyse leaf and/or canopy reflectance, the former feature light-emitting components, while the latter rely on sunlight as a source of light. The principal uses in plant health monitoring are based on spectral wavelengths between 400 and 2,500 nm since reflectance in the VIS, NIR and SWIR is predominantly influenced by photosynthetic pigments, cell structure, and water content. These characteristics may significantly vary in plants growing in unfavourable settings (Mishra *et al*., 2017). While the leaf reflectance pattern in the SWIR region is highly dependent on the light absorbed by leaf water (near 1,450 and 1,900 nm) and on leaf dry matter, the leaf reflectance pattern in the NIR region is highly dependent on the light absorbed by leaf water (near 1,450 and 1,900 nm) and on leaf dry matter (Ge *et al*., 2019).

Spectral reflectance indices (SRIs), which have been used to evaluate morphological, physiological and biochemical components of stress, have been created throughout time by combining reflectance values collected at certain wavelengths in the VIS, NIR, and SWIR domains (El-Hendawy S. *et al*., 2019a). One of the most popular SRIs is the Normalized Difference Vegetation Index (NDVI), which has been demonstrated to significantly correlate with the final yield of several crop species (El-Hendawy *et al*., 2019). The "fingerprint" of a plant can also be determined using the reflectance spectrum. Numerous strategies were used due to the size of the datasets, including multivariate statistical techniques like stepwise multiple linear regression analysis (SMLR) and partial least squares regression (PLSR) (Garriga *et al*., 2017). When the number of predictors (X) exceeds the number of observations (Y) (overfitting) and when many predictors are highly correlated (multicollinearity), SMLR's predictive accuracy suffers. SMLR is beneficial for discovering relationships between spectral reflectance and crop properties (El-Hendawy *et al*., 2019).

2.3. Thermal Imaging

Thermal imaging (thermography) is one of the most often used imaging techniques in the fields of agronomy, environmental science and agri-food (Costa *et al*., 2013). It is possible to

identify stressful situations because of the strong relationships between foliar surface temperature (Tleaf) and leaf gas exchange (CO_2 and H_2O fluxes controlled by stomatal closure or aperture) or stomatal conductance (gs) (Gutiérrez *et al*., 2018). This method includes analysing the temperature of the source crops and figuring out how much water is needed for irrigation, crop water stress, and evapotranspiration (Lopes *et al.,* 2007). By examining the energy generated by the target crop, the technique analyses the actual soil moisture and crop water availability (Kipp *et al.,* 2014). Due to its capacity to gather enormous amounts of data, it is thought to be more effective than other remote-sensing systems at measuring the agricultural water stress of large areas. Due to their effectiveness, thermal infrared devices are frequently employed to detect agricultural water stress. Thermal infrared systems compute an average value for leaf temperature and foliage regions by comparing the temperatures of all target items. A thermal infrared imaging system has both cooled and uncooled cameras. Short temperature fluctuations can be detected by cooled infrared cameras using incredibly sensitive data over small spatial scales (Yang *et al.,* 2006; Gaju *et al.,* 2014; Cormier *et al.,* 2016). Uncooled infrared cameras can be utilised for a variety of research at a minimal cost and are lighter than cooled infrared cameras. They are used in unmanned aerial vehicles and on the ground (UAVs). Thermography also has the benefit of geolocated data acquisition at canopy scale, overcoming the drawbacks of conventional portable infrared thermometers employed at leaf or single plant scales, and providing results on a whole plant basis (Crusiol *et al*., 2020).

2.4. Fluorescence Imaging

By simultaneously gathering a huge number of punctual fluorescence spectroscopic signals recorded with a color-value connection, new technology has made it possible to create images. Thanks to cameras, it is possible to repeat the measurement across time very quickly, resulting in a comprehensive depiction of the crop's spatial-temporal gradients. A UV light source for fluorescent molecule excitation and a charge-coupled device (CCD) camera make up the system's basic components (Sankaran *et al*., 2010). The red, far-red, blue, and green (690, 740, 440, and 520 nm, respectively) wavelength bands of the multicolor fluorescence imaging approach can each produce a fluorescent response from a single UV light source (ranging from 340 to 360 nm). For instance, active ChlF sensors have been successful in detecting soft rot (caused by *Dickeya dadantii*) and powdery mildew (made by *Podosphaera fusca*) infections in zucchini plants (Pérez-Bueno *et al*., 2016; Pineda *et al*., 2017). Agrochemical use, drought and/or salinity, extreme temperatures, pollution and nutrient deficiencies are other stressful conditions that can be investigated using fluorescence imaging. Due to its many uses in plant

stress research, sun-induced ChlF using passive sensors is noteworthy in addition to active fluorescence methods (Bandopadhyay *et al*., 2020).

2.5. Hyperspectral Imaging

The continuous spectral analysis acquisition is the foundation of the hyperspectral camera system. The technique creates a connection between spectral properties and crop health (Timmermans *et al.*, 2018). Its objective is to identify crop responses to numerous environmental factors and offer a quick and accurate estimation of crop water stress. The wavelength band used by the hyperspectral remote-sensing method ranges from 8 to 14 **um** (Kealy and Hook, 1993). The application of atmospheric correction, emissivity and temperature separation techniques is required for hyperspectral crop water stress estimate (Schmugge *et al.,* 2002). The source radiance emission and emission radiated by the surroundings that are reflected from the source's surface make up the spectrum radiance analysis performed by the system for atmospheric correction. It is necessary to be aware of the details and knowledge needed for emissivity and temperature separation. The calculated spectral radiance in the emissivity separation is the parameter of the spectral emissive and obtained environmental temperature of the source target. In order to analyse surface temperatures with a hyperspectral remote-sensing system for crop water stress analysis, it is crucial to keep in mind that radiance is recorded in the n-band wavelength, which is associated with both soil temperature and emissivity characteristics (Alordzinu *et al.*, 2021).

2.6 Multispectral Imaging

Both multi and hyperspectral sensors can load data from a wider and continuous VIS/NIR range, typically from 400 to 1000 nm, with the most advanced systems reaching the 350-2500 nm region. However, the resolution of the measure (i.e., the density of the wavebands in the measure) distinguishes them (Stellacci *et al*., 2016; Maes and Steppe, 2019). While hyperspectral sensors have a resolution of 1 to 10 nm, multispectral sensors have a spectral resolution of about 50 nm (Mahlein, 2016; Stellacci *et al*., 2016). Despite this, multispectral sensors are now most useful in agricultural applications due to their wider availability and cheaper costs. The operating principles of spectral imaging sensors range from filter-based sensors, which only permit light from a particular waveband to pass through, to push broom and whisk broom scanners, which collect the full spectrum on one pixel before moving to another, to the most recent snapshot sensors, which use the same mosaic principle as a common RGB (red-green-blue) camera to allow for faster image recording, which is especially useful in extremely variable and adverse sampling conditions (Thomas *et al*., 2017). The image-based VIS/NIR approach can assess the occurrence of stressful events even at landscape size since it couples spectral information with

spatial and temporal dimensions (Zhang *et al*., 2019). An optical multispectral sensing device consists of a prism, sensor, crate, and lens. The camera system records the external light striking the prism, which divides the light into its minute components. At the end, the sensor produces data for multispectral images. On the other hand, the C-type filter consists of a number of spectrum filters. The filter takes crop images data as quickly as it can in order to offer multi-layer imaging information. High-resolution pixel cameras on multispectral UAV remote sensing systems monitor crop water stress precisely. They are more accessible, more cost-effective, and better indicators of agricultural water stress since they are more inexpensive.

Conclusion:

The changes in the climatic condition all over the world under the influence of global warming is creating unusual weather phenomena often in the form of water deficit or in the form of floods and waterlogging. All the plants are not equally capable in withstanding water stress and their response to the stress also varies. However, they have to pay the price of such tolerance in the form of reduced photosynthesis and resulting in lower biomass yields often caused by the conservative water management scheme adopted by plants. Moreover, plant indicators commonly used to determine crop water status are either destructive, labour intensive or unsuitable for automation, which make it difficult for irrigators to adopt. Remote sensing techniques for monitoring crop water status provide non-destructive, rapid and reliable estimates of plant water status. These techniques help in assessing the crop water stress accurately and thereby predicting the irrigation schedule based on that.

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LABORATORY BIOASSAY METHODS USED FOR TOXICOLOGICAL INVESTIGATIONS AGAINST INSECT PEST

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Abstract:

Insecticide toxicity studies before their administration, upon insect pest is necessary in order to determine the harmful effects associated with them. Laboratory bioassay methods helps in knowing the relative toxicity of several insecticides within a short period of time at comparatively low cost. Bioassays have grown in importance because of their simple, easily adaptable, versatile and sensitive technique for determining toxicity of wide range of chemicals. It gives a comparative predicted toxicological value of the insecticides which can further be used for determining the LC_{50} /LD_{50} and any other lethal concentration/dose. These values therefore provides an improved idea regarding the insect-insecticide as well as plant-insect-insecticide interactions. This helps in carefully opting for the insecticides in order to have maximum effect on target insects without any harm to the natural bio-control agents, plants or to the environment. The objective of this review paper is to give an insight of several laboratory bioassay methods used for toxicological investigations against insect pest.

Keywords: Bioassay, insecticide, toxicity

Introduction:

Bioassay is formed by two words *viz.*, Bios means life and assay means determination. It can be defined as the determination of the effect of chemicals on living organisms. The mainstay of agricultural pest control continues to be chemical synthetic insecticides. Though the emergence of pesticide resistance is a typical occurrence, current developments in science and technology have reignited interest in this issue, leading to the development of resistance risk

assessments for numerous species utilising various bioassay techniques (Durmusoglu *et al*, 2015). The idea of bioassay research is to assess the toxicity of insecticides with various modes of action on the same test species. It is crucial to examine not only the technical and formulated components but also minute quantities of their residues on/in plant and animal tissues because highly toxic insecticides are being used more frequently to control damaging insect pests (Sun, 1963). In order for a bioassay to be successful, the indicator species must be sensitive enough to detect pesticides in even trace levels and responsive to rising concentrations (Chillar *et al*, 2007). The harmful effects of an insecticide on a biological system is dose dependent. LD_{50} (lethal dose) is typically used to express how hazardous a pesticide is to an organism. The figure is generally expressed as dose per unit weight that would be lethal to 50% of the test organism's population (Finney, 1971). The typical unit of measurement for the LD_{50} is milligram per kilogram (mg/kg). When the precise dose initially supplied to the insect cannot be known, then LC_{50} (lethal concentration) is sometimes employed to describe the concentration of the pesticide in the external media that will kill half of the test population (Regupathy and Dhanu, 2001). On the other hand, bioassay techniques can identify hazardous metabolites and are very sensitive, easy to use, and also able to assay novel insecticides. These bioassays are mostly used to choose newer insecticides and determine the dosages that will have the greatest impact on insects, as well as to test for pest resistance (Siqueira *et al*, 2000) and pesticide selectivity to natural enemies (Bacci *et al*, 2009). Bioassays could be affected by some important variables like stage of the insects, choice of insecticide, bioassay response, application method, bioassay environment, food, sample size, sampling, health of the organism, operator skill, *etc*. It is crucial to connect bioassay findings with the pesticide's anticipated field efficacy along with proper selection of the appropriate bioassay method (Ball, 1981). Here the attempt was made to review the different laboratory bioassay methods available to evaluate the pesticide toxicity against insect pests.

Testing procedures

Insecticide doses are usually administered to batches of insects. The insects being tested should be uniform in terms of age, stage, sex, diet, and other factors. It is preferable to have batches as small as consisting of 10 to 20 individuals with replications of about three to five times under precise experimental circumstances. The insect batches should be organised so that each batch represents a representative sample of the population. Since the harmful effect is typically correlated with the logarithm of the dosage rather than the dosage itself, the dosages for testing should be distributed as equally as possible over the mortality range. A dosage response

relationship is typically used to calculate an insecticide's toxicity. The testable animals are randomly separated into a number of groups, usually six.

One of these groups is kept as the control group and will only get solvent treatment. The test substance is administered to the other groups in dosages following geometric progression (e.g., 1, 2, 4, 6, or 1, 3, 9, 27, *etc*.). Depending on the procedure used, the insects may or may not be retained in another container with food after being treated to various insecticide concentrations. At a given period, the length of time that has been calibrated at various time intervals becomes consistent and then the mortality count is collected. This time frame is set as an exposure time frame. Occasionally, in each trial a few insects perish from causes that are unrelated to the use of insecticides. Estimation of the magnitude of this mortality is important. To achieve this, the batch of insects in the control situation (where no insecticide is used) should be exposed exactly the same way as is done to the insecticide treatment batch. The natural mortality among the controls have to be corrected by Abott's formula (Abbott, 1925) as follows:

$$\text{Corrected \% mortality} = \frac{\text{\% survival in the untreated controls- \% survival in the treated insects}}{\text{\% survival in the treated insects}} \times 100$$

The control mortality should be less than 20%; otherwise, the corrected mortality will not be reliable. The mortality in control so obtained will affect the precision of the result.

Preparation of stock solutions

Technical grade (95-99% purity) insecticides are used for the laboratory tests. The active ingredient (a.i) of the insecticides varies so a 100 % stock solution is prepared using the correction factor (CF) below:

CF= 100% divided by % a.i of the insecticides.

For technical insecticide with 99.5 % a.i., CF= 100/99.5= 1.005.

Given the CF, compute the weight of the technical insecticide needed for prepare desired volume and concentration using the formula: Concentration of insecticides x volume x CF

Eg. To prepare 2.5 ml of 10000 µg/ml stock solution, the weight of insecticide needed will be 10000 µg/ml x 2.5 ml x 1.005 = 25,125 µg or 25.125 mg or 0.025 g.

Weigh 0.025g of technical grade insecticide in a 6 ml screw cap vial using the analytical weighing balance and makeup the volume upto 2.5 ml with analytical grade acetone. After preparation secure the cap of the vials with parafilm to minimize evaporation. Store the prepared insecticide dilutions in a refrigerator (4 °C) or freezer (preferably -20 °C). Replace and dispose properly the tips after preparation of an insecticide.

Bioassay methods

Bioassay methods commonly employed for insecticide toxicity evaluation are topical application, potter's tower method, injection method, dipping method (leaf dip and larvae dip), contact or residual method, film method, sandwich method, direct exposure etc.

Topical application

Topical application is a frequently used technique in which the insecticide is dissolved in a relatively non toxic and volatile solvent, such as acetone, and small precise droplets are applied with an operated micro applicator at a chosen location on the body surface on the thorax of individual third stage larvae (Durmusoglu *et al*, 2015). A motor driven topical applicator is available with micrometer-driven precision syringe. The benefits of this method includes its high degree of precision and repeatability, ability to execute a large number of tests in a short amount of time, requirement for only 10–20 insects per replication, ease of use, low cost of equipment, and minimal usage of toxicants and solvents.

Potter's tower method

The potter's tower can be used to evenly sprinkle or dust an insect's body. Potter (Potter, 1941; Potter, 1952) constructed a spray tower where a twin-fluid nozzle set centrally at the top of an open-ended metal tube, where the sprays fall vertically and deposit on a horizontal plane. By placing a Petri dish holding a known number of insects under the bottom of the tower and spraying inside through a nozzle installed on the lower side while maintaining a specific pressure, a potter's tower can be used to apply a topical substance to the entire insect body. This approach simulates conditions of field exposure and is therefore instructive for pest management. The method has shown to be among the most practical for correctly applying known doses of toxins to insects. The potter's tower method's main drawbacks are that it takes a long time to complete and its high cost equipment.

Potter spray tower

After C. Potter, who created this spray apparatus at the Rothamsted Experimental Station in Harpenden, Hertfordshire, England, the Potter spray tower was named (Potter, 1952). The Potter tower is acknowledged on a global scale as the benchmark for laboratory chemical spraying procedures. This kind of equipment is necessary to examine the biological effects of contact poisons on organisms,. The Potters spray tower was created to shield the operator from pesticides and toxicant contamination/exposure.

Principle involved in potter spray tower

The principle underlying in potter spray tower is the constant atmosphereic pressure. A constant flow of 151b/sq.in through the input connection is managed by an exhaust valve and

on/off switch. A sensitive needle valve on the left side of the instrument provides a direct reading on the pressure gauge, or manometer (which is supplied as an extra), which is also used for fine adjustment. The air jack and nozzle head are both operated by this pressure.

Instrumentation

A high grade of stainless steel with a beautiful finish is used to make the tower. The potter's spray tower is 120 cm tall. It has a 20 cc standard sample reservoir capacity. 151b/sq.in is its operating pressure. All controls are easily positioned at the front of the tower, which includes quickly removable atomizers and a pneumatically driven spray table. This air-powered spraying device spreads a uniform layer of spray over a 9 cm diameter circle. Applying a spray solution or suspension on a Petri plate with a fine mist sprayer serves as the insecticide's testing deposit. This tool sprays a predetermined amount in a frame mist that settles on the target surface. In tests with aphids or spider mites, a Petri dish containing leaf substrate can be sprayed by the spraying apparatus.

Injection method

In this method, a hypodermic needle is used to inject the insecticide straight into the insect's body, or thorax. The technique uses a very fine stainless steel needle that is 27 or 30 gauge thick and 0.41 or 0.30 mm in diameter, and it measures the necessary amount of toxicant using a micrometre. For injection in tiny insects, tiny glass needles with a diameter of 0.1-0.16 mm are utilised. The pesticide is frequently administered intraperitoneally (into the body cavity) after being dissolved in propylene glycol or peanut oil. It is important to take precautions to prevent insect bleeding. This technique is used primarily to determine the precise dosage of toxicant required inside the insect's body (Sun, 1963).

Dipping method

Dipping method is advocated when topical application or injection approaches are impractical, such as with red spiders, housefly larvae, stored-product insects, insect eggs, or small plant-feeding insects etc. With the use of a pair of forceps, the insects are scooped up and briefly submerged in either a suspension or an emulsion of the chemical in water (Simon, 2008). For extension specialists and field workers, insect immersion techniques are useful field-based bioassays. The techniques are straightforward and a little more like insecticides that are applied in the field. In a different dipping technique, the leaves are immersed in an aqueous insecticide solution for a predetermined amount of time in successive dilutions of various strengths. Before being employed, the leaves were air dried for an hour after being properly drained of excess solutions (Immaraju, 1990). After a set amount of time, the known populations of insects that are being seen to feed on the problematic leaves are released, and mortality counts are conducted.

Sucking insects that are difficult to remove from the surface of leaves can also be controlled with this technique. Insect-containing leaves are dipped in insecticidal solution for a predetermined amount of time, and population counts are taken before and after the dip. This method makes it possible to verify the efficacy of field doses for pest control by distributing the product equally across the leaf surface (Siqueira *et al*, 2000).

Contact or residual method

In this procedure, the prepared insecticide is diluted in acetone, a volatile solvent and coated within a glass vial. By spinning the container, the solvent is allowed to evaporate, distributing the insecticide uniformly across the entire surface and leaving a residue layer. The concentration of the insecticide solution supplied to the vials affects the dose (Simon, 2008). Insects are released onto the treated surface, where they come into contact with the leftover film. Insects may also be exposed to residual deposits by having the pesticide uniformly applied to leaf, glass, filter paper, wood panels, or other types of building materials and then allowing the material to dry. Potter's tower apparatus is widely utilised for uniform application. Milligrams or grams of active ingredient per square metre (mg or g a.i./m2) are used to describe the deposits. We cannot determine if doses are being delivered effectively using these techniques since they do not simulate field conditions (Bacci *et al*, 2009).

Film method

Insecticide solution is typically applied to glass surfaces, such as Petri dishes, flasks, vials, wide mouth jars, etc., using this technique. Most frequently, petri dishes are used to gauge an insecticide's effectiveness. This method involves coating the inner surfaces of Petri dishes (5 cm in diameter) with one millilitre of solution, gently swirling the dish to ensure equal distribution, and letting the dish air dry at room temperature. The intended test insects are subsequently released onto the container's toxicant film. The known numbers of insects are then exposed for a further 18–24 hours, depending on the results of the recalculation (Sun, 1963; Birah, 2008).

Fumigation method

The fumigation technique is effective against pests in stored goods. The fumigation would be carried out in a sealed chamber with a temperature of 30 $\pm$ 2 ℃ and a relative humidity of 60 $\pm$ 5%. Along with the insects, the insecticide is added to a container that is tightly sealed. Each fumigation test is repeated three times or more, along with a control, and following exposure, the insects are given a modest amount of culture medium for a week before being transferred to a recovery area. At various points after the conclusion of the exposure period, adult mortality is documented (Simon, 2008).

Aqueous solution method

The basic idea behind this technique is to dissolve or suspend the insecticide residue in a small volume of water-miscible solvent (acetone or alcohol). The solution is then exposed to a small number of delicate aquatic creatures, such as mosquito larvae, tiny crustaceans, or fish. By comparing the toxicity of a treated sample to the standard, one can determine how much residue is present. Water should contain as little solvent as feasible, and whether or not it is harmful can be determined through control tests (Sun, 1963). The benefit of this procedure is that the entire organism is constantly in contact with the medium. Some have asserted that the toxicant is administered uniformly, but the suspended substance may settle. High sensitivity is hypothesised to be resulted from the toxicant being circulated and absorbed by the gills or comparable organs (Dewey, 1958).

Photomigration method

Burchfield (Burchfield *et al*, 1952) developed the photomigration method which is another aqueous solution method devised by exploiting the negative phototaxic response to *Aedes aegypti* larvae. The insecticide solution is carefully evaporated to dryness using a mild air stream. The residue is then carefully redissolved in a small amount of acetone, and 50 mL of water is then added. Then, between one and two hundred larvae are contained in a glass trough filled with the aforementioned fluid behind a porous barrier. The light comes on and the barrier is taken down after varied amounts of time. Live larvae move quickly away from the light. A second barrier is kept in the trough after one minute of exposure, and the larvae that are still there are deemed dead. T_{50} (The time needed to inactivate 50% of the test population) and LC_{50} are calculated from a series of dilutions. The amount of insects released and the mortality seen after the exposure period were used to compute the percent mortality corresponding to each dose. The accuracy of the outcome will be impacted by any control mortality. A correction is typically done using the Abott's formula in order to overcome this inaccuracy. By building a dosage-mortality curve that plots the dosage against the % mortality at a specific time point, one can determine how susceptible a population is to a poison. Without considerable testing, it is challenging to establish the asymptotic approaches (infinite ends) in the areas of zero and 100 percent mortality produced by such a plot's sigmoid curve. Plotting the dosage's logarithm against the probit value of percent mortality results in the sigmoid curve being transformed into a straight line in the probit transformation process. The result of this technique of calculation is a straight line, which makes it much easier to calculate the LC_{50}, LC_{90}, or any other lethal concentration/dose.

Sandwich method

This methods involves batch of insects permitted to consume an amount of known insecticides that has been placed between two leaves. This bioassay method is typically used for leaf eating caterpillars. In this case, insects ingest the toxicant through feeding.

Direct exposure

This method involves exposure of the insects to the materials without any extraction. Insects may consume the toxin, come into contact with it, or even breathe it in as vapour. The technique has been used to test the presence of harmful substances in milk, dirt, liquid, and even macerated food. Such insecticide-containing material may be stored in a jar with a known number of insects exposed for 18 to 24 hours before mortality counts are performed.

Utility of bioassay

1. Bioassay is adopted to ascertain the potency of the chemicals as insecticides.
2. It helps to find out the property of synergism, potentiation and antagonism of a compound when used in a mixture with an insecticide.
3. Comparative or relative toxicity of insecticides is worked out on the basis of LC_{50} obtained as a result of bioassay. This gives an index for selecting promising insecticides for field trial against insect pests.
4. Bioassay helps in evaluation of insecticides for their safety to pollinators, predators and pathogens.
5. Bioassay can help the formulators in improving the effectiveness for their formulated products, through changes in solvent, spreader, emulsifier, stickers etc.
6. The quality of marketed insecticides can be checked through bioassay of samples collected and comparing them with standard.
7. The change in values of LC_{50} of an insecticide for an insect with the passage of time indicates variation in susceptibility which helps in detection of resistance if developed in the insect population. Cross resistance to other insecticides, use of synergists and mixed formulations to overcome resistance are also estimated through bioassay.
8. Factors influencing the toxicity of insecticides may also be known through bioassay.
9. Formation of toxic metabolites, not quantitatively, due to use of insecticide can be determined by bioassay.
10. Through bioassay lethal time LT_{50} required to kill 50 % population to test animal. ED_{50} or EC_{50} i.e. the dose or concentration of chemical brings out sterility or other quantitative effects in 50 % test population can also be worked out.

11. Bioassay can also be utilized for estimation of micro quantities i.e. residues of insecticides in different commodities in order to alarm the consumers from hazards associated.

Limitations

1. Requirement of most sensitive organism for particular toxicant.
2. Rearing, handling and maintaining of uniformity of test organism. There may be the complexity of rearing or assay method for a specific organism.
3. Sometimes there is susceptibility of particular organism to plant toxicity or extractives.
4. Standardization of observation time.
5. There may be great variation in results with the change in test organism.
6. Lack of specificity in general though there are some instances in which it is highly specific.
7. Does not tell about the quantities of the different toxic metabolites in case of residue determination.

Conclusion:

For a more accurate assessment of the hazardous of a specific pesticide, it is crucial to understand median lethal dose, lethal concentration, and toxicity. For identifying and researching various agricultural pesticides, bioassay might be a beneficial technique. It can be quick, easy, adaptable, and very sensitive to a variety of toxins. In most cases, expensive equipment or highly skilled labour is not necessary. Bioassay can occasionally be used to identify toxicants, although it works best when the toxin is known.

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SEED PATHOLOGY – A REVIEW

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Abstract:

Seed pathology as an area of plant pathology is relatively new. Paul Neergard is considered the father of seed pathology. Recent developments in the area of seed pathology technology allow for more ecofriendly seed treatments and more reliable seed health testing. Due to economics and new interest in environmental issues, research into the viability of biological seed treatments is becoming more common. The use of International Seed Health Testing Methods allows for the detection of seed-borne pathogens that might go undetected using more conventional means. These types of research will be fundamental in guaranteeing seed health quality standards and achieving inspecting requirements throughout the world. Seed pathology includes the study of diseases and deterioration caused by bacteria, fungi, nematodes, viroid's, and viruses, and physiological and mechanical disorders. The discipline of seed pathology as a sub discipline of plant pathology is relatively new. Seed pathology involves the study and management of diseases affecting seed production and utilization, as well as disease management practices applied to seeds. In this paper, some aspects of seed pathology are discussed: research innovations in detection of seedborne pathogens and seed health testing methods.

Keywords: Seed health testing, Seed-borne.

Introduction:

Seed is the basic unit in crop production technology. It plays crucial role in the healthy crop productivity on the globe and is vulnerable to carry a heavy load of microorganisms which are capable of causing spread of diseases and considerable loss of the yield, in turn adversely affect the global agricultural economy (Bhajbhuje, 2014). In agriculture seeds of many crops are known to carry many pathogenic and non-pathogenic fungi which are commonly known as seed mycoflora or seed-borne fungi. Depending on the presence of fungi either on seed coat or in the seed it is further called as external seed-borne fungi and internal seed-borne fungi. In the year 1869 the first seed pathology laboratory was established in Saxony (Noble, 1951). First British

seed act was passed giving the farmers much security and protection against the seed borne diseases in 1920. About eight/nine thousand years ago, man began to depend on seeds as principle means of carrying his main crops, the cereals, from place to place and from season to season.

Environmental climate of high relative humidity, moderate temperature, seed nutritive content, cloudy weather and high level of seed moisture content of post-harvest crop are proved supportive measure for seed contamination with diverse group of fungal micro-propagules (Ramesh *et al*., 2013). Some fungal pathogens attack matured pre-harvested seeds on standing crops, tissues of embryo, penetrate deep contaminating internal seed coats, endosperm while others contaminate external seed surface in storage as a result of favorable storage environment (Bhajbhuje, 2013). It is premised on the hypothesis that infected seeds are considered highly effective means for transporting diverse pathogenic fungal micro-organisms over long distance (Archana and Prakash, 2013). The fungal population associated with seed coats as surface contaminants elicit response causing seed rot, seed necrosis, seed abortion, elimination and reduction of seed viability as well as seedling damage resulting disease development at later stages of plant growth by systemic or local infection (Gupta *et al*., 2012). Seed mycoflora limits an ability of plants to produce healthy fruit bearing shoots, causing damping – off, stem cancker, collor rot, leaf blight and fruit rot leads to premature defoliation , reduction in size and quality of fruits ,thereby adversely reducing yield potential to the extent of 20% - 30% (Lew - Smith, 2013).

Seed-borne fungi are a serious problem during storage in India. Seed-borne mycoflora is one of the major components reducing the yield. Mycoflora associated with seeds both internally and externally are responsible for seed abortion, mortality of grains, reduction in germination capacity, seed necrosis and at the end cause destructive to serious diseases during different stages of plant growth (Niaz and Dawar, 2009; Patharkar *et al*., 2013; Sontakke and Hedawoo, 2014). Seed infection reduces seed viability and productivity in crops, as well as adds contamination to the edible grains. In the world, about 90% food crops are propagated by seeds (Maude, 1996). However, seeds can be passive carriers of pathogens that are transmitted when the seed hosts are sown and emerge under suitable environmental conditions. Fungi, bacteria, viruses and nematodes can be carried with, on or in the seeds resulting in tremendous yield losses (Neergaard, 1979). During seed harvesting and storage, they may be infested by various pathogenic fungi and that often reduce the yield and the quality as well as quantity of grains (Patharkar and Hedawoo, 2014).

Due to attack of fungi, the color and test of grains has been affected which noticeably reduces its market value. The potential storage life of seed varies from species to species and among the varieties. Thus there is need to understanding genotypic variability in terms of viability of seed during storage. Seed carrying organisms cause manifold loses to the crop and reduces the agricultural productivity. The most common seed-borne fungi on dry beans (*P. vulgaris*) were *Alternaria* sp., *Aspergillu*s sp., *Fusarium* sp., *Botrytis* sp., *Chaetomium* sp., *Penicillium* sp., *Rhizopus* sp., *Cladosporium* sp. and *Trichothecium* sp. (Domijan *et al*., 2005). Seed health testing is one of the conventional methods to detect the presence of seed- borne fungi (ISTA, 1993). The purpose of this testing is to assure the safe movement of seed of different crops for research. It is premised from the hypothesis that many harmful organisms are carried by and moved together with the seeds and these organisms have the potentials to cause severe damage to crop production. Seed health information reveals that the organisms carried by the seeds and the level of infection or infestation will be introduced to another region or country (Archana and Prakash, 2013).

Such information comes from experiments or survey under field conditions where the seed is grown. Therefore, in seed health testing, several methods have been developed to detect the seed-borne mycoflora (Neergaard, 1977). These methods are simple, economic, sensitive, reproducible, efficient and easy for identification of fungi. Hence, the seed health testing is undertaken with the following objectives.

i) Collection of seed samples from different growing area and initial seed health testing by different methods.
ii) Standardization of different seed health testing methods for quick and accurate diagnosis of seed-borne fungi in seeds.

The effect of storage fungi have categorized into – a loss of germinability and discoloration of parts; heating, mustiness, caking and total decay; and changes in nutritional component including production of mycotoxins (Christensen and Gardeon, 1948). Therefore, seed health testing is a prerequisite to minimize losses by assessing the quality of seed before it is sown [International Seed Testing Association (ISTA), 1985]. The magnitude of the problem is further increased on account of shortage of good quality seeds which necessitate the investigation into various aspects of seed pathology. The idea of disease free seeds is therefore vital for maximum oilseed production. If the knowledge of the seed pathogen which is associated externally as well as internally with the seed available, suitable control measures can be adopted to check the disease and crop losses can be eliminated. Thus healthy seeds will be necessary to achieve the goal of our ambitious planners and thereby realizing dream for better tomorrow.

Importance:

Seeds are source of good quality edible oils both from nutritional and cooking quality point of view, and are stable against prolonged storage and heating. India occupies a prominent place amongst the oil seed producing countries of world. In Amravati region, serious problems are observed related to this oilseed. Because, during storage period many seed borne fungi associated and they causes not only reduced the germination but also affected seedling vigor that resulting in low yield. Therefore, seeds are selected for the present study.

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