Multidisciplinary Research in Agriculture and Allied Sciences

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Biocontrol Agents and their Mechanism in Plant Disease Management

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Introduction

Biological control of plant diseases is the suppression of populations of plant pathogens by living organisms. Amongst beneficial microorganisms isolates can be selected which are highly effective against pathogens and can be multiplied on artificial medium. Application of such selected and mass produced antagonists in high densities once or several times during a growing season is called "augmentative biological control.

Plant pathogens and their associated diseases are major limitations to crop production. The use of chemical pesticides has traditionally been the most important option for disease management but they effect environment as well as human and animal health. However, several biological and cultural management options are available to the farmers. Developing an integrated pest management (IPM) system that combines biological, chemical and cultural management options for disease management is efficient, economical and very attractive for better crop quality and environmental sustainability.

Biological products are those that contain naturally occurring beneficial microorganisms, or their derivatives, as active ingredients. The terms "biologicals" and "beneficial microorganisms" are closely associated and will be used interchangeably. Thorough knowledge of biologicals is needed to assist growers in maximizing the effectiveness of biological products in successful disease management. This is a timely topic given the concern about pests developing resistance against chemical pesticide options. Biological products are useful tools that may help delay resistance from developing.

Biological control is nothing but ecological management of community of organisms. It involves harnessing disease-suppressive microorganisms to improve plant health. Disease suppression by use of biological agents is the sustained manifestation of interactions among the plant (host), the pathogen, the biocontrol agent (antagonist), the microbial community on and around the plant and the physical environment. The biological control of plant diseases differs from insect biocontrol in following ways (Table 1).

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Table 1: Differences between disease bio-control and insect bio-control

Sr. No.	Disease bio-control	Insect bio-control
1	Disease control is largely achieved by antibiosis, competition and comparatively less by hyperparasites.	Largely by parasites and predators
2	Antagonists are largely passive and are not mobile. Contact of pathogen is accidental.	Parasites are active, mobile and seek their prey
3	It is a mass effect. For a single species of pathogen a large number of antagonists / competitors available.	Single predator / parasite for single prey
4	This method relies mainly on native organisms	Introduction of parasites / predators from other countries are normally followed.
5	Pathogen free seeds and planting materials are widely used.	Pest free seeds are not used

Advantages

1. Biological control is less costly and cheaper than chemicals
2. Biocontrol agents give protection to the crop throughout the crop period
3. They are highly effective against specific plant diseases.
4. They do not cause toxicity to the plants.
5. Application of biocontrol agents is safer to the environment and to the person who applies them.
6. They multiply easily in the soil and leave no residual problem.
7. Biocontrol agents can eliminate pathogens from the site of infection.
8. Biocontrol agents not only control the disease but also enhance the root and plant growth by way of encouraging the beneficial soil microflora. It increases the crop yield also. It helps in the volatilization and sequestration of certain inorganic nutrients. For example Bacillus subtilis solubilizes the element, phosphorous and makes it available to the plant.
9. Biocontrol agents are very easy to handle and apply to the target.
10. Biocontrol agents can be combined with biofertilizer.
11. They are easy to manufacture.

Disadvantages

Although biological control is advantageous in many aspects, it has the following disadvantages: 1. Biocontrol agents can only be used against specific diseases.

2. They are less effective than the chemicals.

3. Biocontrol agents have slow effect in the control of plant diseases as compare to chemicals.

4. At present, only few biocontrol agents are available for use and are available only in few places.

5. This method is only a preventive measure and not a curative measure,

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6. Biocontrol agents should be multiplied and supplied without contamination and this requires skilled persons.
7. The shelf life of biocontrol agents is short.
8. The required amount of population of biocontrol agents should be checked at periodical interval and should be maintained at required level for effectiveness.
9. The efficiency of biocontrol agents is mainly decided by environmental conditions.

Mechanisms of Biological Control

Direct Antagonism

Direct antagonism results from physical contact and/or a high- degree of selectivity for the pathogen by the mechanism expressed by the biocontrol agents. In such a scheme, hyperparasitism by obligate parasites of a plant pathogen would be considered the most direct type of antagonism because the activities of no other organism would be required to exert a suppressive effect.

Hyperparasitism/predation: A Phenomenon in which one parasite parasitic on another parasite either by direct parasitism or lysis and causes death of the pathogen.

Indirect Antagonism

Indirect antagonism results from activities that do not involve sensing or targeting a pathogen by the biocontrol agents. Stimulation of plant host defense pathways by non-pathogenic biocontrol agents is the most indirect form of antagonism. However, in the context of the natural environment, most described mechanisms of pathogen suppression will be modulated by the relative occurrence of other organisms in addition to the pathogen. It includes competition between pathogen and biocontrol agents for substrate.

Competition: Biocontrol agents competes with plant pathogens for space, organic nutrients and minerals. Most aerobic and facultative anaerobic micro-organisms respond to low iron stress by producing extracellular, low molecular weight (500- 1000 daltons) iron transport agents, designated as Siderophores, which selectively make complex with iron (Fe+) with very high affinity. Siderophore producing strains are able to utilize Fe+ - Siderophore complex and restrict the growth of deleterious micro-organisms mostly at the plant roots. Iron starvation prevents the germination of spores of fungal pathogens in rhizosphere as well as rhizoplane. Eg. Siderophores produced by *Pseudomonas fluorescens* (known as pseudobactinsor pyoveridins) helps in the management of soft rot bacterium, *Erwinia caratovora*. Suppression of *Pythium ultimum* by *Enterobacter cloacae* and *P. putida* colonize the root system in the rhizosphere and a corresponding reduction in Fusarium wilt suppression in cucumber.

Induction of host resistance: Plants actively respond to a variety of environmental stimuli, including gravity, light, temperature, physical stress, water and nutrient availability. Plants also respond to a variety of chemical stimuli produced by soil- and plant associated microbes. Such stimuli can either induce or condition plant host defenses through biochemical changes that enhance resistance against subsequent infection by a variety of pathogens.

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1) Systemic acquired resistance (SAR): SAR is mediated by salicylic acid (SA), a compound which is frequently produced following pathogen infection and typically leads to the expression of pathogenesis-related (PR) proteins. These PR proteins include a variety of enzymes some of which may act directly to lyse invading cells, reinforce cell wall boundaries to resist infections, or induce localized cell death.

2) Induced systemic resistance (ISR): ISR is mediated by jasmonic acid (JA) and/or ethylene, which are produced by applications of some nonpathogenic rhizobacteria.

Lytic enzymes secreted by biocontrol agents: Diverse microorganisms secrete and excrete other metabolites that can interfere with pathogen growth and/or activities. Many microorganisms produce and release lytic enzymes that can hydrolyze a wide variety of polymeric compounds, including chitin, proteins, cellulose, hemicellulose and DNA. Expression and secretion of these enzymes by different microbes and sometimes result in the suppression of plant pathogen activities directly. e.g. Control of *Sclerotium rolfsii* by *Serratia marcescens* appeared to be mediated by chitinase expression and, a b-1,3-glucanase contributes significantly to biocontrol activities of *Lysobacter enzymogenes* strain C3.

Antibiosis: Antagonism mediated by specific or nonspecific metabolites of microbial origin, by lytic agents, enzymes, volatile compounds or other toxic substances is known as antibiosis. Most microbes produce and secrete one or more compounds with antibiotic activity. In some instances, antibiotics produced by microorganisms have been shown to be particularly effective at suppressing plant pathogens and the diseases they cause.

a) Antibiotics: A chemical compound produced by one microorganism that inhibits or kills other microorganisms. Eg: *Gliocladium virens* produces gliotoxin that was responsible for the death of *Rhizoctonia solani* on potato tubers, Colonization of pea seeds by *Trichoderma viride* resulted in the accumulation of significant amount of the antibiotic viridian in the seeds, thus controlling *Pythium ultimum,* Some strains of *Pseudomonas fluorescens* produce a range of compounds, *viz.,* 2,4-diacetyl phloroglucinol (DAPG), phenazines, pyocyanin, which have broad spectrum activity against many plant pathogenic bacteria and fungi.

b) Bacteriocins: Bactericidal substances produced by certain strains of bacteria and are active against some other strains of the same or closely related species. Eg: The control of crown gall (caused by *Agrobacterium tumefaciens*) by the related *Agrobacterium radiobacter* strain K 84 is by the production of bacteriocin, Agrocin K84.

Biocontrol agents

Fungi (Antagonists)	**Pathogen Controlled**
Trichoderma viride	*Macrophomina phaseolina, Pythium, Sclerotium rolfsii*
Trichoderma harzianum	*Fusarium oxysporum f. sp. vasinfectum, Fusarium oxysporum f. sp. melonis, Rhizoctonia solani*
Trichoderma haematum	*Pythium sp., Rhizoctonia solani*
Verticillium laccani	*Uromyces dianthi*
Gliocladium virens	*P. ultimum, Rhizoctonia solani*
Latesaria arvalis	*Sclerotinia sclerotiarum, Rhizoctonia solani, Pythium sp*

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Bacteria	**Pathogen Controlled**
Agrobacterium radiobacter	*Agrobacterium tumefaciens*
Azotobacter chrococcum	*Rhizoctonia solani*
Bacillus subtillis	*Fusarium spp., Pythium spp., Rhizoctonia spp., Sclerotium rolfsii, Streptomyces scabies, Verticillium spp.*
Bacillus thuringeinsis	*Alternaria alternate, Hemilia Vastatrix*
Erwinia herbicola pv. Herbicola	*Erwinia amylovora*
Pseudomonas flurescens	*Fusarium spp., Macrophomina phaseolina, Pyricularia oryzae, Pythium spp., Rhizoctonia solani*
P. cepacia	*Cercospora sp.*
Streptomyces diastaticus	*Pythium aphidermatum*
S. griseoviridis	*Alternaria brassicola, Fusarium oxysporum f sp. dianthi, Rhizoctonia solani*

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Chapter- 2

Biofuel: A Sustainable Source to Mitigate Climate Change

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Introduction

Presently, the term "climate change" is of great interest to the world at large, scientific as well as political discussions. Climate has been changing since the beginning of creation, but what is alarming is the speed of change in recent years and it may be one of the threats facing the earth. Cliimate change has been recognized as a global issue with regional implications and one of the most serious challenges of the twenty first century. It is looming large with immense impact on various aspects.

The United Nations Framework Convention on Climate Change defines "climate change as being attributed directly or indirectly to human activities that alters the composition of the global atmosphere and which in turn exhibits variability in natural climate observed over comparable time periods".

The new definition introduced by IPCC for climate change "a change in the state of the climate that can be identified by the changes in the mean and the variability of its properties and that persists for an extended period, typically decades or longer. Climate change may be due to natural internal processes or external forcing or to persistent anthropogenic changes in the composition of the atmosphere or in land use".

Since 1850, the global use of fossil fuels has increased to dominate energy supply, leading to a rapid growth in carbon dioxide emissions. Data by the end of 2010 confirmed that consumption of fossil fuels accounted for the majority of global anthropogenic greenhouse gas (GHG) emissions, where concentrations had increased to over 390 ppm (39%) above preindustrial levels (Edenhofer *et al.,*2011).

The 29th annual issuance of the report, led by NOAA National Centers for Environmental Information, is based on contributions from more than 470 scientists from nearly 60 countries around the world and reflects tens of thousands of measurements from multiple independent datasets (highlights, full report). It provides a detailed update on global climate indicators, notable weather events, and other data collected by environmental monitoring stations and instruments located on land, water, ice, and in space.

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Report highlights include these indications of a warming planet:

❖ Greenhouse gases were highest on record. The major greenhouse gas concentrations, including carbon dioxide (CO2), methane, and nitrous oxide, rose to new record high values during 2018. The global annual average atmospheric CO2 concentration was 407.4 parts per million (ppm). This was 2.4 ppm greater than 2017 amounts and was the highest in the modern 60-year measurement record and in ice core records dating back as far as 800,000 years.

❖ Global surface temperature was near-record high. The globally averaged surface temperature was 0.30°C to 0.40°C above the 1981–2010 average, depending upon the dataset used. This places 2018 as having the fourth warmest annual global temperature since records began in the mid- to late 1800s. The four warmest years on record have all occurred since 2015. There were also higher and fewer low, temperature extremes than in nearly the entire 68-year extremes record.

❖ Global lower tropospheric temperature was well above average. In the region of the atmosphere just above Earth's surface, the globally averaged lower troposphere temperature was approximately third to seventh highest on record, depending on the dataset used.

❖ Sea surface temperature was near-record high. The globally averaged sea surface temperature (SST) cooled slightly since the record El Niño year of 2016 but was still far above the 1981–2010 mean by 0.33° +/− 0.05°C in 2018. The deeper ocean continues to warm year after year.

Thus, human activity has caused an imbalance in the natural cycle of the greenhouse effect and related processes. These greenhouse gases are trapping heat over Earth's surface, resulting in changes in climatic processes as evidenced by higher temperatures, rising sea levels, increased ocean acidity and ice melting. Facing the reality of human-caused warming, we now look for ways to reduce the problem so that future generations will not inherit a disaster.

In comparison with the stone age or bronze age, the industrial era (1750 to 2050) will be referred to the carbon (C) age or carbon civilization by future generations from 2100 AD and beyond. The use of fossil fuel, since the onset of industrial revolution ∼1750, has drastically disturbed the global C cycle with the attendant impact on the climate change and the increase in earth's temperature along with change in rainfall amount and distribution. The present civilization is hooked on C and is in need of a big time rehabilitation. Breaking the habit will require development of C-neutral or non-carbon fuel sources, and both soil science and agronomy have a major role to play in this endeavor.

If the burning of fossil fuels like coal and oil is not decreased, the earth will very likely heat up even faster, completely changing the world we live in. It is predicted to lead to adverse impacts on human systems like agricultural, health and leave irreversible impacts on the earth and the ecosystem as a whole. Climate change in particular appears to be altering the function, structure and stability of the Earth's ecosystem. We can either adapt to the corresponding changes or try to reduce their impact by significantly reducing fossil fuel burning.

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Biofuel based economy using energy from biomass, solar, wind and other renewable sources and nuclear energy seems to be a viable alternative. Globally biofuels have attracted much attention since they have become a leading alternative to fossil fuel and are produced domestically by many countries.

The term "biofuels" has many meanings, but basically, they are grown fuels (like corn ethanol) that we can use instead of fossil fuels (like petroleum). While biofuels can be any fuel produced from plant material, historically they have been produced from food crops such as corn and soy. But, new technologies are enabling biofuel production from non-edible gases, wood, and other plant waste material.

"A biofuel is a fuel that is produced through contemporary processes from biomass, rather than a fuel produced by the very slow geological processes involved in the formation of fossil fuels, such as oil". The word biofuel is usually reserved for liquid or gaseous fuels, used for transportation.

The word biofuel may refer to the fuels used for the production of electric energy, but in general it refers to liquid fuels used for means of transport.

Classical LCA studies on emissions from biofuel production and processing show that biofuel crops have net green house gas savings between 20% and 90%(Thow and Warhurst 2007). It is reported that replacing gasoline with corn ethanol reduces green house gases by 20% in the 2015 scenario excluding land-use change.

In 2018, worldwide biofuel production reached 152 billion liters (40 billion gallons US), up 7% from 2017, and biofuels provided 3% of the world's fuels for road transport. The International Energy Agency want biofuels to meet more than a quarter of world demand for transportation fuels by 2050, in order to reduce dependency on petroleum. However, the production and consumption of biofuels are not on track to meet the IEA's sustainable development scenario. From 2020 to 2030 global biofuel output has to increase by 10% each year to reach IEA's goal. Only 3% growth annually is expected.

The two most common types of biofuel are bioethanol and biodiesel.

Bioethanol :

Bioethanol is a high octane number biofuel which is produced from fermentation of corn, potatoes, grain (wheat, barley and rye), sugar beet, sugar cane and vegetable residues. The principle fuel used as a petrol substitute for road transport vehicles is bioethanol. Bioethanol fuel is mainly produced by the sugar fermentation process, although it can also be manufactured by the chemical process of reacting ethylene with steam. It is an alcohol made by fermentation, mostly from carbohydrates produced in sugar.

Sugar Fermentation Process :

The hydrolysis process breaks down the cellulosic part of the biomass or corn into sugar solutions that can then be fermented into ethanol. Yeast is added to the solution, which is then heated. The yeast contains an enzyme called invertase, which acts as a catalyst and helps to convert the sucrose sugars into glucose and fructose (both $C_6H_{12}O_6$).

The chemical reaction is shown below:

$$\underset{\textbf{Sucrose}}{C12H22O11} + \underset{\textbf{Water}}{H2O} \xrightarrow[\textbf{Catalyst}]{\textbf{Invertase}} \underset{\textbf{Fructose}}{C6H12O6} + \underset{\textbf{Glucose}}{C6H12O6}$$

The fructose and glucose sugars then react with another enzyme called zymase, which is also contained in the yeast to produce ethanol and carbon dioxide.

The chemical reaction is shown below:

$$\underset{\textbf{Fructose / Glucose}}{C6H12O6} \xrightarrow[\textbf{Catalyst}]{\textbf{Zymase}} \underset{\textbf{Ethanol}}{2C2H5OH} + 2CO2$$

The fermentation process takes around three days to complete and is carried out at a temperature of between 25^0C and 30^0C.

The main sources of sugar required to produce ethanol come from fuel or energy crops. These crops are grown specifically for energy use and include corn, maize and wheat crops, waste straw, willow and popular trees, sawdust, reed canary grass, cord grasses, jerusalem artichoke, myscanthus and sorghum plants. Cellulosic biomass, derived from non-food sources, such as trees and grasses, is also being developed as a feedstock for ethanol production.

Ethanol or ethyl alcohol (C_2H_5OH) is a clear colorless liquid, it is biodegradable, low in toxicity and causes little environmental pollution if spilt. Ethanol burns to produce carbon dioxide and water. It is a high octane fuel and has replaced lead as an octane enhancer in petrol.

Ethanol can be used as a fuel for vehicles in its pure form (E100), but it is usually used as a gasoline additive to increase octane and improve vehicle emissions. By blending ethanol with gasoline we can also oxygenate the fuel mixture so it burns more completely and reduces polluting emissions. The most common blend is 10% ethanol and 90% petrol (E10).Bioethanol is widely used in the United States and in Brazil.

Benefits of Bioethanol

- Bioethanol has been identified as a clean fuel that is mixed with petrol to run automobiles without modifying the engine design.
- Bioethanol is also biodegradable and far less toxic that fossil fuels.
- Bioethanol does not produce SO2 or NOx.
- by using bioethanol in older engines can help reduce the amount of carbon monoxide produced by the vehicle thus improving air quality.

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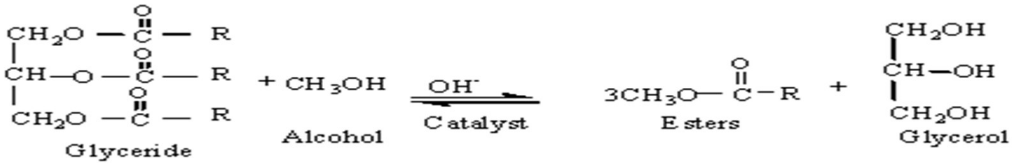
- The road transport network accounts for 22% of all greenhouse gas emissions and through the use of bioethanol, some of these emissions will be reduced.
- By encouraging bioethanol's use, the rural economy would also receive a boost from growing the necessary crops.

Biodiesel : Biodiesel is an alternative fuel similar to conventional or 'fossil' diesel. It is produced from oils or fats using transesterification. The largest possible source of suitable oil comes from oil crops such as rapeseed, palm or soybean.

The Transesterification process is the reaction of a triglyceride (fat/oil) with an alcohol to form esters and glycerol. During the esterification process, the triglyceride is reacted with alcohol in the presence of a catalyst, usually a strong alkaline like sodium hydroxide. The alcohol reacts with the fatty acids to form the mono-alkyl ester, or biodiesel and crude glycerol.

The figure below shows the chemical process for methyl ester biodiesel.



The products of the reaction are the biodiesel itself and glycerol.

Biodiesel can be used as a fuel for vehicles in its pure form (B100), but it is usually used as a diesel additive to reduce levels of particulates, carbon monoxide, and hydrocarbons from diesel-powered vehicles.

Biodiesel is the most common biofuel in Europe. It is produced from oils or fats using transesterification and is a liquid similar in composition to fossil/mineral diesel. Chemically, it consists mostly of fatty acid methyl (or ethyl) esters (FAMEs). Feedstocks for biodiesel include animal fats, vegetable oils, soy, rapeseed, jatropha, mahua, mustard, flax, sunflower, palm oil, hemp, field ennycress, *Pongamia pinnata* and algae. Pure biodiesel (B100, also known as "neat" biodiesel) currently reduces emissions with up to 60% compared to diesel Second generation B100.

Biodiesel can be used in any diesel engine when mixed with mineral diesel. It can also be used in its pure form (B100) in diesel engines, but some maintenance and performance problems may then occur during wintertime utilization, since the fuel becomes somewhat more viscous at lower temperatures, depending on the feedstock used.

Biodiesel is also an oxygenated fuel, meaning it contains a reduced amount of carbon and higher hydrogen and oxygen content than fossil diesel. This improves the combustion of biodiesel and reduces the particulate emissions from unburnt carbon.

Biodiesel is also safe to handle and transport because it is non-toxic and biodegradable, and has a high flash point of about 300 °F (148 °C) compared to petroleum diesel fuel, which has a flash point of 125 °F (52 °C).

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Biodiesel has emerged as one of the most strategically important alternative fuel substitutes to fossil fuel and are considered as an important way of progress for limiting green house gas emissions. According to Britain's National Non-Food crops Centre, total net savings from using first generation biodiesel as a transport fuel range from 25-82%(depending on the feedstock used), as compared to diesel derived from crude oil.

In comparison to conventional diesel fuel, the use of biodiesel results in an overall reduction of smog-forming emissions from particulate matter, unburned hydrocarbons, and carbon monoxide. Sulfur oxides and sulphates, which are major components of acid rain, are not present in biodiesel.

Over 90% of Greenhouse gas emissions from the life cycle of both diesel and biodiesel are from the use phase. Rate of global warming potential from biodiesel production and use is just of 23% of diesel (Prueksakorn and gheewala 2006). This is because the CO2 emissions from combustion of biodiesel in the engine during the use phase are considered Green house gas-neutral as they are of biomass origin and thus absorbed from the atmosphere by the Jatropha plants during growth.

Benefits of Biodiesel :

Biodiesel has several environmental benefits when compared to petroleum-based diesel fuel, such as:

- Reduces lifecycle greenhouse gases by 86 percent.
- Lowers particulate matter by 47 percent, reduces smog and makes our air healthier to breathe.
- Reduces hydrocarbon emissions by 67 percent.
- Biodiesel is rapidly biodegradable and completely non-toxic.
- The fuel produces no net output of carbon in the form of carbon dioxide (CO_2).
- Biodiesel has a higher flash point than fossil diesel and so is safer in the event of a crash.
- Biodiesel can be used in pure form (B100) or may be blended with petroleum diesel at any concentration in most injection pump diesel engines.

Depletion of petroleum derived fuel and environmental concern has promoted to look over the biofuel as an alternative fuel sources. But a complete substitution of petroleum derived fuels by biofuel is impossible from the production capacity because biofuel production will compete with land needed for food production. However, it appears biofuel production especially from biomass residues may be an option for creating economic diversification and provide affordable energy without competition with land needed for food production and environmental degradation.

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Chapter- 3

Different Forms of Potassium in Morena District of Madhya Pradesh

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Introduction

Potassium (K) is an essential element for plant growth which exists in soil in four forms, viz. water soluble K, which is taken up directly by plants; exchangeable –K, held by negative charges on clay particles and is available to plants; fixed –K which is trapped between layers of expanding lattice clays; and lattice –K, an integral part of primary K bearing minerals.

Potassium is essential for various metabolic activities of living cell, transformation of carbohydrates, reduction of nitrates, synthesis of protein and normal meristematic activities where it acts as a catalyst or as a co-factor in enzymatic reaction of living cells. It has been suggested that potassium may also affect photosynthesis maintenance of turgor in plant cells as well as formation of oil and imparting disease resistance.

Balanced nutrition with all the essential plant nutrients supply is pivotal for sustainable and higher crop production. In several long term studies including soybean-wheat on Vertisols (Typic Chromusterts) at Jabalpur, the sustainability of crop yields was threatened in the absence of potassium application. The black soils, though rich in available potassium have generally poor reserves of non –exchangeable potassium (Subba rao *et al*. 2007).

Although most of the Indian soils have been reported to be rich in potassium but intensive cultivation of high yielding verities of crops with optimum application of high rates of N and P tend to deplete the K reserve of soil at a faster rate. To formulate sound fertilizers recommendation, potassium supplying capacity of soil is essential. This will be dependent not only on the available K content of soil, but a sound knowledge of different forms of K and their relationship among themselves is also required.

The major crops of the investigate area are paddy, sesame, pearl millet and sorghum in *Kharif* whereas mustard, wheat and potato in *Rabi.* Under intensive cultivation, readily available exchangeable K is removed by crop. This is followed by further release of exchangeable K from non- exchangeable forms. The dynamic equilibrium is markedly affected when applied soil solutions K is either taken up by plants or leached into the lower

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soil horizons or converted into unavailable forms consequently. Non- exchangeable K is released as exchangeable form when levels of exchangeable and solution K are decreased by plant uptake and leaching. The dynamics of potassium in soil depends on the magnitude of equilibrium among various forms which has relationship with physico-chemical properties of soil.

Different forms of K:

Water soluble K:

Water soluable potassium was estimated in 1:5, soil: water suspension as described by Black (1965).

Exchangeable K:

Exchangeable potassium was determined by flame photometer using 1N neutral ammonium acetate extraction in 1:5 ratio as described by Black (1965).

Non-exchangeable K:

1 N boiling HNO_3 extractable potassium was estimated flame photometerically in 1:10, soil: acid suspension boiled for 10 minutes as described by Black (1965).

Total K:

Total potassium was estimated flame photometerically by digestion with hydrofluoric (48%) and perchloric (70-72%) acid in platinum crucible by the method outlined by Black (1965).

Lattice K:

Estimated by difference between total K and sum of water soluble, exchangeable and non-exchangeable potassium.

Water soluble - K:

Water soluble K status of Morena district (Table-4.6) was found in the range of 4.2 – 19.2 mg kg^{-1} under different villages with the average value of 9.6 mg $kg^{-1.}$ Average maximum value of water soluble K (11.9 mg kg^{-1}) was observed in Mudikhera village of Morena tehsil whereas minimum value (7.9 mg kg^{-1}) found in Visang pura village of Jora tehsil.

Exchangeable - K:

Exchangeable K status of Morena district (Table-4.6) was found in the range of 34.0 – 186.0 mg kg^{-1} under different villages with the average value of 101.1 mg $kg^{-1.}$ Average maximum value of exchangeable- K (115.5 mg kg^{-1}) was observed in Mudikhera village of Morena tehsil whereas minimum value (75.7 mg kg^{-1}) found in Bijlipura village of Porsa tehsil.

Non exchangeable - K:

Non exchangeable K status of Morena district (Table-4.6) was found in the range of 291.3 – 1628.1 mg kg^{-1} under different villages with the average value of 820.0 mg $kg^{-1.}$

Average maximum value of exchangeable- K (1003.6 mg kg^{-1}) was observed in Gidoli village of Porsa tehsil whereas minimum value (570.2 mg kg^{-1}) found in Visang pura village of Jora tehsil.

Table 4.6 : Status of water soluble, exchangeable and non exchangeable forms of potassium in the soils of Morena district.

S.No	Name of Village	W. S.-K ($mgkg^{-1}$)		Ex.- K ($mgkg^{-1}$)		N. Ex. -K ($mgkg^{-1}$)	
		Range	Mean	Range	Mean	Range	Mean
Tehsil : Morena							
1	Mudikhera	6.7-19.2	11.9	64.8-186.0	115.5	552.1- 1585.2	984.1
2	Ganjrampur	6.9-16.0	11.2	67.1-155.4	109.1	571.6-1324.5	896.8
3	Jeengani	5.1-16.2	9.7	57.2-180.7	102.1	455.3-1438.7	812.9
	As a whole	**5.1-19.2**	**10.9**	**57.2-186.0**	**108.9**	**455.3-1585.2**	**897.9**
Tehsil : Ambah							
1	Kuthiyana	5.8-12.8	9.4	64.4-142.9	105.0	516.1-1144.8	840.9
2	Beelpur	4.2-12.2	8.7	46.7-136.0	97.4	374.5-1090.0	778.5
3	Nayapura	5.6-13.2	8.6	62.5-147.4	95.6	491.1-1158.3	751.2
	As a whole	**4.2-13.2**	**8.9**	**46.7-147.4**	**99.3**	**374.5-1158.3**	**790.2**
Tehsil : Porsa							
1	Kasmada	6.1-13.0	8.5	51.7-144.8	89.5	442.6-1239.9	760.8
2	Bijlipura	4.2-15.0	9.3	34.0-122.2	75.7	291.3-1046.3	656.3
3	Gidoli	6.6-16.1	10.5	72.0-175.9	108.4	666.4-1628.1	1003.6
	As a whole	**4.2-16.1**	**9.5**	**34.0-175.9**	**91.2**	**291.3-1628.1**	**806.9**
Tehsil : Jora							
1	Mai	5.2-14.2	10.1	57.2-156.0	110.5	376.5-1443.5	903.7
2	Visangpura	5.7-13.5	7.9	62.5-147.4	86.6	411.6-970.7	570.2
3	Nidhan	5.9-12.4	9.8	64.4-136.2	107.0	493.3-1043.2	809.0
	As a whole	**5.2-14.2**	**9.2**	**57.2-156.0**	**101.4**	**376.5-1443.5**	**760.9**
Tehsil : Kelaras							
1	Torka	6.9-17.0	10.3	75.2-186.0	112.5	576.2-1424.5	861.4
2	Khera	7.1-14.2	10.2	77.9-155.4	111.8	643.0-1282.8	923.1
3	Bhatpura	5.0-15.3	9.8	55.3-167.2	107.1	456.4-1380.1	884.3
	As a whole	**5.0-17.0**	**10.1**	**55.3-186.0**	**110.5**	**456.4-1282.8**	**889.6**
15	**Over all**	**4.2-19.2**	**9.6**	**34.0-186.0**	**101.1**	**291.3-1628.1**	**820.0**

Table 4.7: Status of Lattice and total - K in the soils of Morena district.

S.No	Name of Village	Lattice-K (%)		Total- K (%)	
		Range	Mean	Range	Mean
Tehsil : Morena					
1	Mudikhera	0.66-1.89	1.17	0.72-2.07	1.28
2	Ganjrampur	0.80-1.58	1.20	0.87-1.73	1.31
3	Jeengani	0.56-1.76	0.99	0.61-1.92	1.09
	As a whole	**0.56-1.89**	**1.12**	**0.61-2.07**	**1.22**
Tehsil : Ambah					
1	Kuthiyana	0.79-1.60	1.14	0.86-1.73	1.24
2	Beelpur	0.61-1.58	1.03	0.66-1.63	1.12
3	Nayapura	0.60-1.56	0.97	0.65-1.68	1.05
	As a whole	**0.60-1.60**	**1.05**	**0.66-1.73**	**1.14**
Tehsil : Porsa					
1	Kasmada	0.64-1.39	0.99	0.69-1.53	1.08
2	Bijlipura	0.42-1.51	0.94	0.45-1.63	1.01
3	Gidoli	0.89-1.61	1.24	0.96-1.75	1.35
	As a whole	**0.42-1.61**	**1.06**	**0.45-1.75**	**1.15**
Tehsil : Jora					
1	Mai	0.57-1.83	1.12	0.64-1.98	1.22
2	Visangpura	0.62-1.47	0.94	0.67-1.59	1.01
3	Nidhan	0.68-1.53	1.08	0.73-1.62	1.17
	As a whole	**0.57-1.83**	**1.05**	**0.64-1.98**	**1.13**
Tehsil : Kelaras					
1	Torka	0.45-1.95	0.99	0.53-2.11	1.09
2	Khera	0.81-1.62	1.26	0.89-1.77	1.37
3	Bhatpura	0.79-1.92	1.30	0.84-2.05	1.40
	As a whole	**0.45-1.95**	**1.19**	**0.53-2.11**	**1.29**
15	**Over all**	**0.42-1.95**	**1.08**	**0.45-2.11**	**1.17**

4.3.4: Lattice - K:

Lattice- K status of Morena district (Table-4.7) was found in the range of 0.42 – 1.95 % under different villages with the average value of 1.08%. Average maximum value of lattice - K (1.30 %) was observed in Bhatpura village of Kelaras tehsil whereas minimum

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value (0.94%) found in Visang pura and Bijlipura villages of Jora and Porsa tehsil respectively.

: Total- K:

Total - K status of Morena district (Table-4.7) was found in the range of 0.45 – 2.11 % under different villages with the average value of 1.17%. Average maximum value of total - K (1.40%) was observed in Bhatpura village of Kelaras tehsil whereas minimum value (1.01%) found in Visang pura and Bijlipura villages of Jora and Porsa tehsil respectively.

correlation matrix of different forms of potassium

Water soluble K :

A highly significant (1% level) and positive correlation was found between water soluble K and other forms of potassium. The results (table 4.9) indicate that the coefficient of correlation of water soluble K with exchangeable K (r= 0.953**), with non exchangeable K (r= 0.945**), with lattice K (r= 0.725**) and with total K (r= 0.758**) were observed positive and significantly in the soils of Morena district.

4.5.2: Exchangeable K (NH_4OAc-K) :

The correlation of coefficient between forms of potassium indicated (table 4.9) that exchangeable K had significant positive correlation with non exchangeable K (r =0.965**), lattice K (r =0.741**) and total K (r = 0.774**) in the soils of Morena district.

4.5.3: Non exchangeable K (1N HNO_3 –K) :

Non exchangeable K showed a positive and highly significant correlation (1% level) with lattice K (r =0.761**) and total K (r =0.795**) in the soils of Morena district.

4.5.4: Lattice- K :

A highly significant (1% level) and positive correlation was also found between lattice K and total K (r = 0.998**) in the soils of Morena district.

These relationship indicated that there exited an equilibrium between these forms of K and depletion of one is instantly replenished by one or more of the other forms of K.

Status of different forms of potassium:

Water soluble - K:

Water soluble K status of Morena district was found in the range of 4.2 – 19.2 mg kg^{-1} under different villages with the average value of 9.6 mg kg^{-1}. This form represented 9.49, 1.09 and 0.082 % of exchangeable, non-exchangeable and total-K respectively. Result on the same line with different soil types have also been reported by Padole and Mahajan (2003) and Chandrasekhar rao and Krishnamurthy (2007).

Exchangeable - K:

Exchangeable K status of Morena district was found in the range of 34.0 – 186.0 mg kg^{-1} under different villages with the average value of 101.1 mg kg^{-1} and contributed 0.864

percent of the total K content of the district. Kaskar *et al.* (2001) and Padole and Mahajan (2003) also reported that exchangeable K contributed same line towards total K.

Non exchangeable - K:

Non exchangeable K status of Morena district (Table-4.6) was found in the range of 291.3 – 1628.1 mg kg^{-1} under different villages with the average value of 820.0 mg kg^{-1}, which is contributed 7.00 percent of the total -K content of the district. Chand and Swami (2000) Kaskar *et al.* (2001) and Sharma *et al*. (2009) also reported similar results with different soil type.

Lattice - K:

Lattice- K status of Morena district was found in the range of 0.42 – 1.95 % under different villages with the average value of 1.08 percent and contributed 92.31 % of the total K. Kaskar *et al.* (2001) and Padole and Mahajan (2003) also reported that lattice K contributed same line towards total- K.

Total- K:

Total - K status of Morena district (Table-4.7) was found in the range of 0.45 – 2.11 % under different villages with the average value of 1.17%. Result on the same line with different soil types have also been reported by Padole and Mahajan (2003) and Mandal *et al.* (2011)

Discussion

The amount of water soluble, exchangeable and non-exchangeable-K in different villages of Morena district; ranged from 4.2 – 19.2, 34.0 – 186.0 and 291.3 – 1628.1 mg kg^{-1} with the mean value of 9.6, 101.1 and 820.0 mg kg^{-1} respectively. These forms contributed 0.08, 0.86 and 7.00% towards total-K.Lattice and total-K found in the range of 0.42 – 1.95 and 0.45 – 2.11 % with the mean value of 1.08 and 1.17 % respectively. Lattice – K contributed maximum (92.31%) towards total-K.

Soil pH showed negative relationship with all the forms of potassium but they are not reaching the level of significance except exchangeable-K which show significant coefficient of correlation (r= -0.162*).Morena district shows positive relationship with all the forms of potassium. The coefficient of correlation of organic carbon with water soluble K (r= 0.586**), exchangeable K (r= 0.615**), non- exchangeable-K (r= 0.579**), lattice-K (r= 0.482**) and Total- K (r= 0.500**) were observed which were significant at higher level (1%) .A highly significant and negative correlation observed between different forms of potassium and sand particles and positive with clay content of the soils of Morena district.A highly significant (1% level) and positive relationship were observed between different forms of K, These relationships indicate that there existed equilibrium between these forms of K and depletion of one is instantly replenished by one or more of the other forms of K.The amount of water soluble, exchangeable and non-exchangeable-K in different villages of Morena district; ranged from 4.2 – 19.2, 34.0 – 186.0 and 291.3 – 1628.1 mg kg^{-1} with the mean value of 9.6, 101.1 and 820.0 mg kg^{-1} respectively. These forms contributed 0.08, 0.86 and 7.00% towards total-K whereas, Lattice and total-K found in the range of 0.42 – 1.95 and

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0.45 – 2.11 % with the mean value of 1.08 and 1.17 % respectively. Lattice – K contributed maximum (92.31%) towards total-K. A highly significantly and positively relationship were observed between different forms of K, These relationships indicate that there existed equilibrium between these forms of K and depletion of one is instantly replenished by one or more of the other forms of K.

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Chapter- 4

Forms of Sulphur and their Relationship with Soil Properties

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Sulphur is the fourth most important nutrient after nitrogen, phosphorus and zinc for Indian agriculture. In recent years, sulphur deficiency has been aggravated in Indian soils due to tremendous increase in cropping intensity and adoption of cultivation of high yielding varieties and a drastic shift from low analysis fertilizers to high analysis fertilizers which contain little or no sulphur.

Sulphur deficiency has been found to occur in central India’s soils and low in organic matter. Sulphur requirements of crop plants are quite high and high yielding varieties require higher amounts of sulphur as compared to low yielding varieties of the crops. About 42.3%, Indian soils and 32.0% U.P. soils are deficient in sulphur, it is well accepted that sulphur deficiency in Indian soils is wide spread and major constraint in the way of increasing crop productivity, produce quality and farm incomes (Tandon 2010).

Mostly the sulphur in the soil can be grouped into four forms viz. total-S, organic-S, non-sulphate-S, and available-S. In these different forms of sulphur organic-S dominantly controls the levels of plant available sulphur. The important factors which influence the content and availability of sulphur in soils are organic matter and texture of soil. Sulphate-S represents plant available-S, which is immediate supplier of sulphate ions to the roots for absorption by plants.

The major sources of S in soils are sulphides, sulphates and organic combinations with C and N contents of soils, though a reflection of parent material from which soils originated, is influenced by climate and management practices. Consequently, different soils maintain a wide range of total -S contents which has been found to extend more than three thousand ppm. Since total S does not relate with plant growth, its plant available forms are emphasized more often.

Almost 20 years ago, it was stated that “As intensive farming practices are followed and the use of concentrated fertilizers intensive farming practices are followed and the use of concentrated fertilizers free from sulphur become more popular, the areas which are now

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presumed to contain adequate amount of sulphur may also begin to show sulphur deficiency; In countries such as India, sulphur is one element that must not be overlooked.

Sulphur uptake ranges from 5 to 46 kg ha^{-1} for individual crop to 78 to 80 kg ha^{-1} for intensive annual rotations. Generally, S uptake is 9 to 15% of N uptake and is comparable to P uptake. Sulphur uptake per tonne of grain production is 3 to 4 kg for cereals, 8 kg for pulses and 12 kg for oilseeds (Tandon, 1995). It is, therefore, necessary to have balanced use of sulphur to exploit the genetic potential of newly evolved varieties. The effect on the crop deserves a careful evaluation to sustain the productivity on one hand and soil health on the other.

Under these contexts, a study on the distribution of different forms of sulphur in wheat growing soils of M.P. would help in understanding the availability and depletion of different forms of sulphur. Their interrelationship with some important soil characteristics is considered to be important not only to delineate sulphur deficiency.

Forms of sulphur

1. Total sulphur

This was determined by taking the weighed quantity (1 gm) of air dried soil (ground to pass 0.5 mm sieve) with 10 ml. of digesting solution (100 gm AR KNO_3+ 350ml. concentrated HNO_3 and dilute to 1 liter). Evaporate to dryness on a steam bath and place the breaker in an electric furnace, heat to 500°C and maintain at this temperature for three hours. After cooling, add 5 ml. of 25% HNO_3 and again digest the contents for one hour on steam bath the soluble salts with distilled water and filter the solution through a Whatman No. 42 filter paper.

Dilute to known volume and take a suitable aliquot of the filtrate for the turbidi-metric determination of sulphate (Choudhary and Cornfield, 1966).

2. Organic sulphur

This was determined by taking the weighed quantity (1g) of air dried (20 mesh soil) soil with 1 gm. of $NaHCO_3$ in a high form porcelain crucible ignite the mixture at 500°C in an electric furnace (muffle) for 3 hours. After cooling, transferred the contents of the crucible to a 100 ml flask and add 25 ml of the extracting solution (Dissolve 4.6 gm $NaH_2PO_4.H_2O$ in 1 liter of 2N acetic acid). After the reaction subsides, the contents of the flask were shaken for half an hour. Afterward the solution filtered through a dry whatman No. 1 filter paper. Take a suitable aliquot of the filtrate for the turbidi-metric determination of sulphate (Bardsley and Lancaster, 1965).

3. Available sulphur

This was determined by taking the weighed quantity (5g) of air dried soil with measured quantity of 25 ml 15% $CaCl_2$ extractant and 0.5 gm sulphur free charcoal. The contents of the flask were shaken for half an hour. The solution was filtered through Whatman No. 42 filter paper. 10 ml. of extract was transferred to a 100 ml. conical flask, 1 ml. each of 6 N HCl and 0.5% gum acacia and 0.5 gm $BaCl_2$, $2H_2O$, (50 to 60 mesh) were added to the flask and the flask was shaken gently till the crystals were dissolved, the turbidity thus developed was read on spectrophotometer at 420 nm.

4. Water soluble suphur

Water soluble sulphur is determined by turbidity method in soil extract (Freney 1958). The total-S content was found significant and positively correlated with organic carbon, clay and total nitrogen. The significant and positive correlation of total-S with organic carbon and clay and total-N has also been reported by Aggarwal and Nayyar (1998), Trivedi *et al.* (1998) and Trivedi *et al.* (2000).A significant positive association of total –S with all the other forms of sulphur has been observed in the present investigation. Total sulphur appears to be a function of soil organic matter as both are significantly and positively correlated. This is also due to the fact that in most of the soil, S is a constituent of organic matter (Kumar *et al.* 2002).

Mineralization of S-compounds in soil

The sulphur in soil is being cycled continuously between inorganic and organic forms. The nature of compounds formed and their transformation are influenced by the biologically mediated processes, which, in turn, are affected by environmental conditions. As has been stated earlier in many cases, organic matter is the major source of soil S. Its oxidation to SO_4^{2-} ions is brought about by soil microorganisms and the process is called 'mineralization' . It is this mineralized form in which S can be taken up by plants and microorganisms. Rate of mineralization is affected by such factors as moisture, aeration, temperature and soil pH. The conversion of organic-S to SO_4^{2-} ions is an oxidation process and cannot take place in the absence of oxygen. Proper aeration is, therefore, essential for this process to proceed. Also, this process cannot take place under submerged soil conditions, except in the aerated pockets of the soil.

The formation of end product, SO_4^{2+} ions, is associated with the formation of H^+ ions. Hence, this process lowers the soil pH. This biological transformation in some cases requires very low pH in soil before can be initiated. Under limited S-supply and in the presence of excess of carbonaceous materials, the mineralized-S can be readily used by microorganisms. Carbonaceous materials provide energy to the growing population of microorganisms which consume the mineralized-S. Hence, crop plants may suffer from S-deficiency. This process is called "immobilization". Immobilization is a temporary phase, as on the death of the microbial population, the microbial S is mineralized to SO_4^{2-} which can be utilized by the growing plants. Sulphur in the soil is associated with organic carbon in a fixed C:S ratio of about 140:1. Thus, a favourable C:S ratio is more important than a large store of organic matter for S-response under tropical conditions. It is because the ratio influences microbial decomposition of organic matter.

A major transformation involving S is that of conversion/oxidation of organic S into sulphate. This process is similar to N-transformation in soil. Since this transformation is mediated biologically, extremes of temperature for it are 35-40 ºC. Soil moisture is another important factor. While moisture level of about 60% of field capacity is good for oxidation of

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organic-S to sulphate, excessive moisture decreases the rate of oxidation due to low accessibility of oxygen or aeration.

Sulphur exits like nitrogen, iron and manganese, in nature in more than one oxidation state. Hence, it is subjected to reduction oxidation reactions in soil. These reactions are biochemical in nature and are mediated by autotrophic bacteria belonging to the genus *Thiobacillu*s. Under anaerobic conditions, when the availability of free air is completely cut off and all the soil pore-spaces are occupied by water, as in marshy lands and lowland rice fields, partially oxidized chemicals such as NO 3- and SO42- are reduced and utilized by organisms like *Desulphovibrio* and *Desulphotomaculum*. In the process, these ions are reduced to nitrites, nitrous oxides, sulphites and sulphides. The end product may not always be the foul smelling H2S because under the impeded drainage conditions, iron (Fe-III) and manganese (Mn-IV) are deduced to Fe2+ and Mn2+, which react with sulphites to form relatively less-soluble sulphides of these elements.

In the reduction –oxidation process, SO_4^{2-} is the last ion to undergo reduction after all the nitrate, Mn (IV) and Fe (III) ions have been reduced . This means intense reduction conditions are necessary before SO_4^{2-} is reduced. Under normal conditions of rice-wheat cropping system in light-textured soils low in organic matter, reduction conditions may not reach a stage where reduction of SO_4^{2-} sets in. However, in heavy-textured soils rich in organic matter and where rice-rice system is practised, there is a possibility of sulphide formation. The less-oxidised forms of S such as sulphite (SO_3^{2-}), thiosulphate $(S_2O_3)^{2-}$ and elemental sulphur (S) can undergo reduction relatively more rapidly. Sulphur-deficiency, observed in rice grown under submerged soils, sometimes results from rapid reduction of SO42- to sulphite. Then crop shows a positive response to small applications of SO43- at frequent intervals.

During decomposition of organic matter under normal arable soil conditions, organic sulphur compounds are transformed/oxidised to sulphates. The intermediate products of this transformation are: sulphides, thiosulphates and polythionates. In the presence of excess of aeration, oxidation of sulphides and sulphites can occur through purely chemical processes, while most of the oxidation reactions in soil are biochemical in nature. It is clear that oxidation of organic-S to SO42- through sulphides and elemental S results in the formation of sulphuric acid. This sulphuric acid on ionization, releases H^+ ions in the soil solution, which help in lowering the pH of the soil. Elemental S can be successfully used as an amendment in calcareous soils (Calciorthents) with high pH. Acid formed through oxidation of elemental S helps in dissolving $CaCO_3$ with the resultant decrease in soil pH.

Some fertilizers may contain elemental S such as S-coated urea. Use of such materials may prove useful in alkali soils or alkaline calcareous soils. However, the soils are acidic in nature, use of such sulphur-containing materials is not recommended, as these may increase soil acidity in the long-run. The coastal areas where the soils undergo periodic flooding with sea water may experience extremes of acidity. When such soils are flooded, soil aeration is completely cut off and sulphur is reduced to sulphides by the autotrophic bacteria which use SO42- for its oxygen requirement.

In the process, soil acidity decreases which results in an increase in the pH to near neu- trality. However, when sea water is drained and these coastal soils are prepared for culti- vation, aeration occurs, and sulphides are oxidised to sulphates again. With this oxida- tion, SO, and H' ions are released to the soil solution and pH of the soil may drop to as low as 2.0 to 1.4. These soils are so acidic that it is sometimes uneconomical to cultivate them However, such soils can be utilized for grow- ing wetland rice under flooded conditions. Some salt-tolerant rice varieties like Pokali can be grown under such situations. These soils, which are alternately flooded and drained, are gener- ally fine textured. There are extremes of acid conditions in these soils. Such soils are also called acid-sulphate soils or cat clays. These soils, locally known as 'Kari', occur in Ambalapuzha, Vaikom and Kottayam talukas in the Kuttanad region of the Kerala state. These soils are classified as Sulfaquepts and Sulphaquents.

Adsorption of sulphur in soils containing high amounts of hydrous-oxides of Fe and Al is common, especially in Ultisol, Oxisol and Alfisol. Adsorbed sulphate-sulphur can account for up to one-third of the total sulphur in sub- soils, while the same fraction represents less than 10% of the total sulphur in the surface soils. Sulphate-sulphur adsorption by soils is beneficial since if protects sulphur from leach ing in high rainfall areas.

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Chapter- 5

Modern Concept of Fertility and Fertilizers in Agriculture

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Abstract

Nutrients are removed continuously by the crops from the soil and their replenishment through fertilizers and manures is essential. The ideal ratio of nutrients for optimum growth depends on the crop and soil. When the added fertilizer contains nutrients in less or higher amounts than that required by the crop, the imbalance thus, results in poor crop growth. The application of P along with N increases the nitrogen use efficiency. Sometimes even with NP application, decrease in yield occurs because K becomes the limiting factor. Similarly, with optimum dose of NPK, the deficiency of Zn may appear. Soil test based fertilizer recommendations ensure balanced use of fertilizers and increase yields and profits. Balanced fertilizers encompass, besides major nutrients, secondary and micronutrients whose deficiency appear in different soils in various agro-eco-regions due to intensive cultivation.

Keywords- Soil Fertility, fertilizers rate, Nutrients Integrated Nutrient Management

Introduction

Literally, the world 'fertile' means bearing abundantly and fertile soil is considered to be one that produces abundant crops under suitable environment conditions. A distinction should be made however, between soil productivity and soil fertility. Soil productivity is basically an economic concept and signifies the capacity of soil to produce specified plant or plant part or a sequence of plants under well defined and specified systems of management inputs and environmental conditions, viz. climatic condition. It is measured in terms of outputs or harvests in relation to production factor for a specific kind of soil under a physically defined system of management. Soil fertility refers to the inherent capacity of a soil to supply essential nutrient elements to the plants in adequate amounts and in right proportion for their optimum growth. It is one of the key components to determine productivity. It is defined as the quality of a soil that enables it to provide essential chemical elements in adequate quantities and proportions for the growth of specified plant. The essential nutrient elements comprise the key components of soil fertility vis-à-vis plant nutrition and therefore these are referred to as plant nutrients or nutrient elements.

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Fertility Concept

Types of soil fertility:

❖ Inherent or natural fertility: The soil, as a nature of them, contains more some nutrients which are known as inherent fertility. Among the plant nutrients Nitrogen, phosphorus and potassium is essential for the normal growth and yield of crop. Indian soil contains 0.3 to 0.2 % N, .03 to 0.3 % P and 0.4 to 0.5 % K. Inherent fertility has a limiting factor from which the fertility is not decreased.

❖ **Acquired fertility:** The fertility developed by application of manures and fertilizers, tillage, irrigation etc. is known as acquired fertility. The acquired fertility has also a limiting factor. It is found by experiment that the yield does not increase remarkable by application of additional quantity of fertilizers. So, it is necessary to apply fertilizer on the basis of nutrient content of a soil and it is estimated by soil testing.

Causes of decreasing the fertility*:* Soil is the store house of plant nutrients. Losses of plant nutrients from the soil are the main causes of decreasing the fertility of soil. Plant nutrient are lost from the soil by the following ways.

(i) Removal of plant nutrients by harvested crop: Plant absorbs nutrients from the soil and stores them in their different parts. The crops remove large quantity of N and K and relatively small quantity of P. Four to five percent of total N is lost from soil per acre annually through the harvested crops. The loss can be reduced by adding farm waste materials to the soil.

(ii) Removal of plant nutrients by weeds: Weeds complete with crops for mineral nutrients. Competition begins when the supply of plant nutrients falls below the requirements of both weeds and crops. Weeds by nature of them grow fast and remove the plant nutrients from the soil,

(iii) Losses of nutrients by soil erosion*:* Erosion is the physical removal of top soil by water and wind. Plant nutrients, particularly N remains on the upper layer of soil. When erosion is severe, the nutrient is lost along with soil and the fertility of soil decreases accordingly.

(iv) Losses of nutrients by soil erosion: Fertilizers both straight and mixed are soluble in water and as such hay are liable to loss by leaching in rain water or irrigation water. Leaching loss is more acute in sandy soil and bare soils. N is mainly lost from soil by leaching.

(v)Losses of nutrients by gaseous form: N is generally subjected to loss in gaseous form .Losses of N occur due to denitrification.

Factor affecting soil fertility:

The factors affecting soil fertility may be of two types (A) Natural factor (B) Artificial factor. The natural factors are related to the proper use of land. The factors affecting the fertility of soil are as follows.

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Parent Materials: The property of soil depends on the property of parent rock. If the parent rock contains more nutrients, the soil developed from rock contains more nutrients. The soil developed from calcareous rock contains more P than the soil which is developed from granite rock.

Climate and vegetation: Rainfall and temperature has an effect on soil fertility. In heavy rainfall areas, the nutrients are lost by leaching. As result of which the fertility of that soil becomes low. Besides these, the upper layer is eroded which decreases the soil fertility.

Topography: The fertility of soil is also dependent on the topography of soil. Leaching and erosion is most common in sloppy land. As a result of which, the fertility of that soil becomes low.

Inherent capacity of soil to supply plant nutrient: The nutrient contents of a soil vary according to the nature of soil. The soil which contains much quantity of nutrient becomes more fertility.

Physical condition of soil: Aeration and movement of water is good in soil containing adequate amount of organic matter and this type of suitable conditions of soil is beneficial for the growth of plants. The physical condition of soil should be suitable for the growth and development of plants. It is essential for the proper supply of oxygen in the soil. An improper supply of oxygen is unsuitable for the growth of plants as well as for the proper function of soil organism. Suitable physical condition of the soil increases the water holding capacity of soil which is favorable for the growth of plant. The fertility of soil depends mostly on the texture and structure of soil.

Soil age: The soil developed earlier losses its fertility gradually because the fertility of the soil decreases by the process of leaching and weathering in course of time. Besides this, cultivation of crops without manuring decreases the fertility of soil.

Micro-organism and soil fertility: various types of organism live in the soil. The soil organism brings the unavailable nutrients into the available form.

Availability of plant nutrients: The nutrient of the soil must be in the available form of plants. The plant does not absorb nutrients if it is not suitable in water. Super phosphate applied in acid soil is converted into iron or aluminium phosphate which is not soluble in water. As a result, P remains in the soil in unavailable form to plant.

Soil composition and fertility: The plant absorbs the nutrients from the soil. The nutrient of minerals becomes available by weathering. The soil containing more organic matter becomes more fertile. The sandy soil is less fertile, where as loamy soil is more fertile.

Organic matter and soil fertility: The fertility of soil increases if the soil contains more organic matter. Organic matter contains plant nutrients. Besides this, OM improves the physical condition of soil. Decomposition of OM increases the N content of soil.

Soil erosion*:* erosion is the physical removal of top soil by water and wind. As such it decreases the fertility of soil because the nutrients remaining in upper layer of soil is lost by erosion and the fertility of soil decreases accordingly.

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Cropping system*:* Cultivation of same crop year after year in the same field decreases the fertility of soil. Crop rotation increases the fertility of soil.

Favorable environment for root growth*:* The suitable condition for growth of plant depends on physical, chemical and biological condition of soil. Soil contains 25 % water and 25 % air by its volume and this condition is favourable for good aeration. The bad aeration in the soil is not good for the growth of the soil.

Management of soil fertility:

Efficient nutrients management is the prime concern in the management of optimum soil fertility. This has received the maximum attention n during the last four decades as is evident from the fertilizer consumption pattern and its contribution in increasing crop production. During the early stages, the increase in food grain production was mainly due to increase in area of cultivation but after 1970-71,it has been largely due to increase in the productivity. In a successful management of soil fertility following aspects need consideration:

- Optimum fertilizer rates
- Balanced use of fertilizer nutrients
- Efficient use of fertilizer
- Nutrient needs of cropping system and
- Integrated nutrient management

Future prospects

India's foodgrain requirement to feed the estimated population of 1 400 million by 2025 will be 300 million tonnes (based on rice, i.e. unhusked paddy rice). There will be a corresponding increase in requirement of other crops such as cotton, sugarcane, fruits and vegetables. The country will require about 45 million tonne of nutrients (30 million tonnes for foodgrains and 15 million tonnes of nutrients for other crops) from various sources of plant nutrients, i.e. fertilizers, organic manures and biofertilizers. The further increase in crop production will have to come from an increase in yields as there is limited scope for increasing cultivated area. The yields of the majority of the crops are relatively low and there is great potential for increasing them through the increased use of inputs such as fertilizers. Fertilizer use will remain key to the future development of agriculture. The handling of increasing quantities of fertilizers will put pressure on storage and handling facilities and transport. Products and practices that improve fertilizer-use efficiency will need special encouragement. Fertilizer promotion will have to include activities that promote not only increased rates of use but also better balances between the nutrients and higher efficiency. Attention also needs to focus on the availability of credit, an essential factor in ensuring the availability of fertilizers to farmers. India will continue to be a major importer of raw materials, intermediates as well as finished products. The fertilizer product pattern is unlikely to change in the near future, and urea and DAP will continue to dominate fertilizer production. Attention will need to focus on ensuring the availability of good-quality micronutrient fertilizers.

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Trade in fertilizers since 2005

Source- WTO estimates based on data from UN comtrade (2005-2022)

Optimum Fertilizer Rates:

The amount of fertilizer needed by a soil depends upon its nutrient supplying capacity to crop and the requirement of crop. N is the most limiting factor for crop production in Indian soils because of their poor organic carbon content. The response of crops to N is universal in almost all soils of India, except in hilly region soils containing high OM. The chemistry of applied fertilizers is different for upland crops and submerged rice culture. The low efficiency of N utilization in rice is a matter of concern because rice alone accounts for about 40% of the total fertilizer N consumption in India. Rice, wheat and maize generally respond significantly up to 120kg N /ha through at some places responses up to 150-180 kg N /ha have also been reported (Narang and Bhandari, 1992). A leguminous crop generally requires a starter dose of about 10 kg N /ha as a bulk of the requirement is met through fixation of atmospheric N.

Deficiency of P is also widespread in Indian soils. Soil testing low and medium in P requires the application of P for optimum yields. Wheat crop is highly responsive to P application. In general, it respond s to P applied up to 60 kg P2O5 /ha while in mixed black and red and yellow soils, its response to P continue up to 120 kg P2O5 /ha. Similarly, rice continues ponds generally up to 60 kg P_2O_5 /ha. However, in medium black soil very low in Available P .it responds up to 90 kg P_2O_5 /ha. Response to K application on crops is modified by the amount of reserve K and several other factors.

Crop response to K is large on laterites, red and yellow and mixed red and black soils. Sulphur deficiency is also becoming wide spreading in different soils of India. Differential responses to sulphur application of different crops has been reported. In Punjab and erstwhile UP, 20 kg S /ha to groundnut, linseed, pigeon pea and sorghum, 30 Kg S /ha to ground nut, wheat chickpea, black gram and maize and 40 kg S /ha to green gram and lentil corrected the

S deficiency in crops. In black soils, 30 kg S /ha to green gram, soybean and 50 kg S /ha to potato and 20 kg S /ha to ground nut efficiently corrected S deficiency.

Efficient Use of Fertilizer

Increasing nutrient efficiency is the key to the management of soil fertility. The proportion of the added fertilizer actually used by plants is a measure of fertilizer efficiency. Soil characteristics, crop characteristics and fertilizer management techniques are the major factor that determines fertilizer efficiency.

Soil characteristics

Nutrients status of soil- The response of any crop or a cropping system to added nutrient depends largely upon the inherent capacity of soil to supply that nutrient as per the requirement of crop. Chemical tests have long been used to estimate the nutrient availability in soils to predict the probability of obtaining a profitable response to applied nutrients. On the basis of soil testing, soils are rated, low medium and high in plant nutrient and suitable fertilizer amounts are recommended. In a low nutrient soil, the crop responds remarkably to its application. On the other hand, in a high nutrient soil, the crops may show little or no response. In medium test soil, the response is intermediate. Soil testing helps in adjusting the amount of fertilizer and thus, improves the efficiency of fertilizer use. By demarcating the areas responding differently to different plant nutrients, the right type and proper amount of fertilizer can be applied to them.

Nutrient losses and transformations- The amounts of nutrients estimated by soil tests may not be entirely available to plants because of their leaching, volatilization, denitrification and transformations to unavailable forms. Leaching losses are important for nitrate nitrogen because it is not held by exchange sites in the soil, it is lost. Such losses are of particular significance in sandy soils and in situations if heavy rain or irrigation follows its application. In acid soil, leaching losses of Ca ,sulphate, potassium and magnesium are more common.

Soil organic matter: Soil organic matter content is generally considered as the index of soil fertility and sustainability of agricultural system. It improves the physical and biological properties of soil, protects soil surface from erosion and provides a reservoir of plant nutrients. In the tropics the maintenance of soil organic matter is very difficult because of its rapid decomposition under high temperatures. The cultivation of soils generally decreases its organic carbon content because of its increased rate of decomposition by the current agriculture practices. In cultivated soils, prevalent cropping systems and associated cultural practices influence the level at which organic matter would stabilize in a particular agro-eco-system. LTFEs have shown that the integrated use of organic manures and chemical fertilizers can maintain high productivity and sustainable crop production. Recent studies have indicated that periodic addition of large quantity of crop residue to the soil maintains the N and OM at adequate level even without using legumes in the rotation. The application of FYM, compost and cereal residues effectively maintains the SOM due to incorporation of rice or wheat straw into the soil instead of removing or burning it. Yields are, however, low in residue incorporated treatments due to wide C: N ratio of the residues. This ill effect, however, can be avoidable at least 20 days before seeding wheat.

Soil moisture- Fertilizer application facilitates root extension into deeper layers and leads to greater root proliferation in the root zone. Irrigated wheat fertilized with N used 20-38 mm more water than the unfertilized crop on loamy sand and sandy loam soils and increased dry matter production (Gajri *et al*., 1989). Soil moisture also affects root growth and plant nutrient absorption. Nutrient absorption is affected directly by soil moisture and indirectly by the effect of water on metabolic activities of plant, soil aeration and concentration of soil solution. If soil moisture becomes a limiting factor during critical stage of crop growth, fertilizer application may adversely affect the yield. Fertilizer–use efficiency also depends on a good moisture supply.

Physical condition of soil-Despite adequate nutrient supply, unfavorable physical conditions resulting from a combination of the size, shape, arrangement and mineral composition of the soil particles may lead to poor crop growth and activity of microorganism. Soil N generally increases as the texture become finer. The basic requirements for crop growth in terms of physical conditions of soil are adequate soil moisture and aeration, optimum soil temperature and freedom from mechanical stress. Tillage, mulching, irrigation, incorporation of OM and other amendments like liming of acid soils and addition of gypsum to sodic soils are the major field management techniques that aim at creating soil physical environment suitable for crop growth. Tillage affects water use by crops not only through its effect on root growth but also affects the hydrological properties of soils. Mulching with residues, plastic film etc. influences evaporation losses from soil by modifying the hydro-thermal regime of the soil and affects root growth and rooting pattern. Use of organic mulch also decreases maximum soil temperature in summer and minimum temperature in winter and helps in the conservation of soil moisture.

Nutrient uptake-The total amount of nutrients removed by a crop may not serve as an accurate guide for fertilizer recommendations; it does indicate the differences in their requirements among crops and the rate at which the nutrient reserves in the soil are being depleted. The nutrient uptake may vary depending upon the crops and its cultivars, nutrients level in the soil, soil type, soil and climatic conditions, plant population and management practices. It is estimated that 8 tons of rice grain remove 160 kgN,38 kgP, 224 kg K,24 kg S and 320 g Zn as compared to a removal of 125 kg N,20 kg P, 125kg K, 23 kg S and 280 g Zn by 5 tons of wheat from one hectare field.

Crop rotation-The nature of cropping sequence has profound effect on the fertilizer requirement and its efficiency. The crops requiring high levels of fertilizers such as maize potato may not use the applied fertilizer fully and some amount of the nutrient may be left in the soil which can be utilized by the succeeding crop. P among the major nutrients, is worthy of consideration because less than 20% of the applied phosphatic fertilizer is utilized by the first crop. Similarly, less than 3 % of the applied Zn is used by the first crop. Crops have a tendency of luxury consumption of N and K and may not leave any residual effects unless doses in excess of the crop requirement are applied. On the other hand if sub optimal doses of fertilizers are applied to a crop, they may leave the soil in a much exhausted condition and the fertilizer requirement of the succeeding crop may increase. The legumes leave N rich root residues in the soil for the succeeding crop and thus, reduce its N requirement.

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Fertilizer characteristics and management techniques-The efficiency of a fertilizer varies to a large extent on the type of fertilizer, time of fertilizer application and method of application.

Type of fertilizer- fertilizers differ in both their content and form .In the case of nitrogenous fertilizer, the nutrient may be present in ammonium, nitrate or amide form. Similarly, in case of phosphatic fertilizers, Phosphorus may be present in water soluble, citrate soluble or citrate insoluble form. The nutrient content of a fertilizer mat also differs largely. Thus, the total N content of CAN is 25 % as compared to 46 5 of urea.20.5 % of AS and 82 % of anhydrous ammonia. Nitrogen may also be present as one of the components of the combined fertilizer sources such as 18 % in DAP and 13 % in $KNo_{3.}$ Similarly, SSP will supply only 16 % P_2O_5 as compared to 46 % P2O5 supplied by DAP and triple super phosphate. The K_2O content of KCI is 60 %. The fertilizers may also differ in their water solubility and their ability to get fixed in the soil. Nitrates fertilizers have high water solubility and are subject to leaching in high rainfall areas or due to heavy irrigation, as these do not interact with the soil constituents on their addition to soil and thus, move to greater depths. Generally, all sources of fertilizer N have been shown to be equally efficient in upland soils, but ammoniacal and amide forms of N are more efficient as compared to nitrate source of N for submerged rice soils. Phosphatic fertilizers containing phosphorus in water soluble form like DAP, SSP have been found superior for most of the crops in natural or alkaline soils as compared to citrate soluble or citrate insoluble form. Both SSP and DAP proved equally efficient on the basis of equivalent Phosphate content but SSP has the advantage in soils low in available S and particularly for S loving crops like oilseed and pulses. Rock phosphate which has phosphate in water insoluble form has proved useful in acid soils, rice or for long duration legumes. Nitro phosphate with more than 60% water soluble P have shown promises as efficiently sources of P in alkaline soils. Most of the fertilizer K used in Indian agriculture is imported largely in the form of MOP and only about 1% as SOP. In highly leached acid soils where sulphate deficiency is expected, potassium sulphate should be preferred. For certain crops like tobacco which is chloride sensitive, potassium sulphate should be applied. Straight micro-nutrients carriers like zinc sulphate, ferrous sulphate manganese sulphate and copper sulphate have been found to be superior and economical as compared to other sources of micro-nutrients.

Time of application-Time of fertilizer application is important particularly for those nitrogenous fertilizers which tend to leach with irrigation water or rainfall. Split application of nitrogen is the most common and widely accepted practice for almost all crops. The basic concept is to apply N in two or more splits to coincide with peak period of N requirements of the crop. N application in splits doses is particularly beneficial on light textured soils in increasing the efficiency of applied N. Its application in three split doses at transplanting, tillering and panicle initiation in rice at planting, knee high stage and tasseling stage in case of maize, is usually recommended, In general, crops grown in rainy season should receive N fertilizers in split doses so that the leaching of the nutrient during heavy rains may be avoided and an adequate supply of the nutrient at critical stages is ensured For crops, like wheat, barley brassica sp, which are shown in winter when the rains are scanty, best result are obtained when the entire doses of N is applied as a basal doses in medium to heavy textured soils and on light soils in two equal splits at sowing and at first irrigation. Recent studies have shown that N applied in the form of urea at pre-sowing irrigation even to a loamy sand soil gives higher grain yield of wheat as compared to its application at preparatory tillage and increases both nitrogen-use efficiency (7-34%) and water use efficiency (2-24%) at different nitrogen and irrigation levels (Sidhu *et al*.,1994)

In the case of phosphatic and potassic fertilizers as well as for zinc and copper among micro-nutrient cations, all the quantity applied at sowing gives the best results with most of the crops. However, in the case of iron and manganese where foliar sprays of these nutrients are recommended, initiation of foliar sprays is very important. In the case of manganese, foliar sprays initiated 2-3 days before the first irrigation to wheat have been found to be significantly superior to their application after the first irrigation (Takkar *et al*., 1986). Similarly, foliar application of iron should be initiated as soon as the symptoms of its deficiency appear on crops.

Method of application-Various techniques have been developed to improve the efficiency of applied nutrients. Since, nitrates are easily leached and lost by denitrification, retardation on nitrification of ammonium containing or ammonium producing fertilizers by using nitrification inhibitors in rice fields subjected to intermitted flooding helped in increasing the nitrogen-use efficiency. Chemical nitrification inhibitors as well as indigenous materials such as Neem (*Azadirachta indica*) and karanj (*Pongamia glabra*) cakes have been successfully used except on highly coarse textured soils of Punjab. Soil application of ferrous sulphate in coarse textured soil for rice is less efficient than foliar sprays. However, for upland crops, the soil applied iron has been found to be as effective as foliar sprays.

Integrated Nutrient Management

Integrated nutrient management envisages the use of chemical fertilizer in conjunction with organic manures, legumes in cropping system, biofertilizers and other locally available nutrient sources for sustainable soil health and productivity. The combined application of organic manures and chemical fertilizers generally produces higher crop yields than when each is applied alone. This increase in crop productivity may be due to the combined effect of nutrient supply, synergism and improvement in soil physical and biological properties. FYM constituents an important component of INM for maintaining soil fertility and yield stability. Green manure is a valuable potential source of N and organic matter. *Susbania* sunnhemp and cowpea incorporated before transplanting rice partially meet the nutrients requirement of the crops. In uplands crops, the most efficient time of incorporation of green manure crops has been found to be 15 days before seeding. Besides being a source of P, K, S and micro-nutrients, green manure influences availability of the nutrients through it favourable impact on oxidation-reduction regimes, pH and increased chelation capacity. In permeable coarse-texture soils, green manuring helps correcting the iron chlorosis in rice. The green manuring plays an important role in the reclamation of salt- affected soils and improvement in physical and biological properties of soils.

Evaluation of soil fertility

Evaluation of soil fertility consists of estimating the nutrient-supplying power of a soil. A proper evaluation of the fertility of a soil before planting a crop helps in adopting

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appropriate measures to make up for the short comings and ensuring good crop production. These methods for evaluating soil fertility may be biological or chemical.

Biological Methods:

The biological methods consist of raising a crop or a microbial culture in a field or in a sample of the soil and estimating its fertility from the volume of crop or microbial count. Although these methods are direct estimates of soil fertility, they are time consuming and, therefore, not well adapted to the practice of soil testing. On the other hand, chemical testing of a soil is reasonable rapid and is therefore more handy for advisory work on a mass scale.

Chemical Methods:

The chemical methods for evaluating the soil fertility consist of analyzing a sample of the soil for its content of essential plant nutrients which are expected to be in relatively short supply and whose deficiencies can be corrected by appropriate additions of suitable fertilizers. The approach to analyze a soil for the sample with strong acids or alkalis was given up when it was found to have a poor relationship with plant available quantities of the nutrient. The usual practice today is to obtain an extract from soil by adding to it a small volume of a solution containing mild chemical reagents. Soil is air dried and ground to pass a 2-mm sieve. The nutrient concentration is measured in an aliquot of the extract and results are expressed in terms of the quantity of that nutrient on a per unit mass or volume basis.

Interpretation:

The interpretation of soil test results requires a lot of background information and much intuitive thinking. It is useful to know the variation in soils with respect to pH, OM and soil texture and special problems like water logging or sodicity. Ordinarily there is a greater probability of obtaining a profitable response from fertilization on soils testing low in a nutrients element than soils testing high in that element. However, when difference in any one or more of the aforesaid soil attributes interpretations of soil test results becomes increasingly complex. One way of interpreting the soil test results is to classify them into low, medium and high categories. Such a classification is based on the observation, as already stated, that a low testing soil in the absence of an applied nutrient, produces a very poor crop and when a small amount of that nutrient is added to it, the resultant increase in yield is much more in this soil than what would come about from applying an identical amount of the nutrient to a soil which is high or even medium in its native supply.

Calibration:

The work of determining the calibre of a soil test is known as Calibration. The calibration of soil tests can be represented by a linear equation:

$Y = a + bx$,

where, Y is the yield of a nutrient X or a crop yield, X is the nutrient element whose deficiency is corrected by applying a fertilizer, and b is the yield increase per unit quantity of the deficient nutrient. Depending upon the size of the experiment, more than one nutrients and soil test estimates from more than one soil depths can be related with crop yield in a single multiple regression equation.

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Targeting Crop Yields-Considering the crop yield to be continuous function of plant nutrient supply in the growth medium, calibrations were obtained for different levels of soil fertility. The procedure of creating different levels of fertility artificially in adjacent plots was adopted to ensure homogeneity in soil management and weather whose diversity in experiments performed at different locations and in different seasons usually leads to poor correlations. From such experiments, the nutrients requirement (X) is estimated using following equation:

$$X_f = \frac{X_t\ Y - X_o X_s}{X_r}$$

Where,

X_f = the fertilizer nutrient (kg/ha) to attain the target yield,

X_t = the amount of nutrient (in kg) in the grain and straw of the fertilized crop per mega gram of grain,

Y = the target grain yield in mega grams per hectare,

X_o = the amount of nutrient (in kg) in the grain and straw of the unfertilized crop per hectare per kilo gram of soil test nutrient per hectare,

X_s = the soil test nutrients in the unfertilized control in kilograms per hectare.

X_r = the fractional recovery of fertilizer nutrient in grain and straw, averaged over treatments in the calibration experiments with different quantities of fertilizer nutrients.

Critical values are especially useful when public administration has high concern for increasing crop production in areas producing generally low to moderate yields and adequate profitability of a costly input is vital to the propagation of technology for improved agriculture A set of critical soil test values for use by soil testing laboratories in India as suggested by Muhr *et al*. (1965) are given in Table 1.

Table 1. Critical soil test values for available N, P, and K different

Nutrient	Low	Medium	High
1. Organic carbon (%)	>0.5	0.5-0.75	<0.75
2. Available N (kg/ha)	>280	280-560	<560
2. Olsen's P (P_2O_5 kg/ha)	>22	22-56	<56
3. NH_4Ac-K (K_2O kg/ha)	>110	110-280	<280

(Source: Muhr *et al*., 1965)

Soil test summaries:

Soil testing provides sound information about the fertility and productivity of the soils. This enables the farmer to make the most profitable use of some of the costly inputs in farming. However, neither all farmers have the information or inclination to g*et al*l their soils tested nor is the soil testing service adequately equipped to perform this job on all the millions of fields across the length and breadth of the country. Hence, there is a need to

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compile soil wise and crop wise soil test summaries for all important agro-eco- regions, where already, there is sufficient use of fertilizers or where higher demands in the future are anticipation. It is necessary to have a sufficient number of soil samples as well as representing sampling in order to ensure correct assessment of the prevalent situation. The soil test results are depicted in either of the two ways:

1. Categorizing areas having more than 50% ,25-50 or less than 25% nutrient-deficient soils and

2. Nutrient index value calculated from the proportion of soils under low, medium and high available nutrient categories ,as per represented by expression.

$$NIV = \frac{N_l + 2N_m + 3N_h}{N_l + N_m + N_h}$$

Where, NIV is nutrient index value,N1,Nm and Nh are the number of soil samples falling in the category of low, medium and high nutrient status and are given weight age of 1,2 and 3 respectively.

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Chapter- 6

Physiological Disorder and Post-harvest Management in Cut Flowers

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Introduction

Floriculture is one of the important commercial trades in agriculture as the demand for flowers continues to increase. The production of flowers is estimated at 2151.96 thousand tonnes of loose flowers and 828.09 thousand tonnes of cut flowers in 2020-21. The country has exported 23,597.17MT of floriculture products to the world. 771.41 Crores/ 103.47 USD Millions in 2021-22. The cut flowers production during 2015-16 (5,28,000MT), 2016-17(6,93,000 MT) and 2017-18 (8,23,000 MT)

Good potentialities exist for cultivation of flowering plants. Increasing trends in area and production of flowers has been observed since 2003-04 onwards. In addition to the beautification of the local landscape, great scope exists for export of flowers; and floriculture is important for bee-keeping industry which too provides an alternate source of income to the Indian farmers. The highest production of Flowers was recorded in Tamil Nadu (482.52 Thousand Tonnes) followed by Andhra Pradesh (428.95 Thousand Tonnes)

A flowering crop should also be grown free from toxic wastes, gases, factories that damage leaves and flowers. Flowers damaged by pathogens, insects and mites show high ethylene production, resulting in poor vase-life. Exporters must plan and monitor effective quality control measures from production to post-harvest, storage and transportation.

Table 1. State-wise Area and Production of cut flowers for 2017-18

S. No	State	Production in '000 MT
1	Andhra Pradesh	0.0
2	Arunachal Pradesh	0.0
3	Assam	56.1
4	Bihar	0.1
5	Chhatisgarh	179.6
6	Gujarat	0.0
7	Haryana	4.5

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8	Himachal Pradesh	10.6
9	Jammu & Kashmir	0.4
10	Jharkhand	8.6
11	Karnataka	92.9
12	Kerala	28.2
13	Madhya Pradesh	37.2
14	Maharashtra	57.0
15	Manipur	0.0
16	Meghalaya	0.3
17	Mizoram	2.5
18	Nagaland	8.8
19	Odisha	48.8
20	Punjab	0.0
21	Rajasthan	0.0
22	Sikkim	0.1
23	Tamil Nadu	0.0
24	Telangana	5.2
25	Tripura	0.0
26	Uttar Pradesh	65.2
27	Uttarakhand	11.7
28	West Bengal	204.5
29	Meghalaya	0.5
	Total	**822.7**

Cut Rose

Botanical name : *Rosa spp.* **Family** : Rosaceae

1. Bent neck

Insufficient flower stem hardening or insufficient maturation of the stem tissues below the harvested flower results in stem collapse. The reasons are appearance of plugging materials like pectin, cellulose and microbes, extreme temperatures during shipping or storage and water deficiency in the neck tissues. Premature harvesting and excessive water loss also cause bent neck.

2. Bull head or malformed flowers

The central petals of the bud are only partly developed and the bud appears flat. The causes are lack of carbohydrates for petal development, thrips infestation and low temperature during shipping or storage.

3. Blind wood

Flowers have sepals and petals, but the reproductive parts are absent or aborted. Blind wood is generally short and thin. But it may attain considerable length and thickness when it

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develops at the top of the plant. The causes are low temperature and insufficient light during growth and development, insect pests and fungal diseases.

4. Blackening of petals

The major cause is low temperature (20^{0}C at day and 4^0 C at night).

5. Flower bud abortion

No flower bud initiation occurs or if at all it starts, it does not proceed beyond the initiation of pistils and stamen primordials. The causes are competition among growing shoots for assimilates, deficiency of Boron, night temperatures below 15^{0}C and day temperatures above 28^{0}C. Control measures include application of CCC @ 500 ppm and GA @ 100 ppm or application of Boron @ 30 to 60 ppm.

Cut Chrysanthemum

Botanical name : *Dendranthema grandiflora* (Syn: *Chrysanthemum morifolium)* **Family** : Compositae

1. Leaf yellowing

Chrysanthemum flowers are not affected by ethylene, nor do they produce it. However, exposure to ethylene may accelerate leaf yellowing.

2. Freezing injury

Freezing will occur at temperatures below -1^{0}C. Symptoms include water soaking and collapse of leaves and petals.

3. Floral abnormalities

The common flower abnormality in chrysanthemums is the crown bud, wherein the bud development is severely retarded and the involucral bracts become glossy and enlarged. The plants which are grown in long days after receiving a few short days produce crown buds. Low night temperature (<15^{0}C) during flower development causes pink colouration on white petals. Quilling of florets occurs at low light intensity during flower development and at lower night temperatures. Delayed or no floral induction in chrysanthemum is observed with too long photoperiods and low night temperatures and also due to copper and manganese deficiency. This can be controlled by the application of Manganese sulphate (3 g/l) and Copper sulphate (1 g/l) applied at vegetative, bud initiation and flowering stages.

Many of the abnormalities commonly occur when the period of floret formation coincides with unfavourable conditions. In long days and high temperature, bracts may appear on the receptacle. Sometimes transfer of plants to long days at the period of floret formation encourages the production of more ray florets and so enhances doubleness, while short days enhance singleness. Bleaching of petals may occur due to too high temperature during floral development.

4. Quilling of florets

Petals get twisted and become cup shaped. The peripheral florets lose turgidity. This is due to boron deficiency and low night temperature (<15^{0}C) and can be controlled by applying Borax @ 3g/l during the vegetative, bud initiation and flowering stages.

5. Bleaching of petals

Day temperature above 40^{0}C and night temperature below 15^{0}C causes bleaching of petals.

Carnation

Botanical name : *Dianthus caryophyllus* **Family** : Caryophyllaceae

Calyx splitting

Calyx splitting is an important disorder in carnation, which has been associated with many factors like genetic, environmental, nutritional and other cultural practices. The cultivars with short and broad calyx are more susceptible than the ones with long and narrow calyx. Irregular or fluctuating temperature during flowering also induces calyx splitting. Low temperature below 10°C leads to the development of an extra whorl of petals inside the calyx. The calyx unable to hold these extra growing petals, splits up. Nutritional make up of plants also influences calyx splitting. Low nitrogen, high ammoniacal nitrogen or low boron levels enhance calyx splitting. Closer spacing has also been reported to encourage calyx splitting.

Selection of cultivars that are less prone to calyx splitting, regulation of day (20-25°C) and night (12.5-15.5°C) temperatures and maintenance of optimal levels of nitrogen (25-40 ppm) and boron (20-25 ppm) in the growing medium can minimize this disorder. Spraying borax @ 0.1% at fortnightly intervals will reduce the disorder. Calyx splitting can be reduced by placing a rubber band around the calyx of the flower which has started opening. Foliar application of 0.1 % borax can control calyx splitting. Some varieties such as Espana, Cabaret, Red Corso, Pamir and Raggio-di-Sole are less prone to calyx splitting.

Sleepiness

Sleepiness causes huge post-harvest losses in cut carnation. It occurs due to exposure of flowers to ethylene or water stress. Also, the incidence of sleepiness has been found to be higher when the flowers are stored for a longer period or when they are exposed to high temperature. Spraying of STS 0.4 mM before harvesting the flowers can correct this disorder.

Grassiness

Grassiness refers to failure of plants to produce flowers. This is a genetic disorder which varies from variety to variety. Removal and destruction of affected plants is the only way of correcting this disorder.

Slabside

This disorder refers to uneven opening of flower buds resulting in the petals protruding on one side only, giving an asymmetrical and lopsided shape to the flower. It is

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common during cooler periods. This can be overcome by gradually increasing the temperature to optimum level.

Calyx tip die back

Potassium deficiency and water stress cause tip die back. The disorder commences with browning of the calyx tip and it progresses downwards damaging a major part of the calyx. This disorder is often followed by occurrence of secondary fungal infection which makes the flower unmarketable. Spraying of potassium chloride @ 5g/l two times at 10 days intervals and providing adequate water @ 4.5 l/m^2 can minimize this disorder.

Internode splitting

Splitting of internodes affects the quality of cut flowers. Splitting is due to boron deficiency. Application of borax @ 2g/ m^2 will correct internode splitting.

<u>Gerbera</u>

Botanical name : *Gerbera jamesonii* **Family** : Compositae

1. Flower bending

It is caused by loss of cell turgidity and also by calcium deficiency. It can be controlled by application of Calcium Nitrate @ 0.2%.

2. Double-faced flower

It is caused by imbalance of nutrients and excessive vegetative growth while the flower buds are very small. Maintaining nutrient balance helps to control this problem.

3. Non-uniform flower blooming

It is caused by physical injury to flower stem, pest damage and phytotoxicity. Avoiding application of excess fertilizers can minimize this problem.

4. Short stems

It is caused by high salinity level, moisture stress and low soil temperature. It is controlled by maintaining moisture status in the soil.

<u>Anthurium</u>

Botanical name : *Anthurium sp.* **Family** : Araceae

1. Colour breakdown of spathe

This can be recognized by colour breaks, which may or may not be accompanied by distortion of the spathe. Calcium deficiency is one of the major causes for this disorder. It can be controlled by application of Calcium nitrate @ 25 g/m^2 or lime @ 5 g/plant/month

2. Sticking

Flowers do not open properly and the spathe is stuck. This disorder is variety dependent. Low relative humidity is the cause. During the early stage, the flowers are loosened by hand.

3. Jamming

Flowers get jammed in the sheath since the leaf is wound too tightly around the flower. Cracks develop on the underside of the spathe. It is variety dependent and occurs during arid conditions. It also occurs more frequently in cultivars with long sheaths. Humidifying the top layer of the substrate is recommended to provide a favourable micro-climate.

4. Folded ears

The suspected cause is an environmental-physiological interaction. Temperature below 15^{0}C causes this disorder.

5. Sun burn

Overall fading of the spathe colour or browning of the spadix occurs which is caused by direct sunlight. This can be avoided by efficient management of light.

6. Crooked stem

The suspected cause is an environmental-physiological interaction involving excessive nitrogen feeding and fluctuating wet and dry conditions. Heavy shade levels and lack of pruning may also contribute to crooked stems.

7. Deformed spathes

The suspected cause is an environmental-physiological interaction. The spathe may be wrinkled or curled. Deformed spathes can be caused physiologically by physical and chemical damage early in the development of flowers.

8. Bleached flowers

Impaired colour development occurs in the lobe area of the spathe and in severe cases the entire flower including the spadix may show the signs of insufficient colour development, stunting, distortion and necrosis. The suspected cause of bleaching is an environmental-physiological interaction. It can be controlled by preventing excessive application of ammonium nitrogen, excessive salt build up and temperature fluctuations.

9. Vog spotting

Purple spots that later turn whitish appear randomly on the flower spathe resulting from sulphur dioxide gas emitted during volcanic eruptions and taken in through stomata, causing a localized internal burn.

10. Phytotoxicity

Incorrect fertilizer, sticker or pesticide application rates or methods can cause necrotic or distorted areas on the spathe.

<u>Orchids</u>

Family	**:**	Orchidaceae
Important	**:**	*Dendrobium, Phalaenopsis, Cattleya, Oncidium, Arachnis,*

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genera *Cymbidium, Vanda*

1. Sun burn

Excessive light of above 2400 to 3600 foot candles causes sunburn. It can be controlled by effective shade management.

2. Wilting of floral parts

Rapid temperature changes and high temperature combined with dry air causes wilting of floral parts. Good ventilation can alleviate this problem.

3. Water stress

Water stress causes wilting of *Cymbidium* and *Phalaenopsis*. Low rate of transpiration associated with low uptake of water are major factors involved in such problems.

<u>Gladiolus</u>

Botanical name : *Gladiolus sp.* **Family** : Iridaceae

1. Geotropic bending of spikes

The tips of gladiolus spikes show tendency to bend against gravity if placed horizontally for longer periods. The season is accumulation of IAA on the lower portion of spike which causes asymmetrical elongation of cells. Harvested spikes should always be placed vertically and not horizontally.

2. Tip burn

Discoloration and drying up of leaf tips occurs. High levels of aerial fluorides in the atmosphere is a major cause. Spray of Blitox 50 WP(0.3%) at the initiation of the symptom can control the disorder.

3. Blindness

It results in complete absence of spikes. Zinc deficiency is one of the major causes and it can be corrected by application of Zinc @ 20-100 ppm.

4. Topple

The collapse of a small portion of the internode just beneath the flower occurs and it is called 'sugar stem' or 'wet stem'. It occurs due to Calcium deficiency and it can be connected by application of Calcium nitrate.

Post-Harvest Management

Pre cooling

- Pre-cooling referred to subjecting flowers under cold storage conditions immediately after harvest to brings down the respiration rate and field heat and ultimately to enhance the vase life and quality of cut flowers.
- The pre-cooling temperature varies with the species and cultivars.

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There are seven principal methods of pre-cooling fresh produce:

1) Room cooling, 2). Forced-air cooling 3).Hydro-cooling 4).Ice cooling
2) Vacuum cooling 6). Cryogenic cooling 7)Evaporative cooling

Room cooling and Cold Wall

The simplest method of cooling cut flowers is to stand them in buckets of preservative solution in a cold room.A permanent false wall or air plenum contains an exhaust fan that draws air from the room and directs it over the cooling surface

Vacuum Cooling

Vacuum precooling is based on the principle that water evaporates spontaneously under low vacuum conditions.

Pre-cooling temperature for certain flowers

S. No	Crop	Pre-cooling temp. (^{0}C)	Crop	Pre-cooling temp. (^{0}C)
1.	Rose	1-3	Carnation	0.5-1
2.	Anthurium	13	Chrysanthemum	0.5-4
3.	Gerbera	4	Cymbidium	0.5-4
4.	Dendrobium	5-7	Gladiolus	4-5
5.	Carnation		Bird of Paradise	7-8

Special Treatments

Special treatments include Pulsing, Impregnation, Bud opening, Conditioning for extend the vase life of flowers

Pulsing

The loading of flowers with high concentration of sucrose and anti-ethylene compound for a short duration is known as pulsing.

Impregnation and Bud opening

The ends of stems can be impregnated with high concentration of Silver nitrate (Ag NO3) or nickel chloride (NiCl2) or cobalt chloride (CoCL2) solution for 10 minutes.

Those flowers which are harvested at stage earlier than that of commercial stage require special treatments for opening of flower are treated with some specific chemical solutions known as bud opening solutions.

Conditioning

Conditioning or hardening is a simple process where the flowers are kept standing loosely in a large container of water to restores the turgidity of cut flowers from water stress during storage and transportation.

Role of Special Treatments on Vase-life of Flowers

For evaluation of longevity, temperature of 20-23°C and relative humidity of 40 to 80%, continuous light (12 hours daily) from cool white fluorescent lamps at 1000 to 2500 lux are most suitable conditions.

Germicides used as Floral Preservative

Name of compound	Commonly used symbol	concentration range
8-Hydroxyquinoline sulfate	8-HQS	200-600 ppm
8-Hydroxyquinoline citrate	8-HQC	200-600 ppm
Silver nitrate	$AgNO_3$	10-200 ppm
Silver Thiosulfate	STS	0.2-4 mm
Thiopendazole	TBZ	5-300 ppm
Quaternary ammonium salts	QAS	5-300 ppm
Slowly releases chlorine compounds		50-400 ppm of CI
Aluminum sulfate	Al(SO4)	200-300 ppm

Packaging

It is advantageous to wrap bunches of flowers in paper or cellophane and then, place them in corrugated fiber board cartons to protect flowers against physical damage, water loss and external conditions detrimental to the flowers.

Storage

It results in orderly marketing, reduced retailer's hazards resulting from unforeseen decline for demand, anticipating holidays, improved production efficiency; elimination of greenhouse production in deep winters, saving energy and making possible long term shipment.

Storage

A. Cold storage (i. Wet storage ii. Dry storage)

B. Controlled atmospheric storage (CA)

C. Modified atmospheric storage (MA)

D. Low pressure storage (LPS) / Hypobaric storage.

A. Cold storage

The Cold storage of cut flowers facilitates the adjustment of flowers and other planting material supplies against the market demand and enables the accumulation of large quantities of flowers.

1. Wet storage

- In wet storage, flowers are stored with their base dipped in water or preservative solution for a short time.
- During wet storage, flowers are kept at 3-4oC temperature slightly higher than that used for dry storage

Flowers	Temperature (°C)	Shelf life (weeks)
Antirrhium	4	4
Carnation	4	4
Chrysanthemum	1	3
Gladiolus	0.5-5	10
Gerbera	4	3-4
Lily	0-1	6
Rose	4	4
Tulip	-0.5-0	2-3

2. Dry storage

Dry storage method is used for long term storage. In this method, fresh flowers are harvested in the morning, graded and sealed in plastic sleeves/ bags or boxes to prevent the loss of moisture.

Flowers	Temperature (°C)	Shelf life (weeks)
Anthurium	13	(weeks)
Carnation	0-1	4
Cattleya	7-10	16-24
Dendrobium	5-7	2
Gladiolus	4	2
Lily	1	4
Rose	1	6
Strelitzia	8	3
Tulip	0-1	4

B. Controlled atmospheric storage (CA)

The principles of controlled atmosphere storage for cut flowers are based on the close regulation of three parameters viz. temperature, oxygen and carbon dioxide.

Flowers	CO2 concentration (%)	O2 concentration (%)	Temperature (0C)	Shelf life (weeks)
Carnation	5	1-3	0-1	30
Freesia	10	21	1-2	20-22
Gladiolus	5	1-3	1.5	21
Lily	10-20	21	1.0	21
Rose	5-10	1-3	0	20-30
Tulip	5	21	1.0	10

C. Modified atmospheric storage (MA)

- MA storage is a less precise form of CA storage. The flowers are in sealed bags leads to reduction in O2 and increase in CO2 levels due to respiration of the tissue.
- This increased level of CO2 reduces the biosynthesis of ethylene and hence increases the flower longevity.

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D. Low pressure storage (LPS) /hypobaric storage

- In this storage method, flowers are stored under reduced pressure, low temperature and cooled moist air.

In LPS, gaseous substances like CO_2 and ethylene produced flowers through stomata and intercellular spaces much quicker under low pressure than at normal pressure.

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Chapter- 7

Nano-Grow: Harnessing the Power of Living Particles for Fertile Futures

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Abstract

Chemical fertilizers have played a crucial role in meeting the global demand for food spurred by population growth, with India being a significant consumer. However, the drawbacks of inorganic fertilizers, such as poor nutrient assimilation, environmental pollution, reduced crop yield, and biodiversity loss, necessitate a shift towards sustainable alternatives. Nanofertilizers and nano-biofertilizers, are a promising solution to address the inefficiencies associated with conventional chemical fertilizers. Nanofertilizers, characterized by controlled and gradual nutrient release through nanomaterial coatings, offer a sustainable approach to nutrient management. In comparison to traditional fertilizers, nanofertilizers demonstrate enhanced nutrient use efficiency, reduced leaching, and prolonged nutrient release, resulting in improved crop growth and yield. The study delves into the impact of nanofertilizers on soil microorganisms, acknowledging the importance of soil properties in nanoparticle behavior. Additionally, it introduces the concept of nano-biofertilizers, combining the benefits of nanotechnology and biofertilizers for optimal nutrient release and sustained plant development. The mechanism of metallic nanoparticle synthesis by microorganisms is discussed, emphasizing their role as "nanofactories" and their potential in environmentally friendly nanoparticle production. Furthermore, the responses of plants to nanobiofertilizers are examined, highlighting the synergistic effects on plant growth, stress tolerance, and crop establishment. The activation of various plant mechanisms ensures sustained soil nutrients, contributing to improved agricultural productivity and reduced environmental impact. These innovative solutions not only enhance crop productivity but also mitigate environmental risks associated with traditional fertilizers, offering a path towards more sustainable and resilient agricultural practices.

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Introduction

Chemical fertilizers play a vital role in addressing the increasing global demand for food due to population growth. India, the world's second-largest consumer, annually utilizes over 55 million tons of fertilizers. However, the reliance on inorganic fertilizers raises concerns for future agriculture due to their poor nutrient assimilation, significant nutrient loss through runoff, leading to environmental pollution, decreased crop yield, and the rise of pests, diseases, and pathogens, along with biodiversity loss. The inefficiency of commercial fertilizers is linked to imbalanced fertilization, low nutrient use efficiency, high mineral leaching, low nutrient assimilation potency, insufficient organic matter, and inadequate multi-micronutrients in the soil (FAO, 2015).

In the agricultural landscape of developing nations, nanotechnology emerges as a promising avenue. Nanoparticles, acting as triggers, activate plant defence systems during adverse conditions (Eliaspour *et al.,* 2020). These stimuli, known as nanofertilizers, offer a controlled and gradual nutrient supply to plants, potentially reducing nutrient losses through leaching and volatilization while maintaining chemical stability (Raliya *et al.,* 2017). Nanofertilizers also improve nutrient use efficiency, addressing environmental concerns and showcasing positive effects on the growth and yield of diverse crops (Kumaraswamy *et al.,*2021).

Nanofertilizers

Nanofertilizers involve nutrients coated or encapsulated with nanomaterials, enabling controlled and gradual nutrient release to meet plant requirements (Mena *et al.,*2017). Recognized as "smart fertilizers," they are increasingly considered a preferable alternative to conventional counterparts (Rameshaiah *et al.,*2015; Iavicoli *et al.,*2017). The interaction between nanomaterials and fertilizers enhances nutrient absorption by plants due to the high reactivity of nanomaterials (Prasad *et al.,*2017). Intrinsic factors like particle size and surface coatings, along with extrinsic factors such as organic matter, soil texture, and pH, influence nanoparticle efficiency. Additionally, the absorption route (roots or leaves) significantly affects their behavior, bioavailability, and uptake in crops (El-Ramady *et al.,* 2018).

Nano vs conventional fertilizers

Nanofertilizers offer significant advantages over traditional chemical fertilizers, primarily attributable to their controlled nutrient delivery system. This system, achieved through the encapsulation or cementing of nutrients with nanomaterials, enables a gradual and sustained release of nutrients in crops. This slow-release feature, distinguishing nanofertilizers, ensures a consistent, long-term nutrient supply to plants, spanning 40–50 days compared to the 4–10 days of conventional fertilizers (Chen *et al.,* 2018). Unlike conventional nutrient management, nanofertilizers reduce losses through leaching and enhance nutrient uptake efficiency, minimizing transportation and application costs (Fan, 2014). Their usage in smaller quantities prevents soil saturation with salts, a common issue with conventional fertilizers, and their adaptability allows tailored synthesis based on the specific nutrient requirements of targeted crops (Kah *et al.,* 2018).

Uptake and movement of nanoparticles in plants

Nanoparticles enter plant roots in the rhizosphere through endocytosis, carrier proteins, or plasmodesmata, while foliar spray application allows diffusion through stomata into vascular bundles. Movement within plants occurs through symplastic and apoplastic pathways, dependent on plant type and nanoparticle characteristics (Anjum *et al.,* 2019). However, reports indicate nanoparticle accumulation on primary root cell surfaces, reducing cell wall pore size and affecting root hydraulic conductivity (Martínez-Fernandez´ *et al.,* 2016). Inside the cytoplasm, nanomaterials compartmentalize in organelles, influencing metabolic pathways (Iqbal, 2019).

Effect of nanofertilizers on soil microorganisms

Nanomaterial behavior is influenced by soil properties beyond particle size, affecting interactions with microorganisms and plants. Soil pH, ionic strength, organic matter, and phosphate concentration determine nanoparticle chemical properties (Dimkpa, 2018). Lower pH may lead to dissolution, forming reactive oxygen species with detrimental effects. Nanoparticles interacting with organic matter stabilize in soil, altering surface chemistry, impacting microorganisms and plants. Microorganisms reciprocally influence nanoparticle behavior. Nanofertilizers can have varying effects on soil microorganisms, influencing the microbial community's structure and functions, depending on nanoparticle type and concentration. Research suggests titanium oxide nanoparticles at 1–100 mg/kg were non-toxic, while copper oxide, zinc oxide, and silver nanoparticles exhibited toxicity at similar concentrations (Asadishad *et al.,* 2018).

Nano-biofertilizers

A nano-biofertilizer integrates nano- and biofertilizer technologies, transforming organic fertilizer into nanoscale dimensions (1–100 nm) through specific nanomaterial coatings. By harnessing the synergies of biofertilizers and nanoparticles, nano-biofertilizers effectively enable a gradual and controlled release of nutrients, ensuring sustained delivery for optimal plant development (Thirugnanasambandan, 2019).

Microorganisms in nanoparticle synthesis

Numerous microorganisms, such as fungi, yeast, bacteria, and actinomycetes, demonstrate the ability to synthesize mineral crystals and metallic nanoparticles either within their cells or in the surrounding environment.

Bacterial-mediated synthesis of metallic nanoparticles

Culturable bacteria, resilient in challenging conditions and displaying rapid growth, are readily available (Mukherjee and Nethi, 2019). Studies highlight bacterial-mediated synthesis of metallic nanoparticles with variations in size and shape among different bacterial groups (Pathak *et al.,* 2019). Metal-resistant bacteria utilize distinctive biomolecules, facilitating internal metal ion transport to the cytoplasm. Biosorption, where positively charged metal ions adhere to negatively charged functional groups in bacterial cell walls, occurs externally (Ali *et al.,* 2020). This process involves metal binding through electrostatic interactions, ion exchange, physical adsorption, or complexation (Mukherjee and Nethi, 2019). Reductases, biomolecular agents, aid in reducing metal ions to stable nanoparticles (Ali *et al.,* 2020).

Fungal-mediated synthesis of metallic nanoparticles

Fungi, with superior metabolic capabilities, excel in the production of metallic nanoparticles, surpassing bacteria. They exhibit higher yields and efficiency, generating monodisperse metallic nanoparticles due to abundant biomolecular reducing agents. Metabolites like hydroxyquinoline and napthoquinones aid in electron transport for extracellular nanoparticle synthesis. Metal-tolerant fungi, with robust cell walls, demonstrate high bioaccumulation capacities, allowing substantial metal-binding and intracellular uptake. Similar to bacteria, various fungal types produce nanoparticles of diverse sizes and shapes extracellularly or intracellularly. While extracellular synthesis is advantageous, fungal synthesis requires longer culturing times, ranging from 24 to 120 hours, compared to bacteria (Noor *et al.,* 2020; Nasrollahzadeh *et al.,*2019; Neethu *et al.,* 2019).

Algal-mediated synthesis of metallic nanoparticles

Algae, comprising both microalgae and macroalgae, are pivotal in metallic nanoparticle synthesis due to their abundance and non-toxic reducing agents. This process involves using living or deceased microalgal biomass, or extracting biomolecules through heating, and combining them with metal salt solutions to initiate nanoparticle formation. Algal biomolecules, including pigments and proteins, reduce metal ions to nanoparticles. Metallic nanoparticles produced are stabilized with capping agents present in algae, such as amino groups or carboxyl groups of proteins and polysaccharides. The characteristics of the nanoparticles depend on the type and quantity of algal biomass used (Ali *et al.,* 2020; LewisOscar *et al.,*2016; Fawcett *et al.,* 2017).

Mechanism of nanoparticle synthesis by microorganisms

Metal ions interact with biological components, directing processes like protein corona formation, cellular uptake, and biocatalytic reactions, leading to intracellular and extracellular nanoparticles. In bacterial metal ion reduction, vesicle formation, ion accumulation, oxidation-reduction regulation, and crystal maturation govern morphology (Ramesh *et al.,* 2015). Metal-resistant microorganisms, including bacteria, fungi, and algae, efficiently synthesize metallic nanoparticles due to their resistance mechanisms (Salunke *et al.,* 2016). Reductase enzymes like nitrate reductase, generated by microorganisms, act as reducing agents (Singh *et al.,* 2016). Biomolecules from deceased microbial cells, such as sugars and co-factors, also effectively reduce metal ions to nanoparticles (Sneha *et al.,* 2010). Microorganisms function as nanofactories, synthesizing metallic nanoparticles intracellularly or extracellularly (Salem and Fouda, 2021). In extracellular synthesis, microorganisms are cultured, removed, and the supernatant is added to a metal salt solution for nanoparticle formation (Singh *et al.,* 2016). In intracellular synthesis, the pelleted biomass is washed, suspended in a metal salt solution, and incubated, allowing metal ions to be transported into microbial cells where they are reduced to nanoparticles. Ultrasonication extracts accumulated nanoparticles, with extracellular synthesis proving more cost-effective (Singh *et al.,* 2016).

Responses of Plants to Nanobiofertilizer

The combined action of biofertilizer and nanoparticles intensifies plant responses, enhancing development and yields. This synergy mitigates toxic chemical effects, inhibits rhizospheric pathogen growth, aids bioremediation, and replenishes soil nutrients. Nanobiofertilizers upregulate genes for antioxidants, osmolytes, and stress-related proteins, countering reactive oxygen species' damaging effects. They preserve cell structure, enhance membrane transporter activity and hormone production. Applied to plants, these treatments activate antioxidant systems, protecting cells against stress. Elevated growth hormone levels, like indole acetic acid and cytokinin, coupled with reduced stress hormone production, enhance plant stress tolerance and improve crop establishment under adverse conditions (Vedamurthy *et al.,* 2021; Shcherbakova *et al.,* 2017; Bibi *et al.,* 2022).

Conclusion

Chemical fertilizers disrupt ecosystems and pose health risks, while nanobiofertilizers enhance plant growth, improve nutrition, increase productivity, and fortify resistance against stressors. Activating various mechanisms, nanobiofertilizers ensure sustained soil nutrients, enhancing crop growth and yield. They significantly improve both quantity and quality of agricultural products, even at low concentrations, minimizing bioaccumulation. Contributing to soil fertility, nanobiofertilizers exhibit a unique synergy, surpassing the impact of singular biofertilizer or nanoparticle applications.

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Chapter- 8

Assessing Pod borer, *Penthicoides seriatoporus* Fairmaire Damage in Different Genotypes of Various Spinach Bunch Groundnut

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Abstract

A Field experiment was conducted at Main Dry Farming Research Station, Junagadh Agricultural University, Targhadia (Rajkot) under North Saurashtra Agro Climatic Zone of Gujarat during *kharif* season of the year 2020-21 to 2021-22. The study evaluated twenty genotypes/cultivars in a randomized block design with four replications in one year. In the 2020-21 season, a significant variation in the incidence of damaged pods by pod borer among genotypes were observed. Genotype J-99 exhibited the lowest percentage of damaged pods at 0.81%, while genotypes J-103 (2.96%) and TG- (2.96%) recorded significantly higher levels of damage. During the year 2021-22, among the fourteen genotypes, J-110 (0.61 %) was significantly recorded lower damaged pods, which was analysed simple Randomised block design. However, it was at par with all other genotypes except ICGV-16668 (2.96%) and JB-1487 (3.08). Notably, genotype JB-1487 recorded a significantly higher percentage of damaged pods at 3.08%.

Keywords: Different Genotypes, Pod borer, *Penthicoides seriatoporus* Fairmaire *Spanich* bunch groundnut

Introduction

India stands as the foremost global producer of oilseeds, with the oilseed sector holding a pivotal role in the nation's economy. Contributing to 12-15 percent of the world's oilseeds area, 6-7 percent of vegetable oil production, and 9-10 percent of total edible oil consumption (FAO, 2011), India's significance in this sector is undeniable. The escalating import of oilseed crops, particularly groundnut and mustard, underscores their prominence in the Indian oilseeds landscape. Groundnut is an important oilseed crop of Gujarat grown in both *kharif* and *summer* season spans an extensive area of 61,413 hectares, yielding a production of

1,31,470 metric tons. Nationally, groundnut is a key edible oilseed crop grown in about 12 States. Despite ranking fifth in production and sixth in cultivation area among other crops, groundnut holds a substantial position in Indian agriculture In comparison to other crop, it occupied fifth rank in term of production and sixth rank in area. Gujarat accounts for $1/3^{rd}$ of the total area under groundnut in the country. In term of production, Gujarat contributes around 30 per cent to the country's groundnut production. The cultivation of groundnut in Gujarat is predominantly concentrated in the Saurashtra region, often referred to as the oil pouch of India. Saurashtra accounts for 25 percent of the country's total groundnut cultivation area and 27 percent of its production (*http://www.*wikipedia.org). However, a critical challenge to groundnut productivity arises from the significant damage inflicted by lepidopteran pests, the major ones being tobacco caterpillar, *Spodoptera litura* F. (Noctuidae: Lepidoptera) and pod borer, *Helicoverpa armigera* (Noctuidae: Lepidoptera). Both caterpillars exhibit activity during the vegetative stage, resulting in over 50% defoliation in certain favorable years. The impact of these pests were pronounced, with avoidable yield losses recorded at 48.57 percent in pods and 42.11 percent in fodder (Dabhade *et* al., 2012). Groundnut yields further suffer a significant reduction of 26.74 percent due to pest infestation (Jayewar *et* al., 2017). Addressing these challenges is imperative for sustaining and enhancing groundnut productivity in the Indian agricultural landscape.

Materials and Methods

Feld experiments were conducted at Main Dry Farming Research Station, Junagadh Agricultural University, Targhadia (Dist.:-Rajkot, Gujarat, India) during two consecutive *kharif* season of 2020-21 to 2021-22.

Table: 1 Different genotypes of *Spinach* bunch groundnut (2020-21)

Sr. no.	Genotypes	Sr. no.	Genotypes
1.	J-98	11.	NRCGS-606
2.	J-96	12.	ICGV-16679
3.	J-99	13.	ICGV-16697
4.	J-102	14.	ICGV-16668
5.	J-103	15.	ICGV-16688
6.	J-104	16.	GG-7
7.	J-108	17.	GJG-9
8.	J-109	18.	JL-501
9.	J-110	19.	TG-37A
10.	TG-88	20.	Filler

Each replicate four times in randomized block design with the plot size of (a) gross plot size: Gross plot size: 4.80 m x 2.25 m (6 rows), Net plot size: 4.80 m x 2.25 m (5 rows). The spacing and seed rate were 45 cm X 15 cm and 120 kg/ha, respectively. The crop was fertilized with 12.50-25.00-00 NPK kg/ha.

Table: 2 Different genotypes of *Spinach* bunch groundnut (2021-22)

Sr. no.	Genotypes	Sr. no.	Genotypes
1.	J-108	8	TG-88
2	J-109	9	ICGV-16697
3.	J-110	10	ICGV-16668
4.	J-114	11	GJG-9
5.	JB-1487	12	GJG-32
6.	JB-1488	13	JL-37A
7.	JB-1505	14	JL-501

Each replicate four times in randomized block design with the plot size of (a) gross plot size: Gross plot size: 5.0 m x 2.70 m (6 rows), (b) net plot size: 4.80 m x 2.25 m (5 rows) The spacing and seed rate were 45 cm X 10 cm and 120 kg/ha, respectively. The crop was fertilized with 12.50-25.00-00 NPK kg /ha.

Pod damaged by pod borer, *Penthicoides seriatoprus* Fairmaire in different genotypes of *Spanich* bunch groundnut for two consecutive year of study were recorded.

Results and Discussion

Table 3: Pods damage due to pod borer, *Penthicoides seriatoprus* Fairmaire in different genotypes of *Spinach bunch* groundnut (2020-21)

Sr No.	Genotypes	Pods damage (%)
1.	J-98	2.29* (8.70)
2.	J-96	2.41 (6.83)
3.	J-99	0.81 (5.15)
4.	J-102	2.02 (8.18)
5.	J-103	2.96 (9.90)
6.	J-104	2.29 (8.70)
7.	J-108	1.66 (7.40)
8.	J-109	1.23 (6.38)
9.	J-110	2.02 (8.18)
10.	TG-88	2.96 (9.90)
11.	NRCGS-606	2.03 (8.20)
12.	ICGV-16679	1.66 (7.40)
13.	ICGV-16697	2.08 (8.30)
14.	ICGV-16668	2.02 (8.18)
15.	ICGV-16688	2.75 (9.55)
16.	GG-7	2.03 (8.20)
17.	GJG-9	1.66 (7.40)
18.	JL-501	1.32 (6.60)
19.	TG-37A	2.02 (8.18)
20.	Filler	1.32 (6.60)
	S. Em ±	0.52
	C. D. at 5%	1.47
	C. V. %	12.94

*Arcsine transformed values; Data in parenthesis are original values.

Total twenty different genotypes of *Spinach* bunch groundnut were evaluated against pod borer, *P. seriatoporus* during the year of 2020-21. Data given in Table 3 and Fig.1 revealed that significantly, minimum damaged pods were recorded in genotype J-99 (0.81%) and it was at par with all other genotypes except J-98 (2.29%), J-96 (2.41%), ICGV-16688 (2.75%) , J-103 (2.96%),TG-88 (2.96%) while, significantly, maximum damaged pods were recorded in genotype J-103 (2.96%) and TG-88 (2.96%) which was significantly at par with ICGV-16688 (2.75%), J- 103 (2.41%), ICGV-16697 (2.08%), GG-7 (2.03%), J-102 (2.02%), J-110 (2.02%), ICGV-16668 (2.03%), TG-37A (2.02%) , J-108 (1.66%), ICGV-16679 (1.66%) and GJG-9 (1.66%).

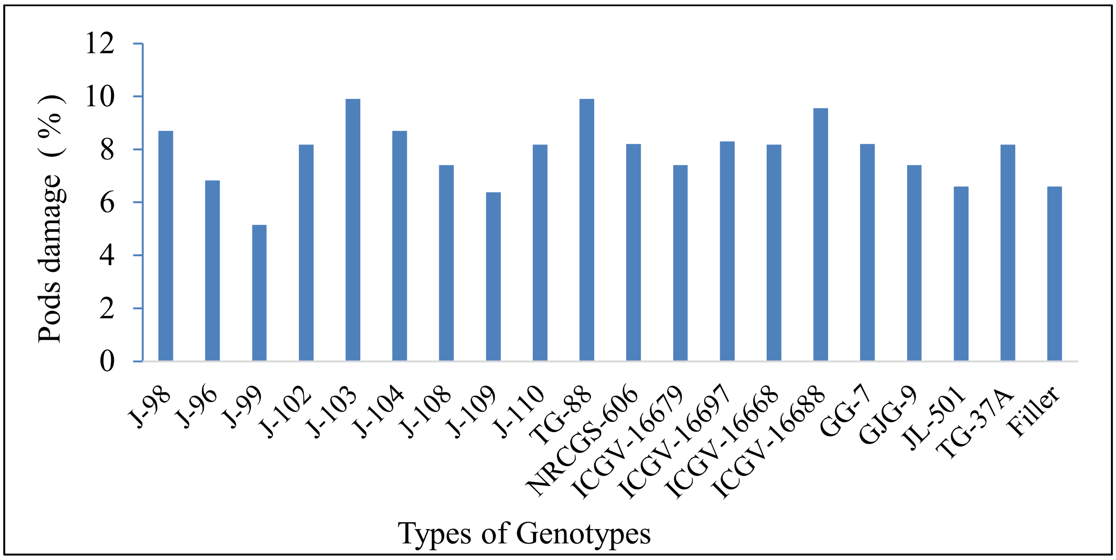

Fig.1 Pod borer damage in different genotypes during the year 2020-21

Table 4: Pods damage due to pod borer, *Penthicoides seriatoprus* Fairmaire in different genotypes of *Spinach* bunch groundnut (2021-22)

Sr. No.	Genotypes	Pods damage (%)
1.	J-108	1.23* (6.38)
2.	J-109	1.41 (6.83)
3.	J-110	0.61 (4.48)
4.	J-114	2.02 (8.18)
5.	JB-1487	3.08 (10.10)
6.	JB-1488	2.29.(8.70)
7.	JB-1505	1.66 (7.40)
8.	TG-88	1.23 (6.38)
9.	ICGV-16697	2.02 (8.18)
10.	ICGV-16668	2.96 (9.90)
11.	GJG-9	2.03 (8.20)
12.	GJG-32	1.66 (7.40)
13.	JL-37A	2.08 (8.30)
14.	JL-501	1.96 (8.05)
	S. Em. ±	0.61
	C.D. at 5 %	1.76
	C.V. %	15.84

*Arcsine transformed values. Data in parenthesis are original value

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Total fourteen different genotypes of *Spinach* bunch groundnut were evaluated against pod borer, *P. seriatoporus* during the year of 2021-22. Data given in Table 4 and Fig. 2 revealed that significantly, minimum damaged pods were recorded in genotype J-110 (0.61%) and it was at par with all other genotypes except ICGV-16668 (2.96%) and JB-1487 (3.08%), while, significantly, maximum damaged pods were recorded in genotype JB-1487 (3.08%) which was significantly at par with genotypes, ICGV-16668 (2.96%), JB- 1488 (2..29%), TG-37A (2.08%), GJG-9 (2.03%). ICGV-16697 (2.02%), JL-501 (1.96%), JB-1505 (1.66%), GJG-32 (1.66%) and J-109 (1.41%).

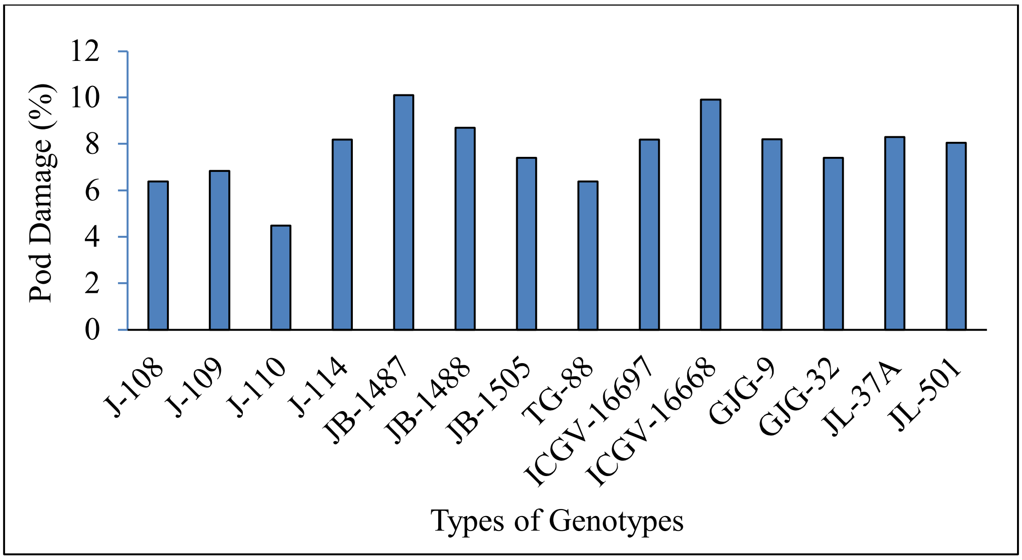

Fig.2 Pod borer damage in different genotypes during the year 2021-22

Overall, results from the year of 2020-21 indicated that significantly, minimum pods was damaged in genotypes (0.81%) and it found best genotypes against damaged by pod borer and in the *kharif* season of 2021-22, J-110 (0.61 %) found superior against pod borer than rest of other genotypes. Similar result was obtained by Kapadia, 1996 also recoded the seed damage in infested pods was 63.5, 73.4 and 42.3% in groundnut; sesame and castor, respectively, and percentage weight loss of damaged seeds was 59.2, 100.0 and 63.0%, respectively.

Conclusion

There were twenty different genotypes of Spinach bunch groundnut was assessed for their susceptibility to pest infestation, particularly the pod borer *P. seriatoporus*, during the 2020-21 period. Among these, genotype J-99 exhibited significantly minimal pod damage at 0.81%. Conversely, significantly higher levels of damaged pods were observed in genotype J-103 (2.96%) and TG-88 (2.96%). In the subsequent year, 2021-22, fourteen different genotypes of Spinach bunch groundnut underwent evaluation for their resistance to pod borer, *Penthicoides seriatoprus* Fairmaire. The results demonstrated that genotype J-110 recorded significantly minimal damage to pods at 0.61%, comparable to all other genotypes except ICGV-16668 (2.96%) and JB-1487 (3.08%). In contrast, genotype JB-1487 exhibited significantly higher levels of damaged pods at 3.08%.

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Chapter- 9

Effect of Primary Nutrients on Growth, Yield and Quality of Cut Foliage *Philodendron xanadu*

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Abstract

Cut foliage is used in abundant for decoration on its own or in group with flowers in bouquets and as indoor potted plants. Evergreen plants with green, silver or variegated leaves are usually used and species with berries are also trending now. This trend set is due to the green, healthy image presented by such products and because of the predicted increase in consumption of floral products. In recent times, the commercial production of cut foliage is increasing substantially. *Philodendron xanadu* is a newly emerging cut foliage crop in ornamental industry which has a great demand due to its attractive green foliage, used for interior decorations. It is grown as border plants, mass planting in outdoor gardens and as indoor plants. It is suitable for growing under shady places. It needs rich, moisture-retentive soil. Philodendrons are heavy feeders. A regular feeding program with a fertilizer will increase leaf size and produces a larger, healthier plant. It is a nice strategy to fertilize more frequently at half strength than to apply one strong dose. For plants to grow and thrive, they need several chemical elements, but the more requirements from external source are primary nutrients *viz*., nitrogen, phosphorus and potassium. Each has its own role in the growth and development of plants. Nutrient such as nitrogen play a major role in the growth and development of plants. Nitrogen helps in increasing vegetative growth by increasing leaf cell number and cell size and ultimately increases leaf area. Nitrogen is especially important in plants to manufacture new cells. Urea is the most suitable nitrogen source for foliar application due its high solubility. Phosphorus is a major component of important metabolic structure involved in energy utilization and storage mechanism. This is also essential for carbon metabolism which increases the biomass production, its partitioning and ultimately the yield of crop plants. Phosphorus is a constituent of chlorophyll and is involved in much physiological process including cell division, development of meristematic tissue, photosynthesis, metabolism of carbohydrates, fats and proteins etc., Potassium is also important for growth and elongation probably due to its function as an osmoticum and may react synergistically with IAA. Moreover, it promotes CO_2 assimilation and translocation of carbohydrates from the leaves to storage tissues.

Keywords: cut foliage, *Philodendron xanadu*, primary nutrients, nitrogen, phosphorus, potassium, urea

Introduction

Floriculture is a fast emerging competitive industry and cultivation of flowers for commercial purposes is common to many countries. It has become one of the high value agricultural industries in many countries of the world (Taj Sajida, 2013). Floriculture is characterized by growing of traditional as well as cut flowers under open field conditions and protected environmental conditions respectively. Other floricultural segments like cut foliage, indoor plants, ornamental plants, turf grass and value added products also contribute their share in the overall growth of the sector. In recent decades there has been increasing interest in floriculture and its products with great potential in the domestic as well as in export market. USA, Netherlands, Germany, U. K and United Arab Emirates were major importing countries of Indian floriculture during 2019-2020. Further India has exported 16949.37 MT of floriculture products to the world for the worth of Rs.541.61 crores in 2019-2020 (Anon., 2020).

Cut foliage or cut greens are attractive in form, colour and freshness are long lasting and in great demand. These are used as fillers along with cut flowers in flower arrangements and elsewhere for increasing aesthetic value. Foliage plants as a pot plants are valued for their foliage beauty, compactness of size and ability to survive under shady conditions. The cut foliages are in demand throughout the year and it peaks during festive seasons. Some of the popular types foliages traded in Indian market are asparagus, ferns, palms, philodendrons, dracaenas, cycads, eucalyptus and cyprus etc., Among them, philodendron is the second largest genus of the Araceae family with many species native to Central America and the caribbean. The name philodendron derives from the Greek word "Philo" which means love or affection and "Dendron" means tree (Pankaj, 2019). The wide diversity in size, leaf shape, colour and growth habits makes philodendron cultivars suitable for interior decoration, plantation in shade, hanging baskets, twiner plant and as pot plants (Madhubala, 2018).

Philodendron xanadu is a very beautiful foliage plant originated in Australia, commonly called xanadu, the leaves are lobbed, medium green, glossy in nature and look like smaller versions of lacy tree philodendron (McConnell *et al*., 2018). It is an ideal plant to use in warm temperature, subtropical and tropical regions. It is commonly cultivated as landscape plant in parts of United States, California, South Africa, Australia and New Zealand. It is prized for its attractive green colour foliage, most suitable for indoor gardening to decorate focal points in living spaces. It is not just good looking and ornamental but also a great air purifier.

There are many ways to increase the yield and quality of cut foliage. Among them nutrient management is most important for the growth and quality. These nutrients may be applied to the soil or they may be applied to the foliage of the plants (Wade Mc.call, 1980). The visual quality of ornamental plants is necessarily linked to an adequate balance of nutrients. Plant height, shape and colouration are the qualitative aspects of ornamental species, directly influenced by mineral nutrition. The nutritional requirements of ornamental species are not yet well established, often resulting in inefficient use of chemical and organic fertilizers, without respecting the needs of each species as well as the proper time for application. This leads to the low quality of the final product, as well as high production costs, which justifies the importance of a nutritional knowledge of the species (Neto *et al*., 2015). The primary nutrients are Nitrogen (N), Phosphorus (P) and Potassium (K). These essential elements are required by the plants in higher quantities and perform crucial functions in plant biology. Nitrogen is necessary for the formation of amino acids, proteins, DNA and RNA. It is essential for plant cell division and vital for plant growth. Phosphorus promotes early root formation and growth, and is involved in photosynthesis, respiration, energy storage and cell enlargement. Potassium is involved in carbohydrate metabolism and translocation of starch, also enhances disease resistance and hardiness. An all purpose NPK fertilizer will provide the nutrients to plant needs for healthy growth. NPK fertilizers are water soluble and can be taken up by the plant almost immediately. Foliar application seems to be promising for ensuring use efficiency of applied nutrients. Foliar spray enables plants to absorb the applied nutrients from the solution through their leaf surface and thus, may result in the economic use of fertilizer (Manasa *et al*., 2015). This paper attempts to review the latest information regarding the role of primary nutrients on growth, yield and quality of cut foliages.

Effect of soil application of primary nutrients on growth, yield and quality of foliage crops

A study conducted by Gul *et al*. (2006) in *Araucaria heterophylla* .revealed that maximum plant height (36.1 cm), stem thickness (1.05 cm), lateral branch length (19.4 cm), internode length (6.2 cm), root length, (25.5 cm), root thickness (0.89 cm), number of roots (11.8) and plant survival (98.3 %) were observed for plants supplied with 2.0 g N plant^{-1}, while the minimum for all the above parameters was found in control (without nitrogen application)

Reports of an experiment conducted by Vijay *et al.* (2009) at University of Rajasthan indicated that application of nitrogen, phosphorus and potassium @ 160 mg kg^{-1} of potting media was found to be best for vegetative growth, leaf chlorophyll content, carbohydrate, protein and sapogenin content of root tuber in asparagus (*Asparagus racemosus*) plants.

Naggar and Nasharty (2009) studied the effect of different growing media and fertilizer rates of NPK (19:19:19) at Alexandria university, Egypt and observed that applying the complete fertilizer of NPK (19:19:19) @ 5 g plant^{-1} at monthly intervals grown in composted leaves medium or its mixture with sand (1:1 v/v) gave the maximum beneficial vegetative growth characters, highest flowering and bulb yield in hippeastrum plants.

The field study by Hussein (2009) at Cairo university concluded that the best vegetative growth of *Cryptostegia grandiflora* was found in plants sprayed with GA_3 at 50 ppm and supplied with 21 g plant $^{-1}$ month^{-1} of the slow release fertilizer (24N: $8P_2O_5$: $8K_2O$).

The experiment carried out at Poznan university points out that the optimal yield of fresh and dry mass of above ground parts of plants, as well as the number and length of leaves, width of leaf blades, length of petioles, leaf colour intensity, decorative value and high suitability of plants for floral green was found in lacy tree philodendron grown in peat moss substrate at the nutrition level containing N 250, P 187, K 312, Mg 187 in mg dm^{-3} (Andrzej *et al*., 2011).

An experiment conducted by Habib (2012) to study the effect of NPK and growing media on growth and chemical composition of fish tail palm and the results revealed that using composted peanut significantly increased plant height and stem diameter. The plants grown in composted peanut or peat moss media with 4 gm NPK at monthly dose gave the best seedling growth.

Youssef and El-Aal (2014) stated that treating *Hippeastrum vittatum* plants with chemical fertilizer (NPK) @ 6 g $plant^{-1}$ improved the growth and chemical composition as compared with untreated plants.

Naggar and Ahmad (2016) conducted an experiment in Abdulaziz university, Saudi Arabia on effect of light intensity, NPK fertilization and their combined effect on growth of *Yucca rupicola*, L. and revealed that planting yucca plants under full sun light with applying 4 g NPK pot^{-1} $month^{-1}$ improved vegetative growth parameters, highest leaf N content and leaf total chlorophyll content.

The research work conducted by Boshra *et al.* (2016) in Egypt concluded that plants treated with NPK (2:1:1) @ 2 g pot^{-1} per 20 cm diameter plastic pot plus spraying the foliage with 1000 ppm of GA3 solution 4 times with 1 month interval improved all vegetative and root growth as well as, the leaf chlorophyll content, total carbohydrate, N, P and K % in cycas plants.

Reports of an experiment by Sohier *et al*. (2017) indicated that the higher values of different vegetative growth characters as well as chemical constituents of leaves were obtained at full dose of NPK chemical fertilizer (20.5% N, 15% P and 48.5% K) in crotons.

Abou Dahab *et al*. (2017) gave a report that application of chemical NPK (Kristalon 19:19:19) @ 2 and 4 g pot^{-1} every three weeks improved all vegetative growth parameters *viz*., plant height, number of leaves per plant, stem diameter, root length, fresh and dry weights of shoots in *Chamaedorea elegans* (parlor palm).

The field trail was carried out in Pakistan to evaluate the effect of fertilization on growth of *Acer mono* plants. Among the nutrient levels, 10 g N and 8 g P were found to yield maximum values of plant height, root collar diameter and root morphology (Muhammad Razaq *et al*., 2017).

Reports given by Mohamed (2018) revealed that growing *Dypsis cabadae* palm plants in a mixture medium contained compost + peat moss + perlite supplemented with NPK (20:20:20) @ 8 g pot^{-1} produced the tallest plant, highest values of fresh and dry weights of roots $plant^{-1}$ and increased number of leaves per plant.

Abirami *et al*. (2018) at Batticaloa in Srilanka studied the effect of graded nitrogen levels on *Cordyline fruticosa* L. var 'Purple Compacta' and observed that the plants treated with 0.5 g N $plant^{-1}$ $month^{-1}$ showed better performance in growth parameters *viz*, plant height, leaf area, plant biomass and gave higher scores in quality assessment while lower performance was observed in the treatment (2.5 g N $plant^{-1}$ $month^{-1}$).

In Dracaena, an experiment was conducted by Syed *et* al. (2009) at Cairo university revealed that NPK fertilizers @ 1 and 2 g alone and NPK fertilizers along with trace elements (Fe, Mn and Zn) @ 50 or 100 ppm increased plant height, stem diameter, number of leaves per plant, leaf area, fresh and dry weights of leaves per plant compared with control. Another study conducted by Ashour *et al*. (2020) suggested that for the higher quality, quantity growth and economic production of *Dracaena marginate* 'Bicolor', the plants could be grown in a medium of peatmoss and supplied monthly with NPK (20:20:20) fertilizer at 2 g plant^{-1}. In another experiment at dry zone of Sri Lanka by Srikrishnah *et al*. (2018) studied the influence of nitrogen levels on *Dracaena sanderiana* and noticed that the highest performances in measured growth parameters such as plant height, leaf area and plant biomass at 1 g and 1.5 g N plant^{-1} month^{-1}. They suggested that nitrogen in the range of 1.11 to 1.15 g plant^{-1} month^{-1} is optimum for the growth of dracaena varieties in 70 % shade level.

Zabotto *et al*. (2019) formulated an experiment to study the effect of nitrogen and phosphorus interaction on ornamental bromeliad. Results showed that plant height, number of leaves, total and leaves fresh and dry mass increase as N concentration increases in modified HA (Hoagland and Arnon) nutrient solution and they concluded that the optimum N concentration in HA solution was 14.5 millimole. Nitrogen was found to be more limiting to silver vase bromeliad growth and development than phosphorus.

Effect of foliar application of primary nutrients on growth, yield and quality of foliage crops

El-Bagoury *et al.* (2008) studied that NPK fertilization @ 1000 ppm along with trace elements (Fe, Zn, and Mn 50 ppm) at two weeks interval significantly increased the mean plant height, stem diameter, number of leaves per plant, fresh weight of foliage (stems and leaves), and leaf chlorophyll content in hedera plants.

An experiment in *Syngonium podophyllum* L. plant, pointed out that higher values of vegetative growth such as plant height, stem diameter, number of leaves, leaf area, fresh and dry weights of plant organs were obtained from plants treated with grow-more inorganic fertilizer @ 2 ml l^{-1} combined with putrescine 100 ppm at 30 days interval (El-Quesni *et al*., 2010).

Research work by Abo-Rekab *et al*. (2010) at Giza, stated that the addition of 2.5 g l^{-1} NPK kristalon fertilizer (19:19:19) at weekly intervals gave the highest values of plant height, number of leaves per plantlet as compared to control in date palm plantlets.

According to Ahmad *et al*. (2012), the concentration of N in most of the ornamental plant leaves is approximately 5% on a dry mass basis and it can reach as high as 7.6%. Foliar application during the growth and development of crops can improve their nutrition balance, which may in turn lead to an increase in yield and quality.

Giampaoli *et al.* (2017) evaluated the effect of urea on the growth of ornamental bromeliad. Plants were submitted to weekly treatments with a solution of 0.0, 0.5, 1.0, 1.5 or 2.0 g urea l^{-1}. From the results, they concluded that the application of 1.5 g l^{-1} urea gave the greatest accumulation of total dry weight and an increased number of leaves.

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Chapter- 10

GMOs for Climate Resilience Farming

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Abstract

Greenhouse gases effect increases at rapid rate due to use of chemicals, fossil fuels and uneven management of residue. That unbalances atmospheric conditions which ultimately affect earth planet populations as plants, humans and animals etc. To avoid these problems and to increase sustainability in farming GMOs developed. As GMOs are genetically modified organisms are useful to develop resistance varieties by inserting gene of interest from other unrelated organisms. GMOs further use to cross with wild relatives to catch desirable genes that tolerant environmental stresses and for many other useful genes.

Keywords: Variety, Wild relative, Gene, Tolerance, Resistant.

Introduction

The first and main objective of crop trait improvement is higher yield. But changing climatic conditions affect this objective as decrease agriculture production, food supply and make economic imbalance. To take this point of view a new technology of gene editing as GMOs developed that insert useful genes in plants to make them able to survive under stress conditions also. And make ecological balance.

What is climate resilience?

It is a trapping and coping of climate changes impact to prevent their affect on growth. In other words, "New challenges occur due to changing in climate conditions with increase of green house gases as CO_2 level etc. that ultimately affect the earth population to withstand under these challenges climate resilience is a useful way" (Espanol, 2022).

What do you mean by GMOs?

Genetically modified organisms are any individual as plant, animal and microorganism whose genome altered at molecular level or engineered artificially in laboratory in order to favor the expression of desired traits as changes their physiological trait expression to adopt desirable ones and survive under worse conditions (Judith *et al*, 2023). GMOs are lower costs, high yield potential with reducing land and environmental foot printing of agriculture (Zilberman *et al*, 2018).

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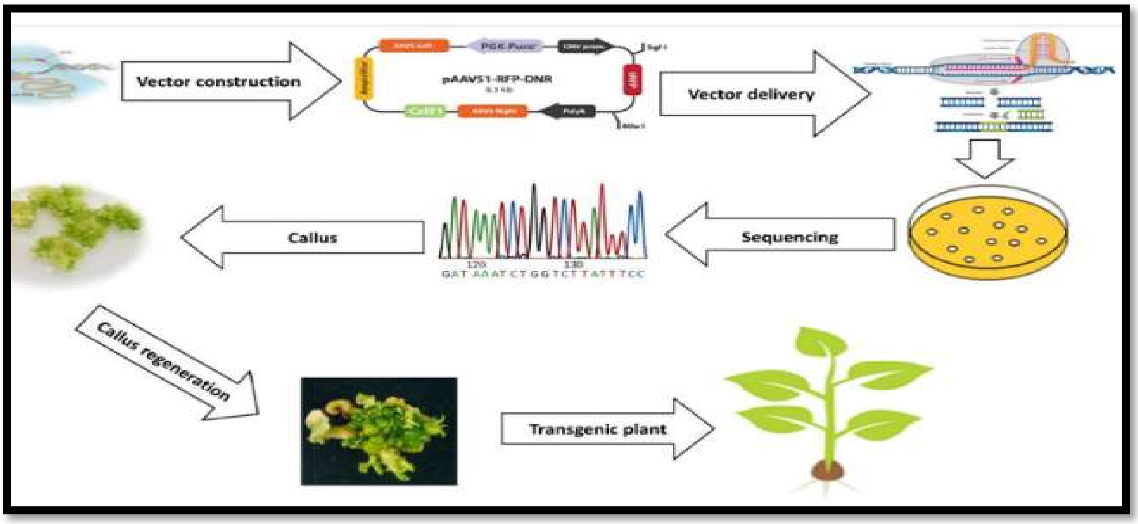

Fig.1- GMOs Construction

How to develop GMOs?

Identify goal of interest (Yield, stress tolerance/resistance and quality trait)

↓

Isolate gene of interest

↓

Inoculation to insert desired information into the DNA of other organism (Gene gun, Vector mediated gene transformation)

↓

New Organism (Anonymous, 2022)

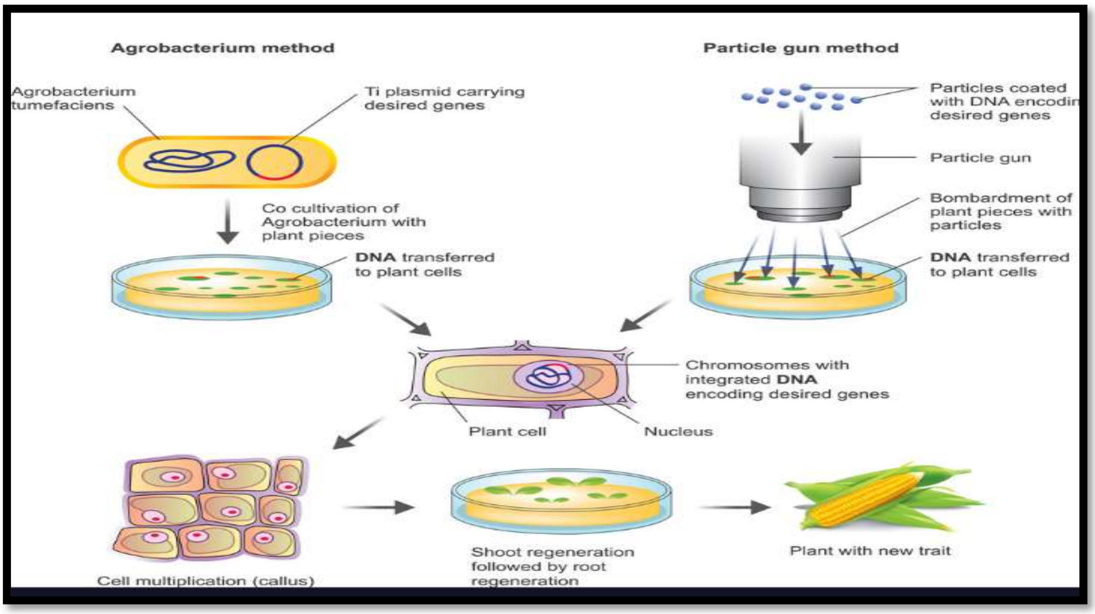

Fig.2- Methods of Gene insertion

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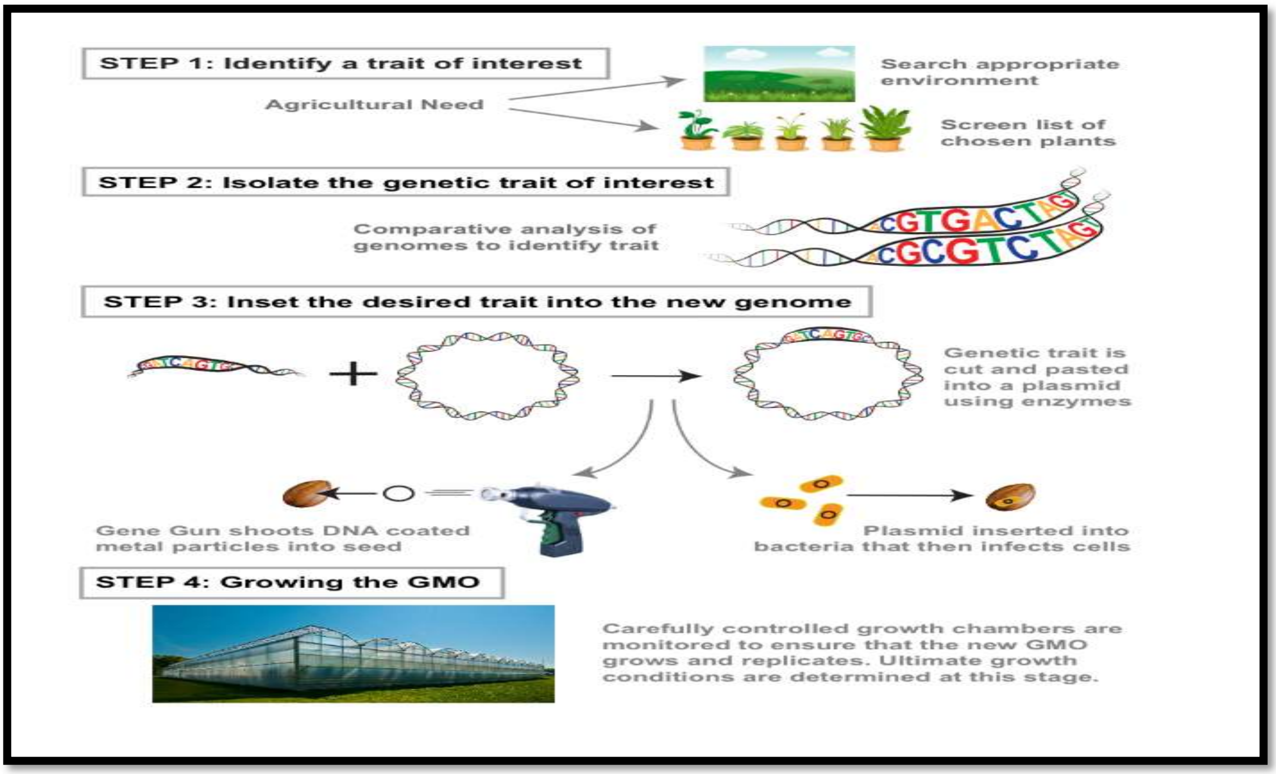

Fig.4- Procedure to develop GMOs

How GMOs helpful for climate resilience farming?

- Abiotic stresses as heat, drought and salinity etc. that reduces crop yield and productivity.

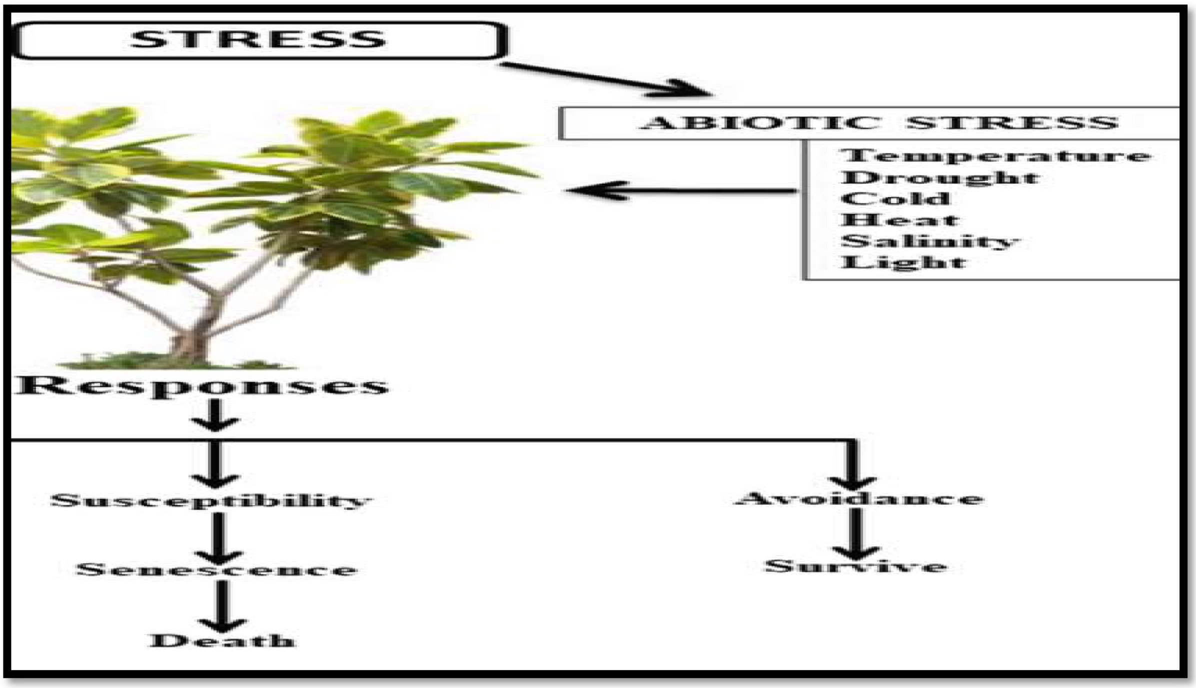

Fig. 3- Climatic stresses

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- These problems are taken by developing designer crops by changing their physiology with number of gene insertion that encode to particular protein and make plants suitable to survive under such changing conditions. As mannitol, Glycine betaine and heat shock proteins employed for abiotic stress tolerance in different plants (Parmar *et al*, 2017).
- **Salt Stress Tolerance Resilience**- Glycine betaine (GB) biosynthetic *codA* , DREBs and Na^+/H^+ antiporters in transgenic plants to conferred stress tolerance phenotype in tomato (Khan *et al*, 2016).
- **Drought Tolerant Resilience**-In rice *OsFTL10* these are flowering locus T-like genes expressed during early flowering to improve drought tolerance (Martignago *et al*, 2020). *P_5C* proline biosynthesis enzyme accumulate during drought stress act as Osmo protectant.
- **Heat Tolerant Resilience**-Sucrose accumulate during heat and drought stress in combination instead of proline to protect plants hyperreactive and susceptible mitochondria from toxins build up during *P_5C s*ynthesis (Atkinson and Urwin, 2012).
- **Water Stress Tolerant Resilience**- Water stress tolerant Glycine betaine and drought tolerant beta gene encodes for choline dehydrogenase (CDH) introduced in sugarcane that acts as osmoprotectant and acclimate sugarcane plants under water stress respectively (Sugihart, 2017).

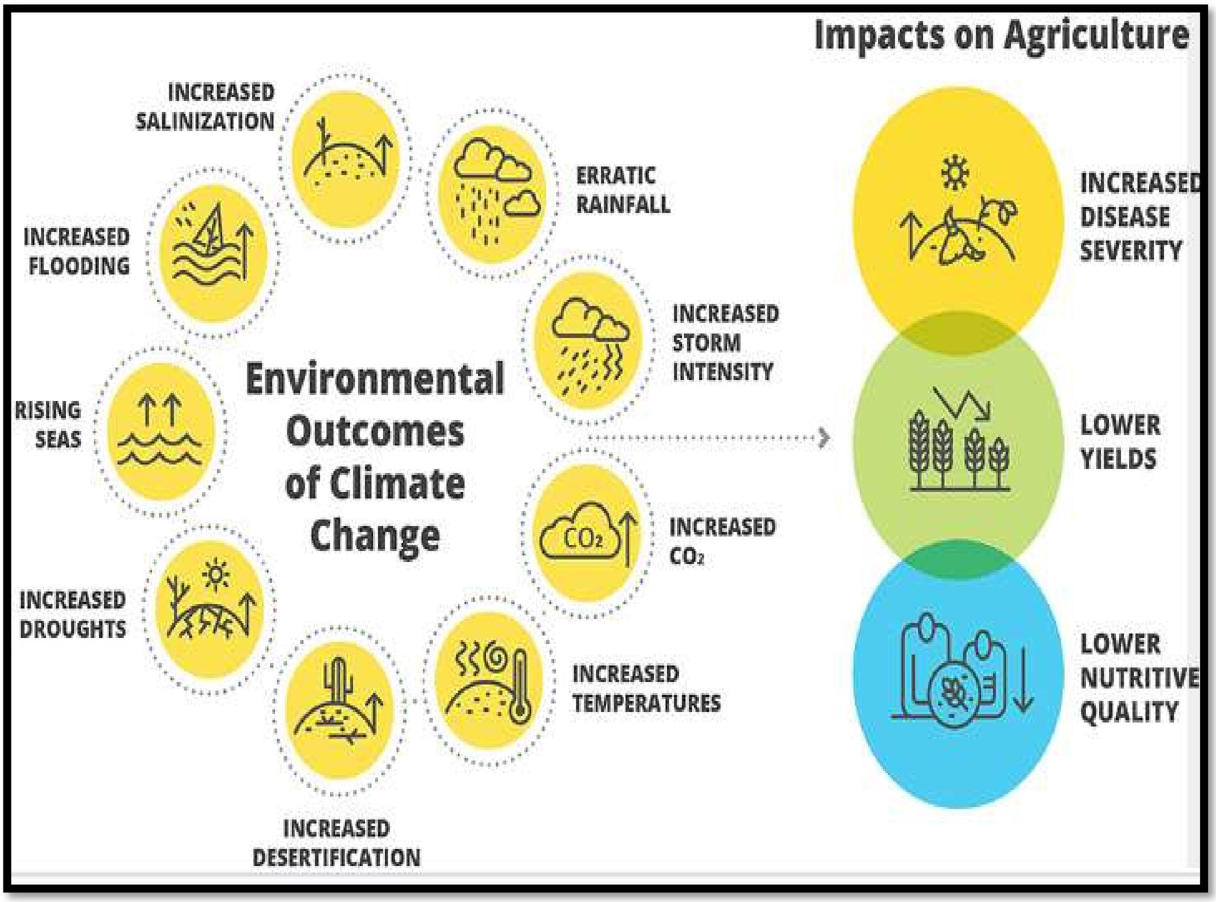

Fig. 5- Effect of Climatic Changes on Farming

Advantages of climate resilience farming

- **Reduce poverty-** Production of crop plants increase that fulfills demand of food supply. And Income sources ultimately increases with production due to management practices considerations.
- **Saves life-** Supply balanced diet to each and every individual. And make ecological balance.
- **Deliver strong economic returns-** Source of economic return increases with high yield objective and their product supply also increases that rapidly increase economic returns.
- **Reduce worse effect of climate**- Climatic effect as high temperature cause heat stresses, drought, and storms and increase sea level to withstand under these affects climate resilience farming is key point.

Achievements

Crop	Gene	Trait
Tomato	*Coda*	Drought and salt stress tolerance
Rice	*OsFTL10*	Drought tolerance
Maize	*CP4EPSPS*	Drought (Benevenuto *et al*, 2017)
Sugarcane	*Beta*	Water stress tolerance

Conclusion

If stress affects increases rapidly on earth planet here's a no chance to survive without vegetation due to lack of food supply and income sources. To manage this strategy GMOs are useful practice that plays important role by inserting desirable gene so, plants stand under adverse conditions.

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Chapter- 11

Endophytic Fungi: Mechanisms and Applications as Biocontrol Agents

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Abstract

Control of plant diseases by biological methods has transformed into a distinct field of science. Fungal endophytes are a group of microorganisms which can colonise in all the plant parts without overt negative symptoms/harmful effects in the plants in which they live in for their survival. The endophytic fungal isolates have been identified by many researchers, and they are reported to reduce the growth and activity of a surfeit of plant pathogens and pests. Scientists and researchers are increasingly drawn to this field due to its potential as a substitute for synthetic fungicides. The primary objective of this chapter is to chart the progression in endophytic fungal research and provide updated insights to assist researchers in future investigations. The chapter will commence with an introduction to endophytes and their diverse nature, followed by an exploration of the principal processes governing the modes of action in disease control. It includes different sections, namely, competition, antibiosis, insect deterrence, ISR and mycoparasitism. The chapter ends with future thrust which will summarize the future niche research areas on endophytic fungi.

Keywords: Endophytes, Biocontrol, Competition, Antibiosis, Mycoparasitism

Introduction

Plant diseases cause biotic stress to plants which results in economic loss for farmers. They spoil food through toxin production during storage. The wilful and excess urge of farmers to deal with the diseases rose the need of discovery of several fungicides and bactericides. The excess application of these fungicides and bactericides resulted in environmental degradation which ended up imperilling the health of people. Numerous plant pathogens are reported to develop resistance to these chemicals and have made plant disease management difficult. In order to get rid of these risks, bio control measures gained greater importance.

Fungal endophytes are very common and highly distinct microorganisms that live inside plant tissues, but usually stay asymptomatic[1]. In recent past, researchers defined endophytes as ‘endosymbionts’ which inhabit the inner parts of the plant tissues and do not damage or inflict diseases which could be isolated through adherence of aseptic methods[2].

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Mechanisms of endophytic fungal as potential biocontrol agents

Understanding the intricate dynamics among endophytes, host plants, and pathogens is crucial for effectively selecting and utilizing endophytes. This involves delving into the fundamental biology governing their interactions and studying the physiological processes at play. There are four established control principles applicable to Biological control agents and, consequently, to endophytes: (1) competition for resources, (2) inhibition via antibiosis, (3) mycoparasitism, and (4) triggering the plant's defence system for induced resistance. Often, multiple mechanisms operate simultaneously.

This chapter's goal is to assess the evidence necessary to pinpoint the mechanisms behind the biological disease control exhibited by endophytic fungal isolates during their endophytic behaviour.

Induced systemic resistance (ISR)

ISR involves the active fortification of a host plant's defenses, spurred by external factors, be they living organisms or environmental influences[3]. This process initiates the production of a mobile chemical signal within the host, prompting a resilient reaction against pathogenic threats. Consequently, in this chapter, we designate the shield activated by beneficial endophytic fungi, reliant on stimulating the plant's defense mechanisms, as induced resistance.

Throughout pathogenic or endophytic colonization, fungal effectors—typically small, cysteine-rich proteins—are secreted. Their role lies in fostering compatibility with the host plant by regulating defensive responses and altering physiological conditions[4,5].

The root endophytic isolate *Serendipita indica* dwells inside wide range of monocots and leguminous plants and has shown to confer enhanced growth and tolerance in a variety of plant systems[6,7,8].

Antibiosis

Antibiosis is defined as the suppression of pathogens that can be accredited to compounds directly produced by a bio control agent. Many natural products having antimicrobial properties have been isolated from endophytes, comprising of alkaloids, phenols, flavonoids, steroids, peptides, quinones, terpenoids, volatile organic compounds and polyketides where, terpenoids and polyketides are the most purified anti-microbial metabolites[9,10].

Endophytic fungal isolate recovered from *Ocimum sanctum* and *Aloe vera* was evaluated for their antifungal activity against *Fusarium oxysporum*. Out of 18 fungi isolated, AVR1 and AVR3 showed antagonistic activity against *Fusarium oxysporum*. AVR1 and AVR3 were found positive for cellulase and chitinase production respectively. The results of the research suggested that endophytic fungi associated with *O. sanctum* and *Aloe vera* are potential agents for antimicrobial activity and a substantial source of enzymes[11].

In some incidences, host and endophyte have some parts of a specific metabolic pathway in common and contribute partially to the metabolite production or one partner induces metabolism or metabolises products of the other partner[12]. Another study reported the

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function of an antifungal protein secreted by *Epichloe festucae* in the control of *Sclerotinia homoeocarpa* in *Festuca rubra* ssp. *rubra*[13].

Mycoparasitism

Mycoparasitism is defined as the condition where fungi directly obtain nutrients from other fungi. This definition includes a range of sequences, from a biotrophic interaction where in host stays alive and parasite gains nutrients from the host tissue to a necrotrophic interaction where the parasite kills the host to subsist on the dead cells.

The mycoparasite will have direct contact, penetrate the hyphae of the pathogen, coil around the hyphae and ultimately damage/cleave the pathogen hyphae. Instances of mycoparasitism by endophytic fungal isolates includes the work[14], which has reported parasitism of *Geotrichum* sp. (isolate EFds104-16) on *Thanatephorus cucumeris* by penetration mechanism followed by hyphal coiling around pathogen and break down of cytoplasm. Besides, production of the extracellular cell wall degrading enzymes, *viz.,* β-1,3-glucanases and chitinases were also demonstrated.

Competition

Competitive exclusion of microbes is a deciding factor for the formation of the plant microbiome and a potential mechanism by which host-dwelling endophytes can prevent colonisation of host by pathogens[15].

Pseudomonas aeruginosa FP6, earlier isolated from rhizosphere soil was screened for its siderophore producing ability on CAS agar plate. The strain when assessed for its *in vitro* antagonistic activity against *Rhizoctonia solani* and *Colletotrichum gloeosporioides*, with and without addition of $FeCl_3$, showed a significant decrease in *R. solani* growth with $FeCl_3$ supplementation compared to the control, indicating the role of siderophore mediated antagonism of *R. solani*. Antifungal activity was not influenced by $FeCl_3$ in the case of *C. gloeosporioides*, which revealed the presence of other antagonistic mechanisms[16].

However, competitive exclusion is not very important for endophytes[17] as bio control agents because colonisation at the expected point of entry the pathogenic microbe needs to be compact.

Insects' deterrence

Most clavicipitaceous fungal endophytes increase resistance of host plants to insect feeding; the benefits arise partially from the production of alkaloidic mycotoxins loline and peramine which are normally associated with resistance to insect pests.

Many fungi conventionally known as entomopathogens such as *Isaria farinose*, *Clonostachys rosea*, *Acremonium* sp. and *Beauveria bassiana* have been isolated as naturally occurring endophytes from asymptomatic plant tissues. For instance, *Metarhizium robertsii* infects and kills soil born insects, generates fungal mycelia from dead insects and subsequently, establishes an endophytic association with the plant roots, thus improving nitrogen translocation[18].

Conclusion

According to Pesticide action network, UK, excess use of chemical pesticides leads to poisoning of soil microbes, earthworms, bees, and other pollinators. Pregnant women and infants are the most sensitive groups in this regard. Taking into consideration the health and environmental effects of chemical pesticides, it is evident that a new concept in agriculture is urgently required. This new concept should lower the application of chemical pesticides, and should result in health, environmental, and economic benefits. One of the methods is the use of bio-control agents. Endophytes provide one such control measure.

In recent years, substantial research works has focused on utilizing endophytic fungi to manage crop diseases. Technologies for analyzing the mechanisms of action have evolved considerably in the past decades, presenting advanced molecular methods for thorough examinations of the interaction between endophyte, host, and pathogen. 'Omics' techniques, such as genomics, transcriptomics, proteomics, and metabolomics, prove highly efficient in studying these interactions. They help decipher the impacted pathways in different microorganisms and their potential synthesis of metabolites, facilitating a comprehensive understanding of the contributions made by all involved entities in these interactions.

To summarize, it's essential to evaluate the dynamics of the plant-endophyte relationship and the necessary conditions for effective research and establishment. This involves identifying the roles played by fungi and the compounds vital for reducing diseases. Additionally, studying the responses triggered in plants due to this interaction is crucial. This understanding will aid in creating markers to gauge the effectiveness of biocontrol agents. It also enables testing the impact of plant genetics, existing microbial communities, and environmental factors, paving the way for a systematic approach to discovering new endophytes with desirable traits.

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Chapter- 12

The Evolution, Ecology and Mechanisms of Infection by Gram-Positive, Plant-Associated Bacteria

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Abstract

Gram-positive bacteria, a diverse group of microorganisms characterized by their thick cell wall structure, have evolved over millions of years, adapting to various ecological niches and establishing intricate relationships with their hosts. This review delves into the evolutionary trajectories of Gram-positive bacteria, examining the genetic and physiological adaptations that have shaped their diversity and resilience. The exploration of their ecological roles highlights their significance in ecosystems ranging from soil and water to the intricate microbial communities within the human body.

Keywords- Gram positive, Gram negative, Plasmids

Introduction

Gram-positive bacteria are prominent members of plant-associated microbial communities. Although many are hypothesized to be beneficial, some are causative agents of economically important diseases of crop plants. Because the features of Gram-positive bacteria are fundamentally different relative to those of Gram-negative bacteria, the evolution and ecology as well as the mechanisms used to colonize and infect plants also differ. Across the Bacteria domain, there is variation in the structure of the cell envelopes, and responses to Gram staining do not always follow phylogeny. Most members of Actinobacteria react positively to Gram staining, but the phylum also includes mycolic acid-containing genera of bacteria that do not (Bouchek *et al.,* 2016).[1]

Gram positive vs Gram negative: Plant pathogenic bacteria

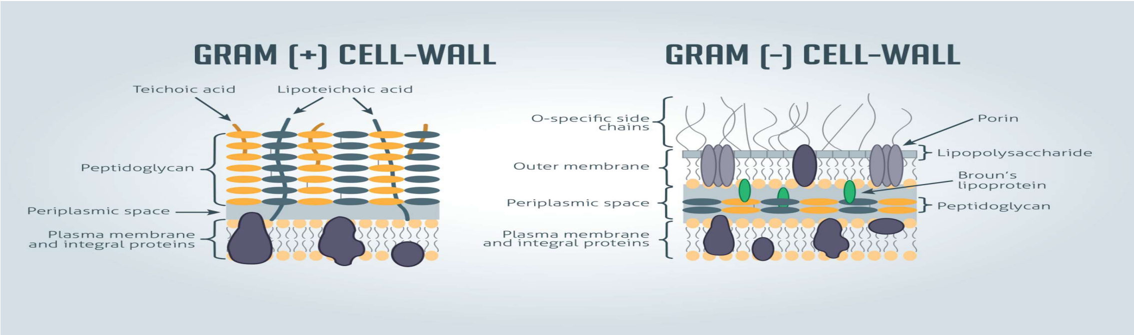

Fig. 1 : Difference between Gram positive and Gram negative bacteria

Gram positive

Bacteria have cell walls composed mostly of a substance unique to bacteria known as peptidoglycan, or murein. These bacteria stain purple after Gram staining.

Ex : *Clavibacter, Streptomyces, Rhodococcus.*

Gram negative

Bacteria have cell walls with only a thin layer of peptidoglycan and an outer membrane with a lipopolysaccharide component not found in Gram positive bacteria. These bacteria stain red or pink after Gram staining.

Ex : *Xanthomonas, Pseudomonas*

Table 1: Differences between Gram positive and Gram negative

Structural compositions	Gram positive	Gram negative
Cell wall	Cell wall thick and homogenous	Cell wall thin and trilayered
Murein	Accounts for 40-90% of cell wall (100A°)	1-10% of cell wall
Techoic acid	Present, TA are main surface antigens	Absent, lipopolysacchrides (LPS) are main surface antigens
Lipids	Lacks except Mycobacterium and Corynebacterium	Present
Sesitivity to antibiotics	More sensitive to antibiotics like penicillin	Less sensitive to wall attacking antibiotics like penicillin
Gram staining	Deep purple (crystal violet)	Pink/red (safranin)

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Lifestyles of Gram-Positive, Plant-Associated Bacteria

❖ ***Clavibacter* : A Group of Xylem-Limited Bacteria**

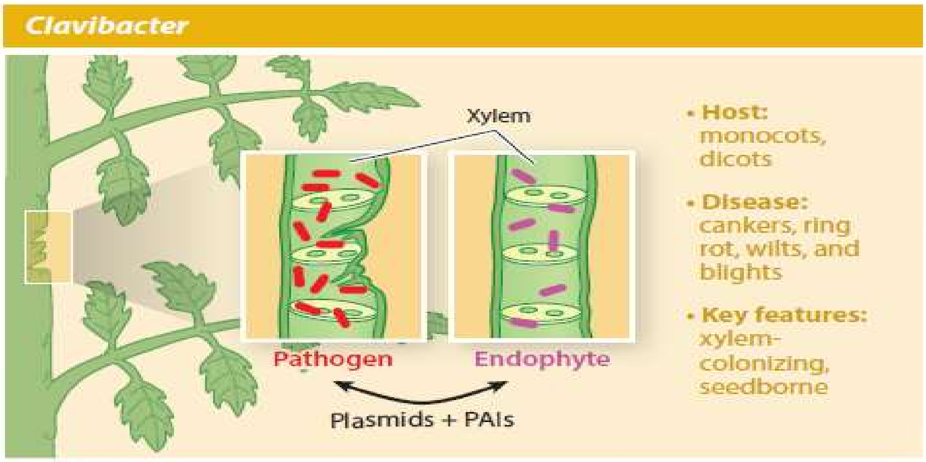

Fig. 2 : *Clavibacter* : A Group of Xylem-Limited Bacteria

Clavibacter proliferates in xylem vessels, resulting in systemic infection Symptoms include wilting, canker, necrosis, and leaf spots. Owing to its effectiveness as a seedborne pathogen, *Clavibacter* has a high risk of adverse consequences, and some species have been classified as quarantine organisms in Europe and other countries. Although the genus is most known for its pathogenic members, multiple *Clavibacter* strains have been isolated as asymptomatic endophytes of monocot and dicot plant species. Endophytic *Clavibacter* cells colonize the internal tissues of the plant and confer no conspicuous symptoms or negative effects on plant fitness (Creason *et al*., 2014)[2]..

Plasmids are a significant source of variation in the Clavibacter genus.

Clavibacter. michiganensis ssp *michiganensis* possesses two plasmids, pCM1 and pCM2, both of which are implicated in virulence, and their absence results in a delay in or complete loss of the ability of the bacteria to cause disease symptoms. The main virulence genes on the plasmids of NCPPB382 are celA (pCMI), a cellulase, and pat-1 (pCM2), a serine protease. Plasmid-free derivatives of *C. michiganensis, C. sepedonicus, and C. capsici* fail to induce disease symptoms, demonstrating that plasmids are required for virulence on solanaceous hosts.

❖ ***Streptomyces* : Taxa of Root-Associated Bacteria**

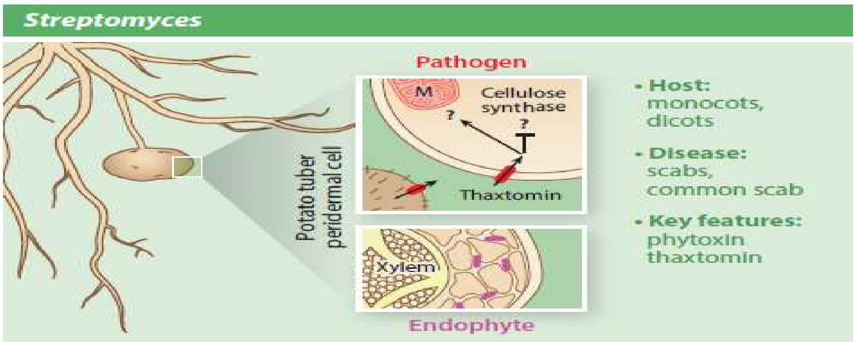

Fig. 3: *Streptomyces* : Root-Associated Bacteria

Streptomyces is a large and diverse genus with more than 800 named species. This group is best known for the production of secondary metabolites. Twenty species are known to include strains that cause common scab disease in plants. Some of the more commonly studied pathogenic species include *Streptomyces scabiei* (syn. *Streptomyces scabies*), *Streptomyces turgidiscabiei,* and *Streptomyces acidiscabiei*. Diseases of potato and other root crops caused by this group are prevalent, endemic, and costly because of the expression of raised or pitted lesions that make the tubers nonmarketable (Hogenhout and Loria, 2018)[3].

Thaxtomin: the root of scab diseases.

Thaxtomin is the most important virulence molecule associated with plant-pathogenic. lineages of *Streptomyces* . Studies suggest that the thaxtomin locus is necessary for expression of common scab disease, as disruption of txt and other genes associated with thaxtomin biosynthesis eliminates thaxtomin production, and mutants fail to elicit symptom formation on plants. It is a host specific toxin inhibit the cellulose synthesis. The thaxtomin locus is located within a txt pathogenicity island (PAI) and some strains of *Streptomyces* modified to carry the txt PAI gain the ability to cause symptoms typical of common scab disease.

❖ ***Rhodococcus*: Root and Leaf-Associated Bacteria**

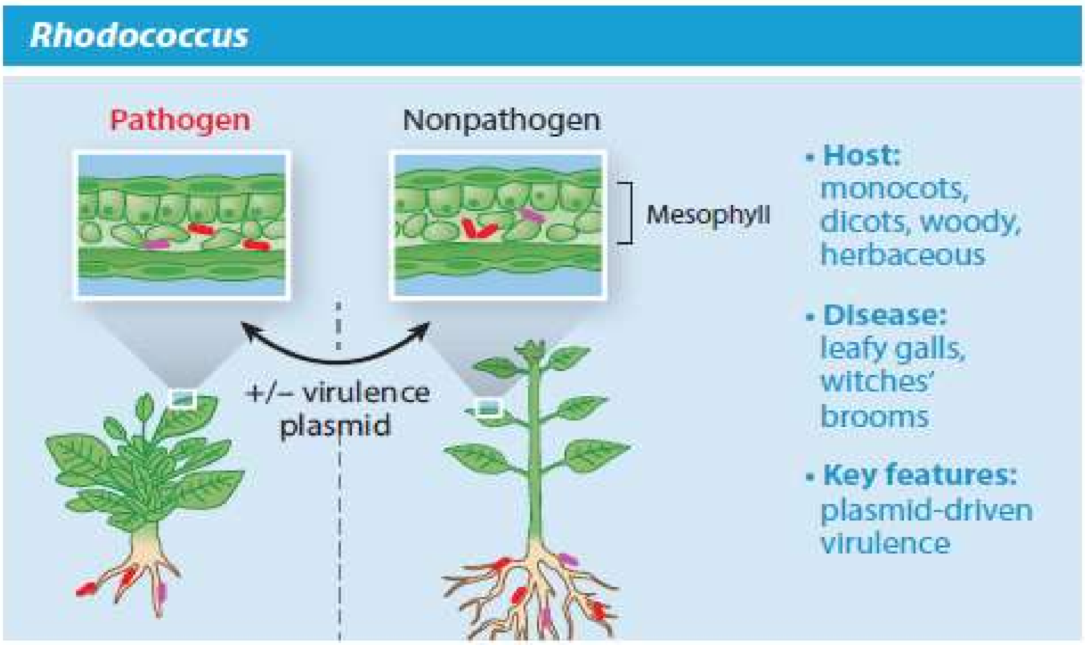

Fig. 4 : *Rhodococcus*: Root and Leaf-Associated Bacteria

The *Rhodococcus* genus is genetically diverse, and its members have a cosmopolitan distribution. They are mycolic acid-containing bacteria and have enzymes belonging to the carbohydrate esterase family 1 (CEI) that are necessary for the biosynthesis of their cell walls. *Rhodococcus* has been predominantly studied for their potential uses in bioremediation and biofuels. *Rhodococcus* cause proliferations of differentiated shoots called leafy galls or witches' brooms. Pathogenic *Rhodococcus* can be distinguished from nonpathogenic lineages by the unique presence of a cluster of three virulence loci. In the majority of sequenced pathogenic lineages, these virulence loci are harbored on a plasmid (Hwang *et al*., 2017)[4].

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❖ ***Rathayibacter :*** **Gumming disease of grasses**

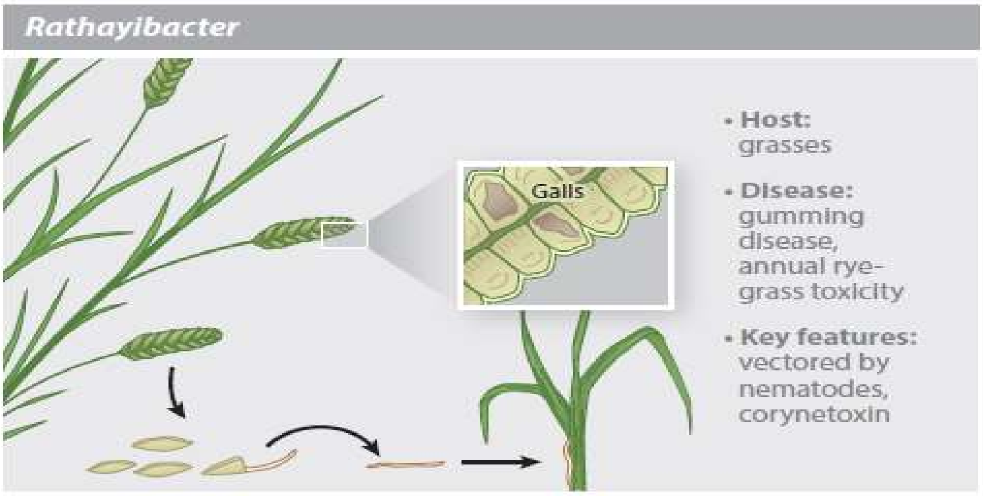


Fig. 5 : *Rathayibacter :* Gumming disease of grasses

Rathayibacter genus cause gumming disease of grasses. These bacteria are vectored by juvenile parasitic nematodes of the subfamily Anguininae and gain access to the ovaries of grasses. The bacteria are hypothesized to require the nematode to first establish seed galls that they then inhabit, as it is not as capable of colonizing plant hosts when inoculated directly. *Rathayibacter* may then outcompete the nematode to occupy the pre-established gall. The relatively low inventory of CAZymes may reflect the reliance of *Rathayibacter* on nematodes for plant colonization.

Rathayibacter toxicus is the most infamous species of *Rathayibacter*. Grazing animals that consume grasses contaminated with *R. toxicus* can succumb to annual ryegrass toxicity, which is associated with often lethal neurological disorders. This toxicity is due to corynetoxin, a member of the tunicamycin family of nucleotide antibiotics that inhibit the first step of protein glycosylation in eukaryotes.

❖ ***Leifsonia*** **: A Group of Xylem-Limited Bacteria**

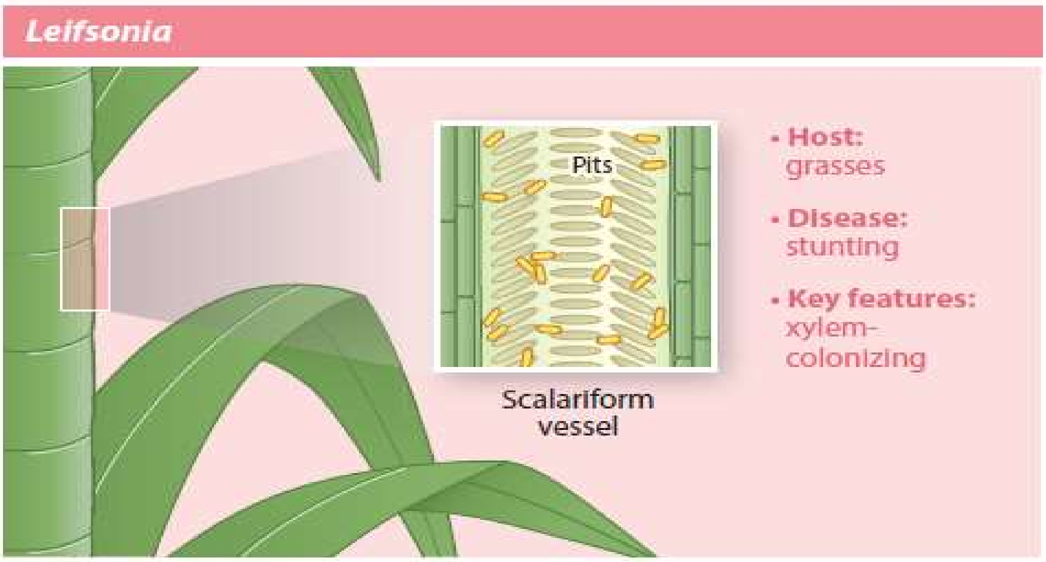


Fig 6 : *Leifsonia* : Xylem-Limited Bacteria

There are 11 species of *Leifsonia*, with only two subspecies of *Leifsonia xyli* recognized as plant pathogens. *L. xyli* subsp. *xyli* is the causal agent of ratoon stunting disease of sugarcane and to significant economic losses worldwide. Pathogen adheres to the surface of scalariform xylem vessels and interferes with water transport, leading to the reduction of height and diameter of sugarcane. *L. xyli* subsp. *cynodontis* colonizes the xylem of Bermudagrass, causing Bermudagrass stunting disease. Given that these pathogens and those in the genus *Curtobacterium* are similar to *Clavibacter* species in having xylem-limited lifestyles, it was somewhat unexpected that the examined genome sequences had so few predicted CAZymes. However, the decay in the genome of *L. xyli* subsp. *xyli* and the fastidious nature of this pathogen could also account for the lower number of predicted CAZymes. In many grasses, *L. xyli* subsp. *cynodontis* is an endophyte (Thapa *et al*., 2019)[5].

❖ ***Curtobacterium*: Xylem-Limited Bacteria**

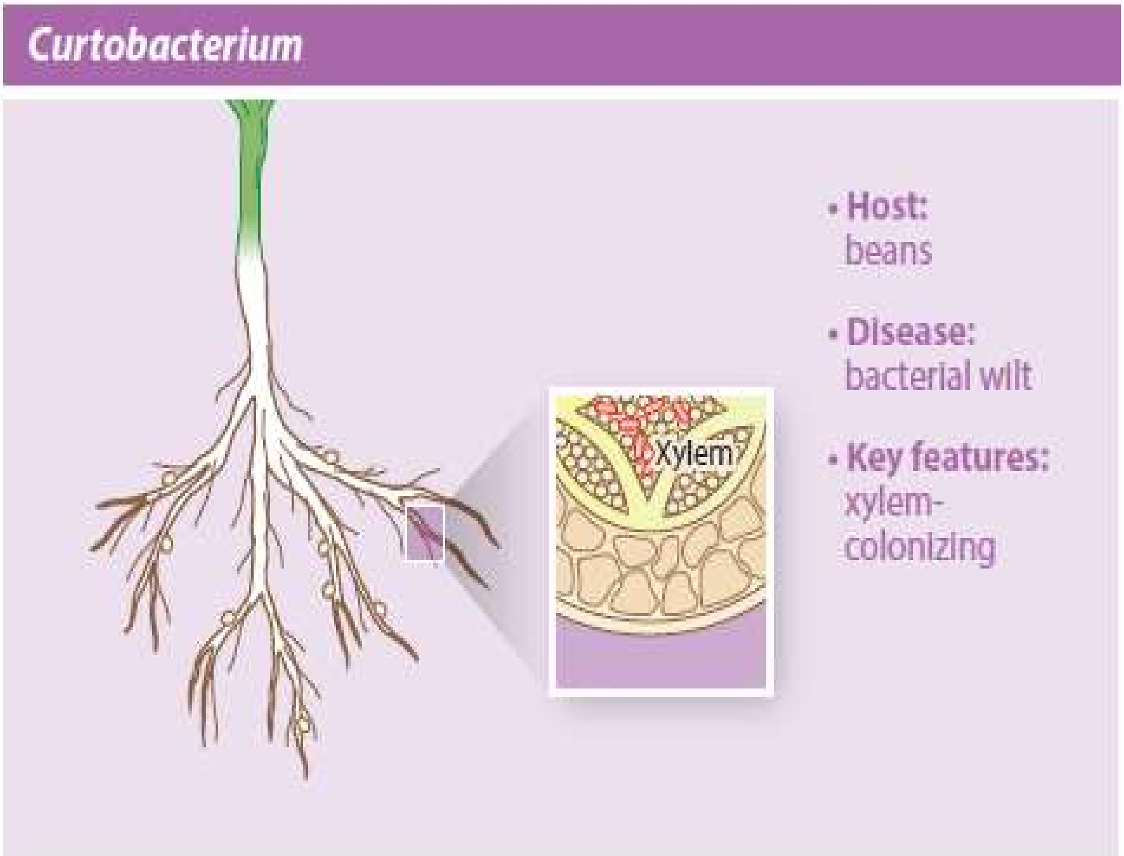
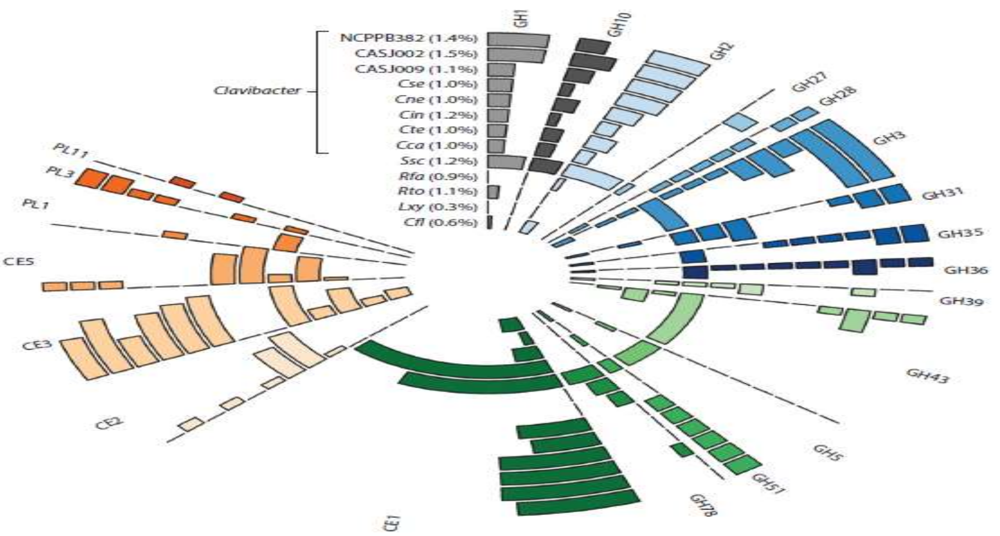


Fig. 7 : *Curtobacterium*: Xylem-Limited Bacteria

Curtobacterium flaccumfaciens is a seed-transmitted vascular pathogen causing bacterial wilt of common bean. This disease is the most important bacterial disease of dry beans in the United States and is a quarantined pathogen in many countries. Like other groups of bacteria discussed in this review, the outcomes of the symbioses of members of *Curtobacterium* with plants are variable.

Fig 8 : Distribution of predicted plant cell wall–degrading enzymes across Gram-positive bacteria

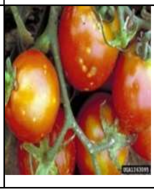


Clavibacter michiganensis subsp. *michiganensis* (NCPPB382, CASJ002), *Clavibacter* tomato endophyte (CASJ009), *Clavibacter sepdonicus* (Cse; ATCC33133), *Clavibacter nebraskensis* (Cne; NCPPB2581), *Clavibacter insidiosus* (Cin; R1–1), *Clavibacter tesellarius* (Cte; DOAB 609), *Clavibacter capsici* (Cca; PF008), *Streptomyces scabiei* (Ssc; 87.22), *Rhodococcus fascians* (Rfa; D188), *Rathayibacter toxicus* (Rto; WAC3373), *Leifsonia xyli* (Lxy; CTCB07), and *Curtobacterium flaccumfaciens* (Cfl; UCD-AKU). The percentage of predicted enzymes was calculated relative to the total number of encoded proteins. The bars represent total numbers of predicted enzymes within each category and scale from 1 (shortest bar) to 32 (longest bar) predicted enzymes.

Abbreviations: CE, carbohydrate esterase; GH, glycoside hydrolase; PL, polysaccharide lyase.

Table 2: Difference between the Gram positive plant associated bacteria

General characters	***C. michiganensis ssp. michiganensis***	***Streptomyces scabies***	***Rhodococcus fascians***	***Rathayibacter tritici***	***Leifsonia. xyli subsp. xyli***	***Curtobacterium flaccumfaciens***
Colony color	Yellow to orange 	White to creamy white 	Cream to yellow 	Yellow color 	Creamy white 	Yellow color 
Ecology	Seed and soil associated	Soil and tuber associated	Phylloplane associated	Phylloplane associated	Sett associated	Phylloplane associated

Virulence factor	pCM1 and pCM2 plasmids	Thaxtomin, *nec1* and tomA genes	Fas and att genes	Corynetoxin	CAZymes	CAZymes
Host	Tomato, potato and pepper	Potato	Tobacco	Rye grass and wheat	Sugarcane and bermuda grass	Common bean
Symptoms						

Evolution of Virulence

Horizontal gene transfer (HGT):

HGT was first discovered in 1928 by Grifith, who demonstrated that virulence factor was transferred between pneumococcal strains in mice. Horizontal gene transfer (HGT) refers to the transmission of genetic material across the genomes of biological organisms by processes other than fertilization. HGT is a universal phenomenon observed in bacterial, fungal, and eukaryotic genomes. However, it occurs infrequently and only serves as an alternative process for the exchange of genetic material between distantly related species. HGT is relatively more common in prokaryotes than eukaryotes. Studies have shown that approximately 81 % of genes have transferred through HGT, in 181 sequenced prokaryotic genomes.

The HGT phenomenon in endophytes, which highlights an aptent biological mechanism for their evolutionary adaptation within the host plant and simultaneously confers "novel traits" to the associated microbes, remains less studied. Studies in endophytic bacteria revealed its role in toluene biodegradation and disease reduction in wheat and corn

The mechanisms of HGT:

HGT differs from other mechanism of genetic transmission since it does not involve parent- offspring inheritance, and therefore, might occur between distantly related species. Furthermore, the transfer of genetic material is rapid, rendering it an important mechanism for microbial adaptation to new environmental niches. The HGT phenomenon has been widely studied in bacteria and archaeal genomes, however, this is not the case in eukaryotes.

HGT in the evolution of prokaryotes:

The role of HGT in prokaryotic evolution depends on the amount of genes transferred, their integration in microbial genomes, and the phylogenetic relationship between different species. Successful HGT is mediated by the transduction, conjugation, and transfection processes and depends on the stable integration of the transferred genetic material. The differences between the transferred genes might be observed due to the barriers limiting gene transfer and various selective forces acting on HGT.

Conjugation:

The process of transfer of DNA from donor to recipient via direct contact between donor cell to recipient cell.

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- In 1946 Lederberg and Tatum discovered conjugation in *E. coli*. In conjugation the donor bacterium contains conjugative or mobilizable plasmid.
- Recipient cells have specific receptors-site for conjugation and donor cells have special appendages called sex pilli.
- Minute portion of the donar DNA passes through the sex pilus into the recipient cell.
- The recipient cell after accepting the DNA divides by.

Transformation:

- The process by which DNA released by the donor bacteria into the environment is acquired by the recipient bacteria.
- Fredrick Griffith first time demonstrated in *Streptomyces pneumonia*.
- The compatibility is due to the presence of specific proteins in the recipient cell.

Transduction:

- The process of DNA transfer from donor cells to recipient cell with the help of virus bacteriophages.
- The bacteriophages acquire the bacterial DNA at the time of packaging and when such phages infect another bacterial hosts the recombination takes place in bacterial genome.

Mechanisms of infection by Plant associated bacteria

Enzymes, toxins, and growth regulators, probably in that order, are considerably more common and probably more important in plant disease development than polysaccharides. It has also been shown that some pathogens produce compounds that act as suppressors of the defense responses of the host plant. Among the plant pathogens, all except viruses and viroids can probably produce enzymes, growth regulators, and polysaccharides.

Enzymes Pectinases and pectolytic enzymes are pectin methyl esterases (PME's), polygalactouronases (PG's) and pectin lyases (PL's).

- **Pectin methyl esterases** (PME's): Breaks ester bonds and removes methyl groups from pectin leading to the formation of pectic acid and methanol (CH3OH).
- **Polygalacturonases** (PG's) : Split pectin chain by adding a molecule of water and breaks the linkage between two galacturonan units. These enzymes catalyze reactions that break a- 1,4-glycosidic bonds.
- **Pectin lyases** (PL's) : Split pectin chain by removing a molecule of water from the linkage, thereby breaking it and releasing products with unsaturated double bonds. These pectin enzymes can be exopectinases (break only terminal linkage) or endopectinases (break pectin chain to random sites). Pectin degradation results in liquefaction of the pectic substances and weakening of cell walls, leading to tissue maceration. Ex: Soft rot bacterium, *Erwinia caratovora* subsp. *caratovora*
- **Cellulose**: Cellulose is a polysaccharide, made Cellulose is insoluble in crystalline form (native form), and soluble in amorphous form (modified cellulose). The enzymatic breakdown of cellulose results in final production of glucose molecules.

- **Cell wall proteins**: Cell wall proteins are similar to other proteins, except that they are rich in aminoacid, hydroxy proline. Five classes of structural proteins are found in cell walls: extensins, proline-rich proteins (PRP's), glycine-rich proteins (GRP's), Solanaceous lectins and arabinogalactan proteins (AGP's). Proteins are degraded by means of enzymes, proteases or proteinases or peptidases.
- **Lipids**: Various types of lipids occur in all plant cells. The most important ones are phospholipids and glycolipids. These lipids contain fatty acids, which may be saturated or unsaturated. Lipolytic enzymes, called lipases (phospholipases, glycolipases) hydrolyze lipids and release fatty acids.
- **Starch**: Starch is the main reserve polysaccharide found in plant cells. It is a glucose polymer and exists in two forms: amylose, a linear molecule and amylopectin, a highly branched molecule. Starch is degraded by enzyme.

The term toxin is used for a product of the pathogen, its host, or pathogen host interaction which even at very low concentration directly acts on living host protoplasm to influence disease development or symptom expression.

Examples: Tabtoxin or wild fire toxin: *Pseudomonas tabaci*

Phaseolotoxin: *Pseudomonas syringae* pv. *Phaseolicola*

Different secretion systems in plant pathogenic bacteria

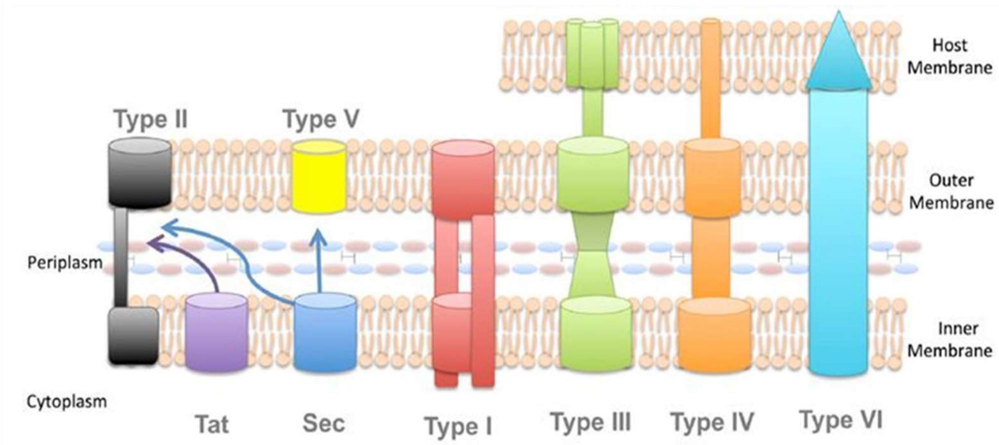

Fig. 9: Secretion systems in plant pathogenic bacteria

Type I-SS is present in almost all plant pathogenic bacteria and carries out the secretion of toxins such as hemolysins, cyclolysin, and rhizobiocin. They consist of ATP-binding cassette (ABC) proteins and are involved in the export and import of a variety of compounds through energy provided by the hydrolysis of ATP.

Type II-SS is common in gram-negative bacteria and is involved in the export of various proteins, enzymes, toxins, and virulence factors. *Ralstonia* and *Xanthomonas*, which have two type II-SS per cell, use them for secretion outside the bacterium of virulence factors such as pectinolytic and cellulolytic enzymes.

Type III-SS is the most important in terms of pathogenicity of the bacteria in the genera *Pseudomonas, Xanthomonas,* and *Ralstonia*. The primary function of type III-SS is the transport of effector proteins across the bacterial membrane and into the plant cell. Genes that encode protein components of the type III-SS apparatus have a two-third similarity at the amino acid level and such genes are called hypersensitive response conserved (Hrc) genes.

Type IV-SS transports macromolecules from the bacterium to the host cell. The proteins transferred are very similar to those responsible for the mobilization of plasmids among bacteria (Tiwari and Bae, 2020)[6].

Type V-SS autotransporter is found in *Xylella* and *Xanthomonas* and contains genes that encode surface-associated adhesins. Similar autotransporters exist in mammalian pathogens and are important for adhesion to epithelial cells.

Type VI-SS John Mekalanos in 2006 identified type VI secretion system in *Pseudomonas aeruginosa* and *Vibrio cholera.* The type VI-SS is recently characterized secretion system that appears to constitute a phage tail spike like injectisome that has the potential to introduce effector proteins directly into the cytoplasm of host cells, analogous to the Type III-SS and Type IV-SS machineries.

Type VII-SS Mycobacteria use type VII SS to secrete proteins across their complex cell envelope. It is also known as the ESX pathway in mycobacteria. Although, Gram positive bacteria have only a single membrane, some species, most notably the mycobacteria, have a cell wall that is heavily modified by lipids, called a mycomembrane. As a result, the genomes of these species encode a family of specialized secretion systems collectively called type VII-SS.

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Chapter- 13

Rhizosphere Engineering: An Efficient Strategy for Sustainable Agriculture

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Abstract

The rhizosphere inhabits multitude of microorganisms. The intricate plant–microbe community is of paramount significance for crop health. Manipulating or engineering the rhizosphere- microenvironment plays an crucial role in sustainable agriculture *via* shaping the plant health and nutrient availability. Rhizosphere engineering is a holistic and sustainable approach to agriculture that leverages the interactions between plants, roots and soil microorganisms to enhance crop productivity while minimizing adverse environmental impact. It aligns with the principles of precision agriculture and ecological sustainability, making it a promising strategy for the future of agriculture. By modifying soil properties and fostering specific microbial populations, this approach holds promise in reducing dependency on synthetic fertilizers and minimizing environmental impacts. This chapter highlights the multifaceted aspects of manipulating the rhizosphere, emphasizing its potential to revolutionize the approach to plant-soil interactions.

Keywords: Rhizosphere engineering, rhizosphere, microbiome, PGPR

Introduction

Food insecurity is a severe problem that is projected to get worse when the world's population approaches nine billion people by the year 2050 (Kumar and Dubey, 2020). The world's food supply has been negatively impacted by the fast growth in human population, biotic and abiotic stressors brought on by climate change, and land shortages. Because of these, global agriculture is under tremendous pressure, making its sustainability crucial.

The plant microbiome represents an area of active ongoing research efforts and represents a promising approach to increase crop productivity and optimize agricultural management strategies (Li *et al*., 2020; Taye *et al*., 2019). According to Bakker *et al.* (2012), the functioning of soil microbial communities, particularly those in the rhizosphere, is crucial to agricultural systems' productivity. Research has demonstrated a robust correlation between rhizosphere microbiome assembly and plants. The rhizosphere inhabits thousands of species of microorganisms including bacteria, fungi, archaea, algae, protozoa, viruses etc. The intricate plant–microbe community is of paramount significance for crop health (Li *et al.*, 2020). Through improving nutrient uptake, preventing stress (biotic and abiotic), releasing compounds that promote plant growth, and triggering a systemic immune response, they control plant productivity and health. These rhizosphere's microbial activity influences the microbial component by altering the root exudates that plants emit, including their composition, quality, and amount (Philippot et al., 2013), resulting in the selective recruitment of associated microbiome members, which helps them to cope up with numerous adverse climatic conditions.

This complex cocktail of root-secreted exudates mediate the interactions occurring in the rhizosphere and acts as chemo attractants and repellants to shape the root microbiome and also through adjustments to osmotic pressure, ionic balance, and redox potential, this intricate mixture of molecules plays a role in altering the chemical and physical properties of the soil. (Philippot *et al.*, 2013). The intimacy of this interface between plants and their environment is essential for the acquisition of water and nutrients and for beneficial interactions with soil-borne microorganisms. Hence, understanding the structure and function of the rhizosphere will allow us to tackle plant–microbe interactions precisely, which may act to increase plant health and productivity. Modifying the rhizosphere helps to increase beneficial microbes that increase nutrient availability and reduce biotic and abiotic stresses, as a result, it increases plant health. Therefore the creation of a "biased rhizosphere" through rhizosphere engineering is a novel approach to meet sustainable agricultural production under adverse climatic conditions (Savka *et al*., 2013; Bano *et al*., 2021).

Rhizosphere Engineering

Rhizosphere engineering aims to direct the plant-microbe interaction towards multitude of enhanced beneficial outcomes including mineralization and organic matter decomposition, nutrient cycling, tolerance to drought, salinity, temperature, other abiotic stresses and resistance to diseases (Hakem *et al*., 2021). This may ultimately reduce our reliance on agrochemicals by replacing their functions with beneficial microbes, biodegradable biostimulants or transgenic plants.

The three key components of the rhizosphere are plants, microbes and soil (Hakem *et al.*, 2021). Plant productivity can be increased by engineering or manipulating any one of these or all of these three elements. The two millennia-old practice of adding soil amendments can influence the rhizosphere functioning for plant growth promotion. Soil amendments such as biochar, silicon (Villegas *et al*., 2017), zeolites (Jakkula and Wani, 2018), plant residues, coal fly ash, cattle manure, and sewage sludge have been used (Dessaux *et al*., 2016). Plant engineering is beneficial for soil restoration, plant growth, nutritional quality, and pathogen resistance, but its application remains limited because of lack of social acceptance and concerns regarding environmental sustainability and human health (Hakem *et al.*, 2021).

Another component, plant-growing rhizobacteria have been engineered to inhibit the synthesis of stress-induced hormones like ethylene, which slows root growth, as well as to produce antibiotics and lytic enzymes active against soil-borne root pathogens. (Bano *et al.*, 2021). By introducing beneficial microorganisms or fostering the growth of specific microbial strains, researchers seek to establish a symbiotic relationship between plants and microbes. Finally, rhizosphere engineering aims at manipulating the rhizospheric microbes and/or plants in such a way that beneficial PM interaction supersedes harmful ones leading to increased productivity (Hakem *et al.*, 2021). Beyond agriculture, rhizosphere engineering holds promise for environmental remediation and ecosystem restoration. The deliberate modification of the rhizosphere can enhance plant tolerance to pollutants, facilitate soil remediation, and contribute to the establishment of resilient ecosystems in degraded areas.

There are mainly three approaches for rhizosphere engineering. They are:

- ✓ Microbiome based rhizosphere engineering
- ✓ Plant based rhizosphere engineering
- ✓ Meta Organism based rhizosphere engineering

Microbiome-based approach of rhizospheric engineering

Engineering and manipulation of the microbiome is one of the most important strategies for engineering the rhizosphere. (Del *et al.*, 2022). Microbes are a promising solution for sustainable agriculture because they can positively affect plant growth and mitigate the majority of the issues involved in contemporary agriculture. A diverse microbial community in the rhizosphere is often associated with improved plant health and resilience. Engineering strategies may aim to enhance microbial diversity by promoting the growth of beneficial microorganisms. The ability to manage and manipulate the entire rhizosphere microbiome is limited due to the complexity of the microbiome; however, the most effective and sustainable way to alter the microbiome is the inoculation of artificially multiplied microbes. (Del *et al.*, 2022). Several commercially synthesized products, comprising one or more strains of bacteria or fungi in the form of biofertilizers, are accessible to enhance plant growth and promote sustainability.

In this regard, next-generation sequencing and advanced quantitative and qualitative molecular methods such as qPCR, TRFLP, FISH and DNA arrays have allowed scientists to develop a better understanding of the structural and functional composition of soil microbial communities (Del *et al.*, 2022). Such advanced technologies can be used to identify and isolate the suitable microbial targets that are the primary drivers for the associated host plant fitness (Shayanthan *et al.*, 2022). Only a small percentage of the rhizosphere microbiome's plant-growth-promoting rhizobacteria are found to be pathogenic or harmful to plants; the majority of these microbes interact beneficially with plants and support their growth and survival (Kumar and Dubey, 2020). By increasing the availability of nutrients to plants, inhibiting pathogens, and enhancing soil structure, these PGPR bacteria contribute significantly to plant productivity and, in turn, play a crucial role in sustainable crop production. They are also utilized in the bioremediation of contaminated soils and mineralize organic pollutants (Bibi *et al.*, 2018).

Further, strategic manipulation of the rhizospheric microbiome requires an advanced understanding of the mode of action of beneficial root-associated microbiota and identification of key root exudates, which can play integral roles in mediating plant disease suppression and crop improvement (Pascale *et al*., 2020). Recently, the focus on the microbiome-based approach of rhizospheric engineering has undergone a paradigm shift from the application of bioinoculants to modulating of rhizospheric soil microbiome majorly by two approaches, viz. application of synthetic microbial communities/consortia (SMC) (Shayanthan *et al*., 2022). or acclimatization of the rhizospheric soil microbiome to any adaptive trait using plant-mediated artificial selection/multi-passaging approach over successive plant growth cycles.

Modernized approach: constructing a synthetic microbiome

Synthetic community development, which refers to the controlled design and establishment of microbial communities with specific functions, is one option for mitigating the uncertainties associated with consortia inoculation (Shayanthan *et al*., 2022). The objective is to promote an increase in community stability through synergistic interactions between its members. SynComs, are usually constructed following top-down approach (engineering of plants to release specific inducers of microbial genes and/or modify the rhizobiome populations) and bottom-up approach (manipulating the resident microbiota). The fundamental idea is to retain some of the crucial interactions between the microorganisms and their hosts while simplifying the original microbial community (Shayanthan *et al*., 2022). Synthetic communities, so-called SynComs, are usually constructed following a bottom-up approach (Tosi *et al*., 2020) involves the identification of keystone microbial taxa, interactive network development and then the use of combinations of microbial isolates as a composite inoculum.

In this regard, based on functional screening of 1,893 microbial strains isolated from root-associated compartments of soybean plants, Wang et al. (2021) built three synthetic communities (SynComs). Significant plant growth and nutrient uptake were facilitated by the functional assembly of SynComs in both nitrogen/phosphorus nutrient deficiency and sufficiency scenarios. According to additional field trials, the application of SynComs significantly and steadily increased plant growth, improved the uptake of nitrogen and phosphorus, and ultimately raised soybean yield.

Plant mediated rhizosphere engineering:

"Plant-mediated rhizosphere engineering" refers to the manipulation and modification of the rhizosphere—the soil region influenced by plant roots—through various plant-related activities. Principle involved in plant mediated rhizosphere engineering is manipulating the plant and its production of exudates in order to recruit a desirable or beneficial microbiome (Dubey *et al*., 2021). Engineered plants secrete specific exudates that attract specific microbes. Engineered plants release specific inducers of microbial genes, which have an effect not only in stimulating plant growth but also on the health of the plant and the soil through pollutant remediation (Gunarathne *et al*., 2019). “Rhizodeposition”, term refers to the process by which plants deposit organic materials into the rhizosphere, influencing the microbial community and nutrient cycling. The composition of rhizodeposits can vary among plant species and can have specific effects on soil microbial communities.

Plants can be directed to secrete specific exudates that attract specific microbes. Plant cultivars, or “designer plants”, are created by incorporating breeding/engineering strategies to attract and maintain a stable and efficient microbiome and, ultimately, tolerance against abiotic stress such as drought and salinity, as well as biotic stress such as pathogens (Del *et al.*, 2022). A study compared plant cultivars with varying levels of resistance to wilt-causing diseases and revealed that some disease suppressant bacteria were more abundant in the rhizosphere of resistant cultivars (Lebeis *et al.,* 2015). Plant rhizosphere may be modified by engineering plants to release microbial signal molecules like isoflavonoids or lipooligosaccharides which induce microbial gene expression in the rhizosphere (Dubey *et al*., 2021). This method can be effectively utilized in ensuring nodule occupancy by the appropriate rhizobial species in leguminous crop plants by utilizing nodule-specific compounds as growth enhancers (Savka *et al*., 2002).

Plant-mediated strategies involve the manipulation of plant characteristics through two distinct methods: genetic engineering and plant breeding. It's an intriguing strategy to use plant breeding techniques to select a particular microbial community because the primary goal of this technique is to boost crop yield by giving plants resistance to a range of stresses (Dubey *et al*., 2021). Therefore, when microbiome selection was incorporated into plant breeding programs, important taxa and functions were targeted. The quantitative trait locus (QTL) mapping technique can be used to search the host genome for specific gene pools associated with distinct phenotypic traits (Dubey *et al*., 2021). Once a suitable genes pool determined in the plant genome, advanced genome editing tools such as site-specific transcription activator- like effector nucleases (TALENs), zinc finger nucleases (ZFNs) and clustered repeatedly interspaced short palindrome repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) can be incorporated for site-directed manipulations to modify the traits for recruiting the desired rhizosphere microbiome (Dubey *et al*., 2021). These tools are used to target key genes involved in biosynthetic and metabolic pathways at the transcriptional and translational levels. Numerous success stories have demonstrated that the CRISPR/Cas9 system is the most advanced technology for knocking out or inserting key genes across multiple plants. Bano *et al.* (2021) successfully developed wheat cultivar resistant to powdery mildew disease by knocking out the *TaMLO* gene (associated with fungal pathogens colonization) found in its protoplasts. Many more achievements have been made with the CRISPR/Cas9 system as a genome editing tool, and there is still much more to come.

Meta Organism based rhizosphere engineering

The multicellular eukaryotic organisms together with their associated microbial components have been referred to as ‘holobiont' (Compant *et al*., 2019). In plants, the microorganisms are acquired by vertical transmission from the host plant to its offspring via seeds and/or by horizontal transmission from the soil environment. The associated microbial population is referred to as ‘second genome' of the plant because of their significant contributions to affecting the essential life traits of the host plant and determining its overall fitness (Compant *et al*., 2019). The metaorganism-based approach involves co-engineering of plants as well as the associated microbial communities for facilitating a particular metabolic pathway. For example, plants are engineered to produce compounds and inoculated bacteria are engineered to degrade these compounds.

Challenges and future Perspectives

While the potential benefits of rhizosphere engineering are evident, challenges remain. The complexity of plant-microbe interactions, the variability in soil conditions, and the long-term effects of these interventions require further exploration. Future research should focus on refining techniques, understanding ecological consequences, and developing practical applications for diverse agricultural and environmental contexts.

Conclusion

The literature suggests that rhizosphere engineering produces efficient and sustainable crop yields to meet the needs of an expanding global population at a time when agriculture is confronted with a variety of environmental challenges. Rhizosphere engineering stands at the forefront of innovative approaches to sustainable agriculture and ecosystem management. Therefore, the emerging field of rhizosphere and ecosystem engineering offers an exciting and powerful opportunity to attain sustainability in agriculture and microbiome-based rhizosphere engineering showed immense potential in offering services toward plant ecosystem productivity. Before using PGPR inoculants in intensive farming practices, it is imperative to comprehend all of their key features. As research in this field advances, the prospect of harnessing the power of the rhizosphere for improved plant growth and ecological sustainability becomes increasingly promising.

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Chapter- 14

New Insights into the Role of Siderophores as Triggers of Plant Immunity

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Abstract

Iron deficiency hinders several metabolic and physiological aspects in plants and human beings. The crucial role of iron has been well-acknowledged for several redox reactions of different physiological mechanisms of plants like respiration and photosynthesis mediated electron transport systems. In the presence of heavy metals, it causes destruction in membrane bound ferric reductase enzyme, and thereby declines the Fe uptake in plant. This Fe deficiency exhibits young leaf chlorosis. Hence, inoculation of plants with siderophore producing bacteria prevent iron deficiency even under heavy metal polluted conditions. Siderophores facilitate several functions of plants such as respiration, photosynthesis bioremediation.

Keywords- Siderophores, Bioremediation, Fe uptake, Defense

Introduction

The adequacy of the agricultural soil deteriorates owing to exposures to adverse environmental conditions such as salinity, drought, heavy metal stress, etc., which induces plant stress and reduces plant growth productivity which will trigger food scarcity in the future. Iron deficiency is a communally observed phenomenon in CaCO3 (calcium carbonate)-rich desert soil at high pH. The availability of iron in soil mostly depends on the range of pH, and the character trait of saline (pH 7.2–8.5) and alkaline (pH > 8.5) soil (Aguado-Santacruz *et al*., 2012)[1] showed iron deficiency due to less solubility of iron at high pH.

The plant growth reduction mediated stress due to nutrient imbalance in degraded soil is a common phenomenon; the increase of degraded soil due to salinity, drought, heavy metal, *etc.* are reported by several workers. Out of numerous detrimental factors, the lack of available iron for the plant is one of the major factors. Several pieces of research demonstrated that plant growth-promoting rhizobacteria (PGPR) may be a promising tool for mitigating the adverse effect of degraded lands; for instance, in saline soil drought conditions and heavy metal conditions.

Importance of Iron in Plant growth

Iron (Fe) is an essential element for the growth of almost all living microorganisms because it acts as a catalyst in enzymatic processes, oxygen metabolism, electron transfer, and DNA and RNA syntheses. Soil degradation is a natural and anthropogenic phenomenon that reduces soil nutrients, mediated by soil salinization, drought and heavy metals contamination. Iron plays a crucial role in chlorophyll biosynthesis by maintaining electron flow in CO2 fixation through (PS)-II-b6f/Rieske (PS)-I complex.

Plant stress under Iron deficient condition

Iron deficiency hinders several metabolic and physiological aspects in plants and human beings. The crucial role of iron has been well-acknowledged for several redox reactions of different physiological mechanisms of plants like respiration and photosynthesis mediated electron transport systems. Iron also participates in several enzymatic activities such as peroxidase, catalase, cytochrome, oxidase, *etc*. Also, Fe plays the role of a co-factor in the synthesis of many plant hormones like ethylene and ACC deaminase.

Iron plays a crucial role in chlorophyll biosynthesis by maintaining electron flow in CO2 fixation through (PS)-II-b6f/Rieske (PS)-I complex. Iron plays a remarkable co-factor in the electron transport chain of plant photo-system. In photosystem (PS)-I, iron is required to form three 4Fe-4S in clusters, Cytochrome-b6f (Cyt-b6f) requires iron for Risk subunits as a cluster of 2Fe-2S, and photosystem (PS)-II requires iron as a cofactor for cytochrome.

Iron is essential for leghemoglobin and nitrogen-fixing machinery in the leguminous plant. The deficiency of Fe leads to several disorders in the plant by altering the redox and enzymatic reactions and shows primarily a symptom of wilting and chlorosis which leads to a lowering of plant growth and productivity (Fig. 1). Several researchers reported that root growth of the plant is hindered under Fe deficient soil, altering the function of the gene responsible for iron uptake. Iron stress also triggers a plant's reactive oxygen species-mediated Fenton reaction. The Fenton reaction elaborates the interplay of Fe^{2+} and H_2O_2 (hydrogen peroxide) to generate hydroxyl radical ($OH*$), which is one of the reactive oxygen species.

Iron stress also leads to necrosis in tissue, blackening of roots, and an overall decrease in plant growth. Degraded soil due to salinity increases ionic and osmotic stress and reduces plant growth and productivity. Ionic stress induces the influx of Na^+ ions, resulting in the efflux of K^+ ions in soil, while osmotic stress accumulates the NaCl concentration in the rhizospheric soil. Soil salinity induces nutrient imbalance and iron deficiency. Both iron and NaCl stresses induce reactive oxygen species (ROS), which directly causes injury to the plant tissue and salinity damages the base and cross-correlation of double-stranded DNA.

More salinity and iron deficiency affect the morphological traits such as a decrease in root length, plant size, variety of leaves, flowering of plants, and decrease in the plant's pigment chlorophyll content, resulting in reduced photosynthesis, hence poor plant growth reduces the crop productivity. So, to overcome all these adverse condition PGPR plays important role by

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enhancing the nutrient uptake from the soil by different strategies *viz.,* P-solubilization, siderophore production, ACC deaminase *etc*.

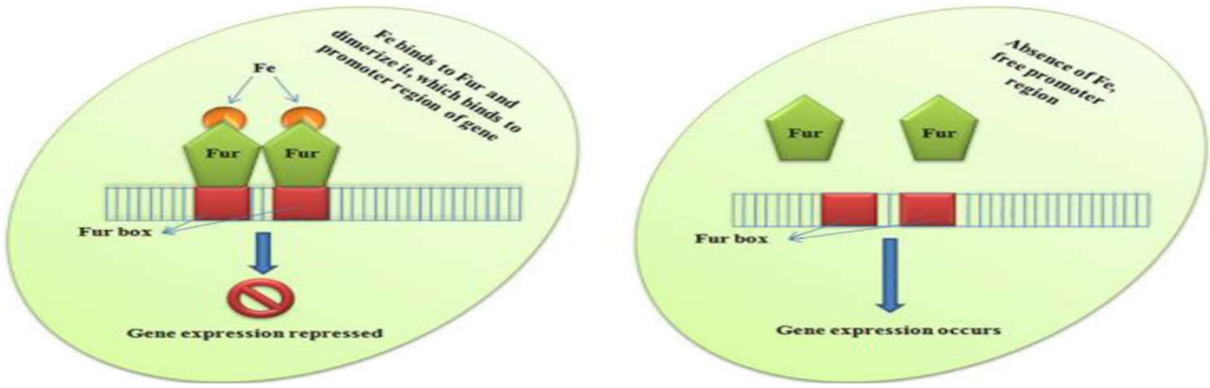
What is siderophores?

Siderophores facilitate several functions of plants such as respiration, photosynthesis bioremediation plant growth promotion and phytoremediation of heavy metals. Siderophores are also produced by non-ribosomal peptides bonds and multidentate iron-chelating compounds that solubilize and chelate organic and inorganic forms of compounds in soil.

The term is derived from the Greek wordssidero meaning "iron" and phore meaning "carriers" or ironbearing compounds that uptake insoluble iron from different environmental sources. Primarily siderophore-producing bacteria release iron-binding proteins, such as permeases and ATPases, that chelate the ferric iron (Fe^{3+}) and transport Fe^{3+}ions in the cell membrane in gram-positive bacteria and disrupting osmotic balance, ultimately leading to cell lysis and the demise of the targeted insect (Alhendawi *et al*., 1997)[2].

Gene regulation of Siderophore production in Bacteria under Iron source

In Gram-negative bacteria, iron regulation is carried out by the Fur (Ferric iron- uptake regulator) protein. In many Gram-positive bacteria (*e g. B. subtilis*), Fur protein is also found. Fur protein in E. coli has a molecular weight of 17 K Da and acts as a transcriptional repressor and is activated by divalent iron which acts as a corepressor. When Fur protein binds to the Fur binding region ('Fur box' which is 19 bp- inverted repeat sequences and located at the upstream of the iron transport gene), it negatively regulates transcription of iron- transport gene.



Iron abundance condition **Iron deficient condition**

Fig 1- Gene regulation of Siderophore production in Bacteria under Iron source

Mechanism of siderophores in biocontrol of Plant Pathogens

Siderophore produced by a microorganism can bind iron with high specificity and affinity, making the iron unavailable for other microorganism or pathogen, thereby limiting their growth. Microbial siderophores may stimulate plant growth directly by increasing the availability of iron in the soil surrounding the roots or indirectly by competitively inhibiting the growth of plant pathogen with less efficient iron uptake system.

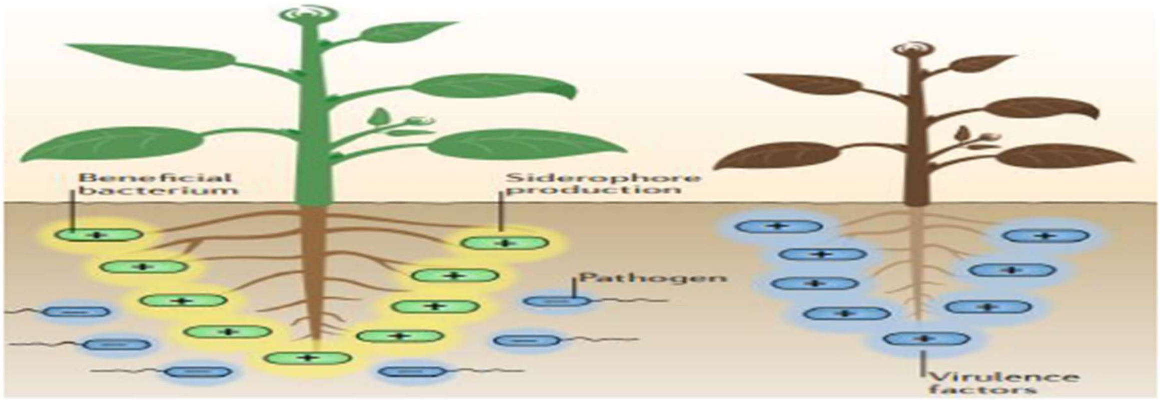


Fig 2- Siderophore-mediated plant protection

How fungal siderophores serve iron to plants?

Fungi that serve the plants for iron by four different mechanisms for siderophore mediated Fe transport systems (i) the shuttle mechanism – here the Fe3+-siderophore complex after coming in contact to the plant cell membrane, is crossed the cell membrane where the Fe3+ is dislodged from the ligand and is reduced by enzyme, and the siderophore becomes free and then comes back to outside and recycled *e.g*., transporting ferrichrome by *Ustilagomaydis* (Bhojiya *et al.,* 2022)[3], (ii) the taxicab mechanism, in this case the Fe3+is released from the membrane of plant from the extracellular sideropohorewhere it is transported across the cell membrane to intracellular ligands *e.g Rhodotorula* species (iii) the hydrolytic mechanism, here the whole Fe3+siderophore complex after coming in contact to plant membrane, is passed into the cell through membrane trans porter and Fe3+become free by several reductive and degradative processes (Crowley, 2006)[4].

Siderophore mediated social interactions

Cooperation: Because secreted molecules can be shared between cells, siderophore production is often considered a form of cooperation. Cooperative behaviours involve an actor generating benefits for another individual, with such interactions having been at least partly selected for this purpose.

Cheating: Although cooperation can generate substantial fitness benefits for all individuals involved, it might not be evolutionarily stable. The reason is simple: every cooperactive act can be exploited by cheaters that save the costs of cooperation but freeride on the cooperative benefits generated by others. The tug of war between cooperators and cheaters can result in the so-called tragedy of the commons in which cooperation, although maximizing group benefits, collapses due to exploitation by selfish cheaters.

Competition: The discussed scenarios of cooperation and cheating typically involve interactions between closely related individuals that share the same siderophore system. However, bacteria often live in diverse consortia with interacting strains that likely have different siderophores, each requiring a specific cognate receptor for iron uptake (Ermakova *et al*., 2019)[5].

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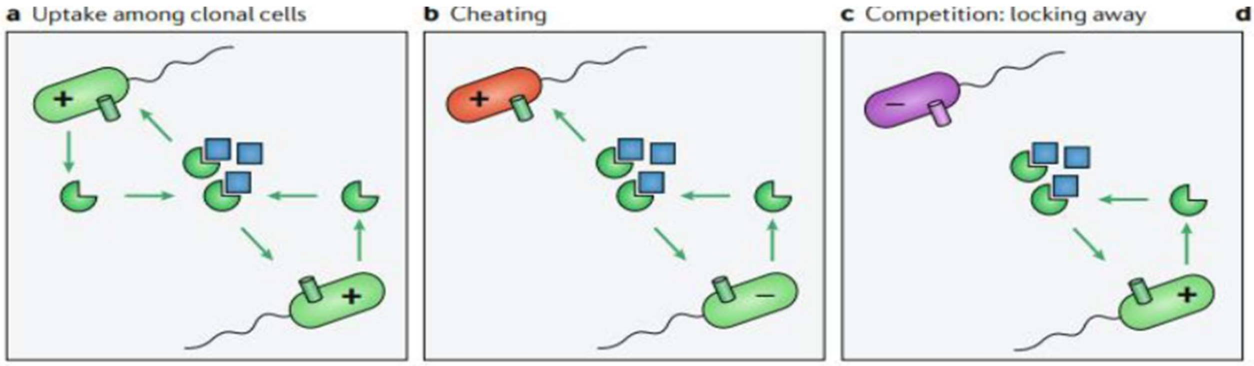
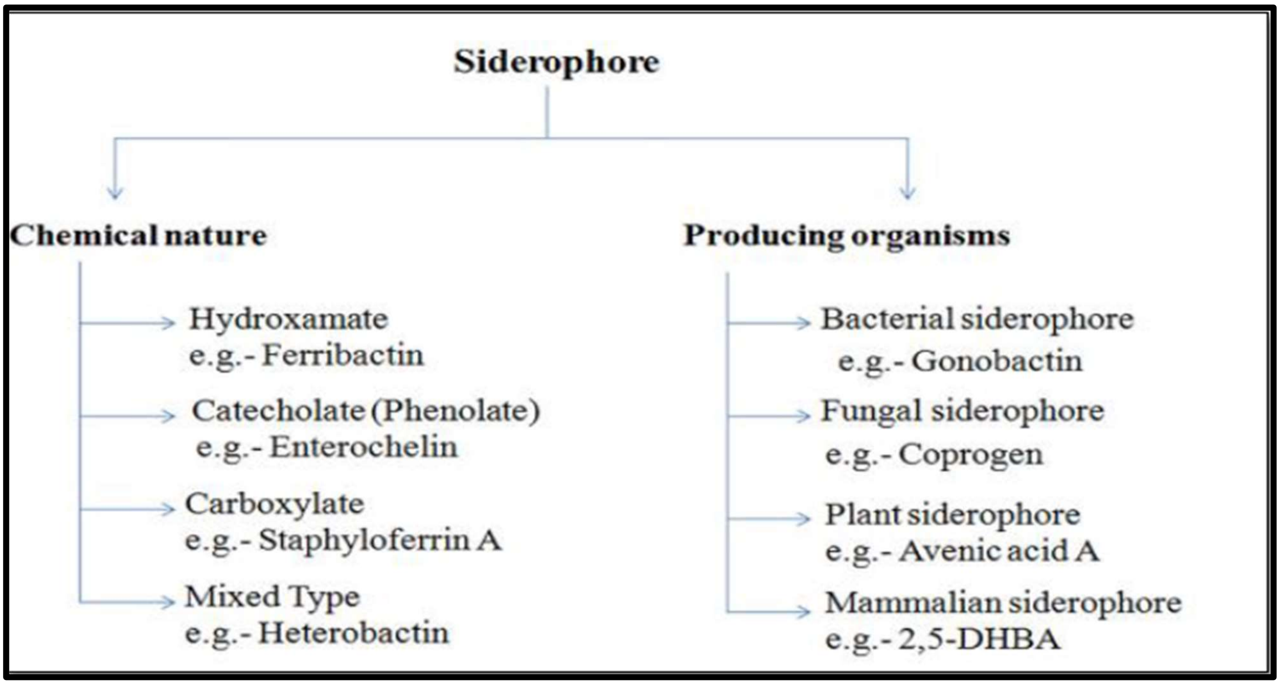


Fig 3 - Siderophore mediated social interactions

Classification of Siderophores



(A) Based on Chemical Properties and Structure

I. Hydroxamate Siderophore

Hydrophilic siderophores of bacteria are made up of acylated and hydroxylated alkylamines, whereas those of fungus are made up of hydroxylated and alkylated containing N5-acyl-N5-Hydroxyornithine or N6-acyl-N6-Hydroxylysine are the components. All other hydroxamates possess peptide linkage, with the exception of fusarinine C generated by *Aspergillus nidulans* (*e.g*., TAFC), which contains ester bonds (Lalitha and Nithyapriya, 2021)[6]. Between two oxygen molecules from each hydroxamate group and iron, a bidentate ligand formed. Each hydroxamate may form a hexadentate octahedral complex with ferric ion that has a binding constant between 1022-1032 M1 (Fig 4).

II. Catecholate Siderophore

It's solely found in bacteria. Catecholate and hydroxyl groups make up this molecule, which binds Fe3+ to neighbouring hydroxyls or catechol endings. It's made up of dihydroxybenzoic acid (DHBA) and an amino acid. Lipophilicity, complex stability and pHresistance are its unique characteristics. By supplying two oxygen atoms for iron chelation forming a hexadentate octahedral complex (Fig. 4).

III. Carboxylate siderophore

Few bacteria, such as *Rhizobium meliloti's* rhizobactin, and fungus such as members of the Mucorales family of Zygomycota possesses carboxyl and hydroxyl groups for iron uptake. It is made up of citric acid or β -hydroxyaspartic acid that binds to iron, like staphyloferrin A, which is made up of one D- ornithine and two citric acid residues joined by two amide bonds and excreted by *Staphylococcus aureus* (Fig. 4).

IV. Mixed Siderophores

Some species, such as *Rhosococcus erythropolis*, create mixed siderophores that include both catecholate and hydroxamate groups, such as heterobactin.

(B) Based On Producing Organism

I. Bacterial Siderophore

Enterobactin is a catechol siderophore produced by *E. coli* that has the greatest affinity for the Fe (III) ion of any known siderophore. *Streptomyces*, a Gram-positive spore-producing member of the *Streptomycacetaceae* family, generates desferrioxamine siderophores such as desferrioxamine G, B and E (Fig. 4).

II. Fungal Siderophore

Aspergillus fumigatus and *A. nidulans* generate 55 siderophores that are quite similar. *A. fumigatus* uses the hydroxamate siderophores fusarinine C and triacetyl fusarinine C to capture extracellular iron, ferricrocin, a hyphalsiderophore, for intracellular iron distribution and storage and hydroxyl ferricrocin, a conidial siderophore, for conidial iron storage, germination and oxidative stress resistance. Linear fusigen, an ester-containing siderophore, is found in large numbers in two ectomycorrhizal basidiomycetes, *Laccaria laccata* and *Laccaria bicolor*, as well as coprogen, ferricrocin, and triacetyl fusarinine C. The brown-rot fungus *Wolfiporia cocos*, a basidiomycota member used in Chinese medicine, also produces catecholate siderophore (Osman *et al*., 2019)[7].

III. CyanobacterialSiderophore

Dihydroxymate type siderophores, such as schizokinen and anachelin H, are known to be produced by cyanobacterial species. *Anabaena oryza* produces a siderophore that functions as a biological sequestering agent for cadmium metal ions, with promising outcomes for agricultural productivity increase.

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IV. Plant Siderophores

The mugineic acid family includes phytosiderophore (PS) forming a hexadentate Fe-PS complex in members of the poaceae family. Two amine-N, two carboxylate-O and one a-hydroxy carboxylate site create a compact octahedron in which the core Fe (III) atom remains bound in these siderophores.

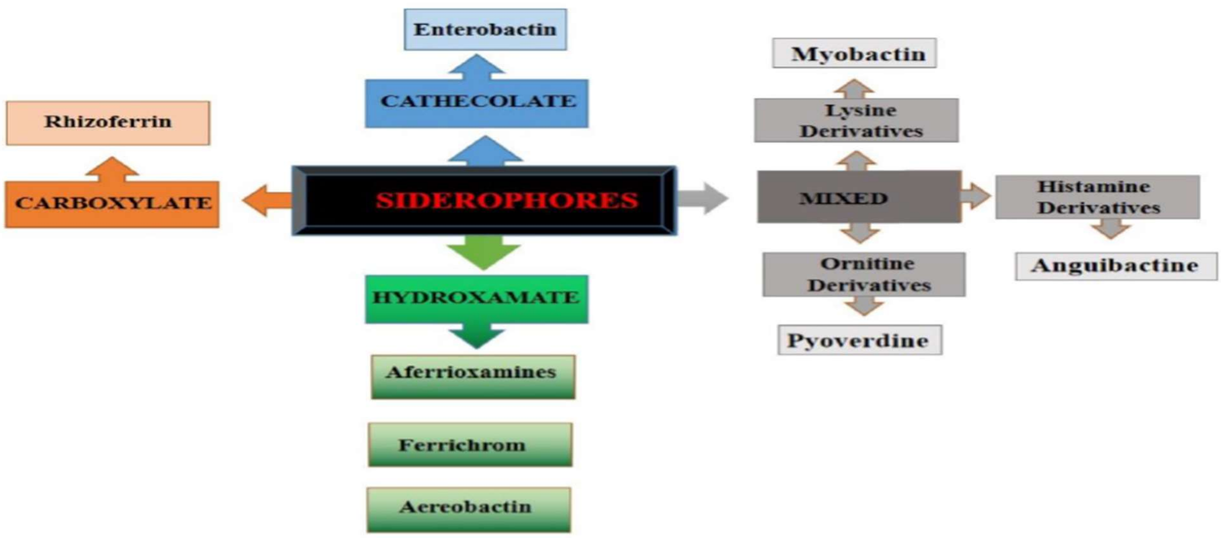

Fig 4- General group of siderophores

Role of siderophores in nature

Fig 5- Functions of siderophores

Siderophore in plant protection

Although different plant species are able to mobilize immune programmes in response to siderophore treatments, different patterns and mechanisms seem to emerge. For instance, in maize, the effect of coprogen seems to be more reminiscent of 'priming' whereas, in Arabidopsis, the coprogen effect resembles direct elicitation. In the studies described above, oxidative stress has been reported in several instances. ROS production is associated with the combination of pyochelin and pyocynanin in tomato cells, with the siderophore Psb374 in tobacco cell cultures, and with siderophores in *A. thaliana*, in which differential expression of ROS metabolism-linked genes is also observed. Modifications in the cellular redox balance may trigger a diffusible signal causing the systemic spread of defence response. For example, in the study by Singh *et al*. (2008)[8] on tobacco plant cells, there were only direct cellular effects of siderophores, although the systemic signalling effect of metal mobilization in entire plants was not monitored (Fig. 6).

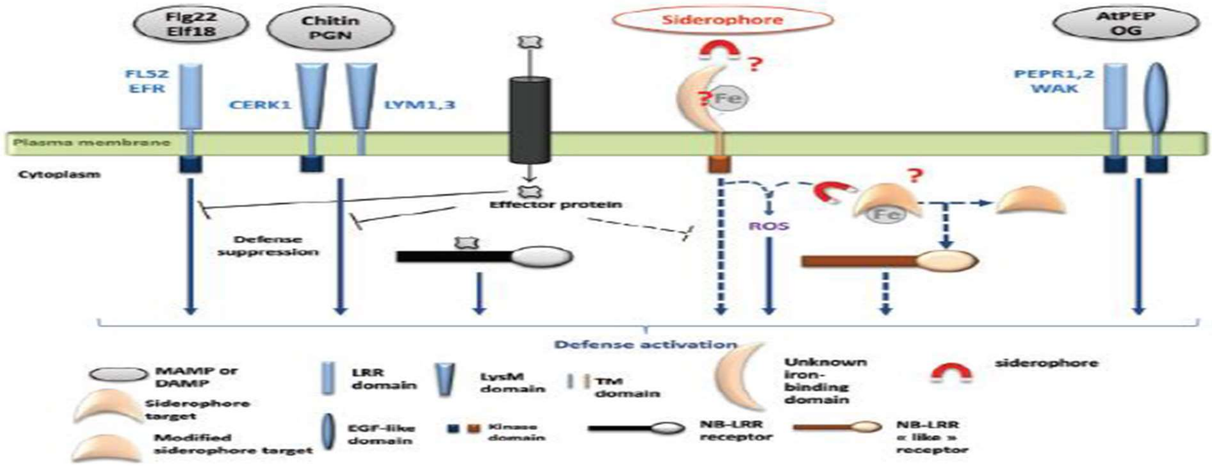

Fig 6- Mechanism of siderophore mediated defence activation against plant pathogens

Siderophore and IAA

IAA, is a plant growth hormone, produced by both microbes and plant, plays a vital role in plant growth and development. It is well documented that heavy metals possess a negative effect against this hormone. This, causes destruction of IAA, and thereby causes growth inhibition in plant. The oxidative degradation of IAA has been reported in metal-stressed plants for instance, auxin degradation was reported in leaves of pea (*Pisum sativum* L.) seedlings treated with Cd and Cu.

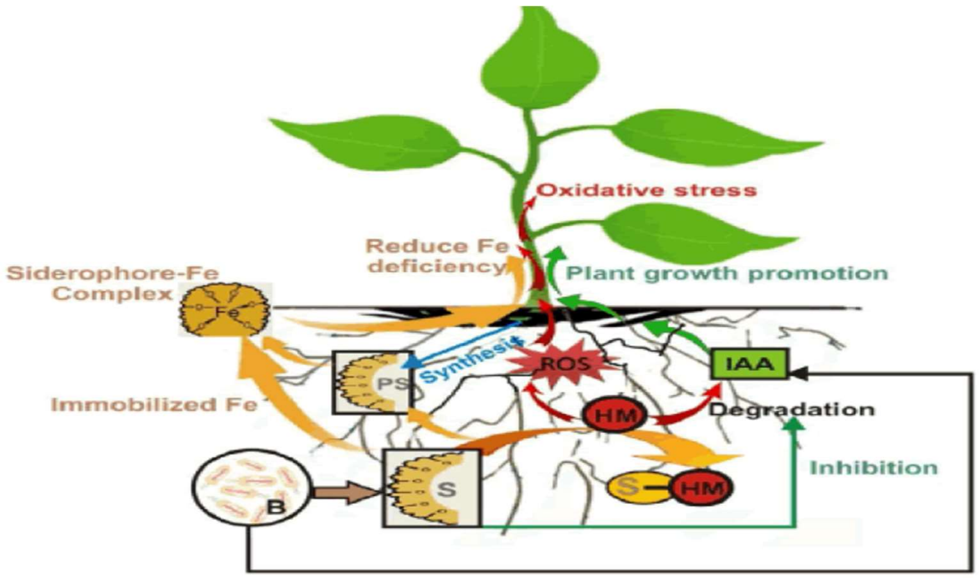

Fig 7- The role of siderophores on plants under different stress

Biosynthesis of siderophore group of Non-ribosomal peptides

The mugineic acid family includes phytosiderophore (PS) forming a hexadentate Fe-PS complex in members of the poaceae family. Two amine-N, two carboxylate-O and one a-hydroxy carboxylate site create a compact octahedron in which the core Fe (III) atom remains bound in these siderophores.

Synthesized by Non-ribosomal peptide synthetases (NRPSs) / Polyketide Synthase (PKS) also produced by pathways independent of NRPS and polyketide synthases. Consists of multi enzyme assembly line in which amino acids, carboxy and hydroxy acids (Stolte et al., 2016)[9] built into peptidic precursor molecule, later modified by other enzymes to form final siderophore. One such modification is formation of heterocycles through the cyclization of cysteine side chains (Fig. 8).

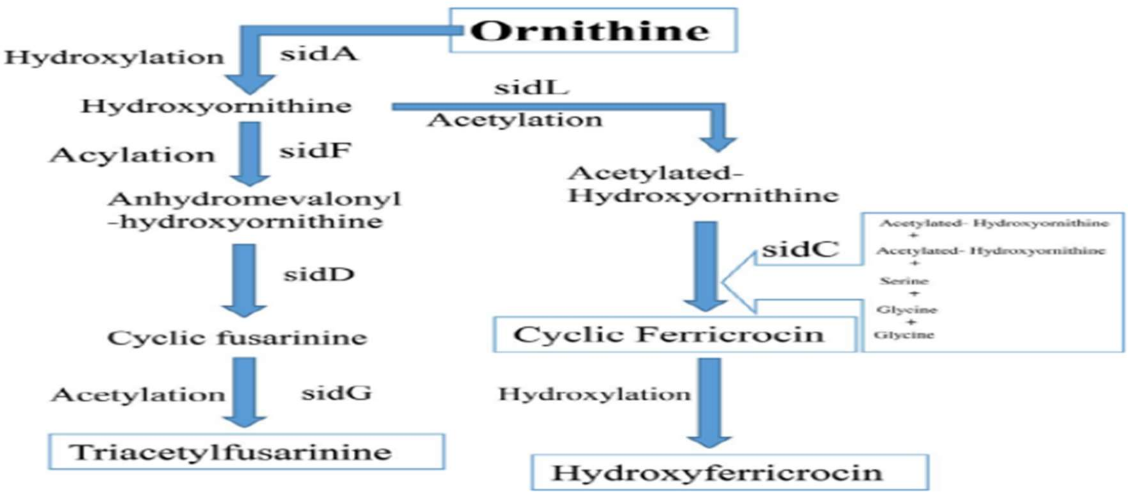

Fig 8- Biosynthesis pathway of triacetylfusarinine, ferricrocin and hydroxyferricrocin (Upadhyay and Chauhan, 2022)[10].

Conclusion

A proportional relation exists between growth performance and yield of plants; however, a big challenge arises in this proportional relationship due to the rapid rise in degraded land across the globe. The utilization of degraded land for agricultural practices becomes an issue for researchers to meet global food production for the future with eco-friendly and sustainable technology. Degraded land poses several detrimental impacts on plant growth and induces plant stress by less cycling of available nutrients and disruption in the metabolic function of the plant. The review discussed the influence of iron-deficient soil on plant and their management through eco-friendly products i.e., siderophores. The diverse chemical nature of siderophores can chelate Fe^{3+}, which is produced by siderophore-producing rhizobacteria, and plant roots commonly known as bacterial siderophore (BS) and plant siderophore (PS).

In the rhizospheric microenvironment, both BS and PS synergistically facilitate iron uptake in the plant from iron deficient soil mediated by reduction and chelation strategies. The utilization of siderophore-producing rhizobacteria can effectively maintain the iron level in plants and induce plant growth performances under degraded soil effectively when their selections meet compatibly with plant roots specifically. Future research requires the selection of the perfect candidate for siderophore-producing rhizobacteria, for a specific plant in degraded soil that would be useful for plant stress management and plant productivity at the field level.

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Chapter- 15

Role of Insect Gut Microbiota in Pesticide Degradation

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Abstract

Insect pests cause significant agricultural and economic losses to crops worldwide due to their destructive activities. Pesticides are designed to be poisonous and are intentionally released into the environment to combat the menace caused by these noxious pests. According to recent findings, microbes that live in insect as symbionts have recently been found to protect their hosts against toxins. A defensive strategy against pathogens and pesticides has emerged through the symbiotic relationship established between pests and diverse microbes. Insect's gut provides unique conditions for microbial colonization and resident bacteria can deliver numerous benefits to their hosts. Insect digestive tracts are very different in shape and chemical properties, which have a big impact on the structure and composition of the microbial community. Insect gut microbiota has been found to contribute to feeding, parasite and pathogen protection, immune response modulation and pesticide breakdown.

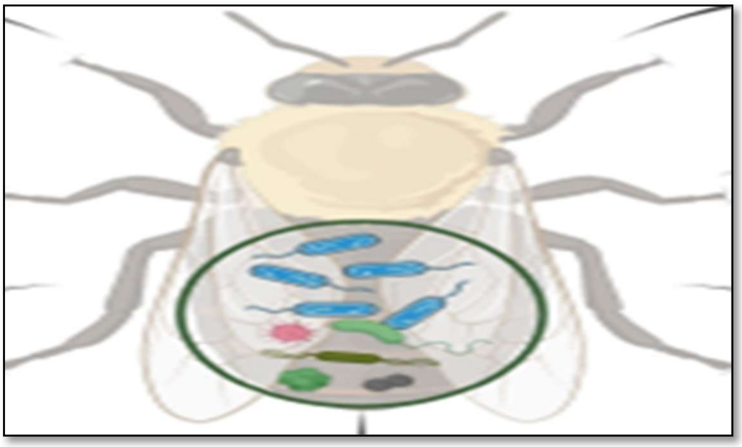
Keywords: Insect gut microbiota, Pesticide degradation, Enzymes, Symbionts

Introduction

Physical, chemical and biological methods are applied to degrade pesticides, but as compared to other methods, biological methods are considered more efficient, eco-friendly and less expensive (Engel and Moran, 2013). In particular, employing microbial species and their purified enzymes makes the degradation of toxic pollutants more accessible and converts them into non-toxic products by several metabolic pathways. Insect gut microbiota tries to degrade toxic compounds by changing their toxicity, increasing the production and regulation of a diverse range of enzymes. These enzymes breakdown into their derivatives and microbial species utilize them as a sole source of carbon, sulfur and energy. The resistance of pesticides in insect species is developed by metabolic mechanisms, regulation of enzymes and the expression of various microbial detoxifying genes in insect gut (Jaffar *et al*., 2022).

Microbes are known to degrade a wide variety of pesticides, coupled with their potential to confer detoxifying abilities to insects *via* symbiotic partnerships. Detoxifying microbiota that can be isolated could be used in bioremediation or to treat pesticide poisoning. The multifaceted roles of gut microbiota in host's physiology, modulation of immunity and toxin degradation open a new arena to exploit the potential of these microbes for sustainable pesticide degradation (Siddiqui *et al.,* 2022).

Insects are the world's most diverse and abundant animals in terms of species diversity and body mass in all ecological habitats (Nagarajan *et al.,* 2022). Their numerous interactions with beneficial microbes are essential for survival and diversity. Microbes that are living in the guts of insects play a vital role in the biology and behavior of their hosts, including assisting in the digestion of recalcitrant food components, upgrading nutrient poor diets, modulating the immune response, and protecting from predators, parasites, pathogens, and disease vectors. Other functions include facilitating plant specialization, governing mating preference and reproductive systems, and contributing to inter and intraspecific communication. Most insects are thought to be in symbiotic partnerships with microbes, with estimates ranging from 15 to 20% of the total (Zhou *et al.,* 2021).



(Siddiqui *et al.,*2022)

The role of microorganisms, particularly gut microbes, in insect function is important from various viewpoints, including agriculture, ecology, and medicine. Few insects are good laboratory models for studying microbe populations and their associations with hosts, especially immunology and metabolic associations. Entomological studies of parasitic and mutualistic connections have focused on social insects like ants, which have evolved diverse interactions with other species at various levels, including individual and community interactions. These interactions can occur between bacteria and different insects and plants (Moreau, 2020). Symbiotic bacteria can affect the efficacy of disease vectors or their developmental time, making them possible targets for disease control. Microorganisms allied with pollinators and herbivores, and insects that feed on them are likely to impact the agricultural crop's health substantially. Insects and their gut microbial populations play vital

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roles in the nitrogen cycle and the decomposition of plant material in natural and human-impacted ecosystems.

Insect gut structure and functions

The elementary structure of the intestinal system is alike among insects, even though they have a variety of alterations connected with adaptation to diverse feeding styles and environmental conditions. The digestive tract is divided into three basic regions: the foregut, the midgut, and the hindgut. The foregut and hindgut originate from the embryonic epithelium and are protected from pathogens by an exoskeleton of chitin and integument glycoproteins. This exoskeleton is shed at each ecdysis, separating the gastrointestinal lumen from the epithelia. When divided into functionally different subgroups, the foregut is frequently distinguished by another diverticula or crop for impermanent food storage.

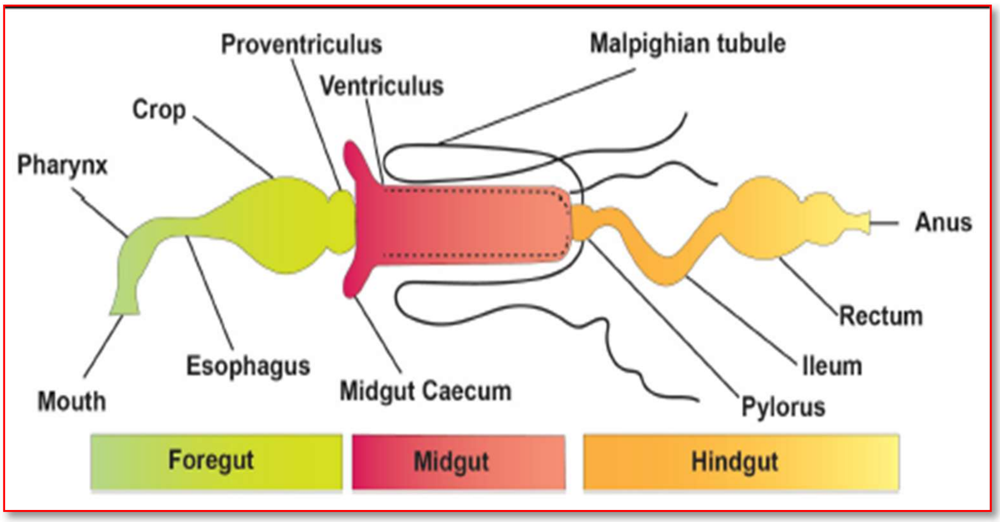
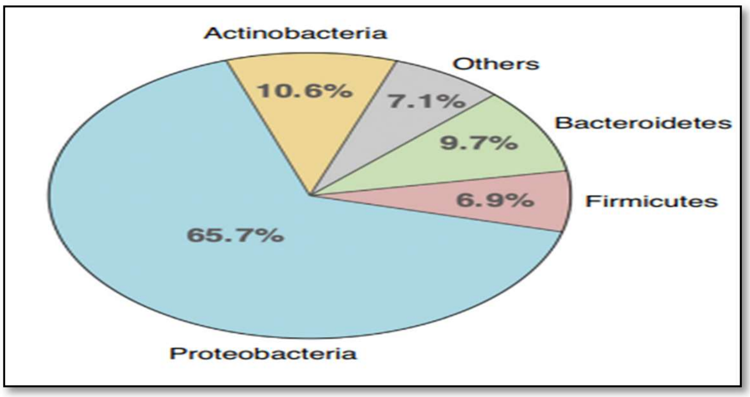


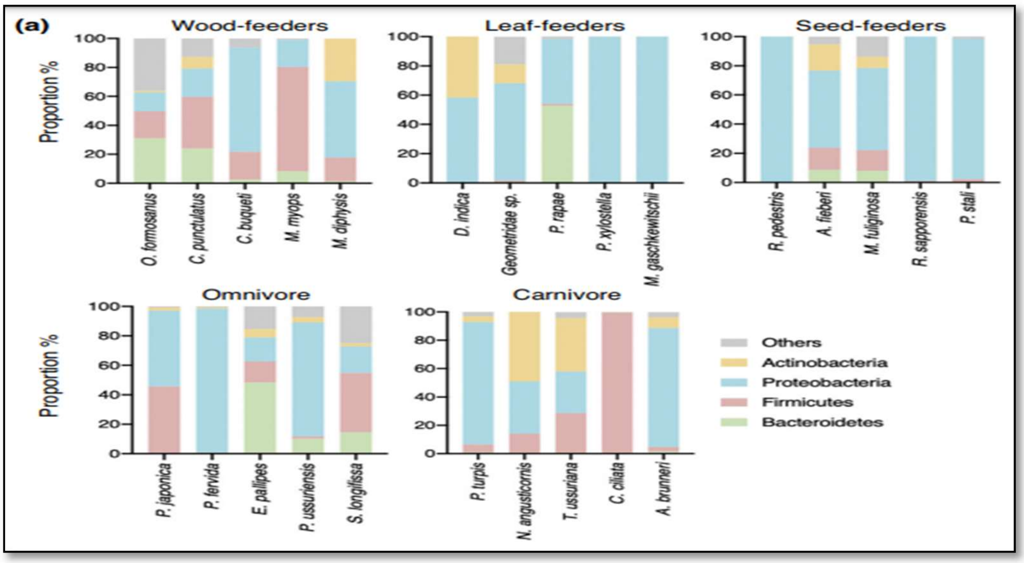
Fig- Insect gut structure and functions(Siddiqui *et al.,*2022)

Insect gut microbiome composition

A wide range of parameters can influence gut microbiota composition, including insect growth, biochemical changes in different intestinal areas, and the insect's ability to obtain available resources. The hindgut of insects, which serves as an extension of the body cavity, is one of these structures that collect dietary waste. Therefore, it provides a great food environment to the gut microbiota, encouraging their proliferation and diversification. The insect gut microbiome includes protozoa, fungus, archaea, and bacteria.



Protists occupy almost 90% of the hindgut of subterranean termites for example, lower and higher termites' guts include bacteria and archaea. Scientists revealed that the digestive regions of adult workers of honeybee (*Apis mellifera*) are dominated by a diverse group of nine bacterial species (five of which are *Snodgrassellaalvi* and *Gilliamellaapicola*, two species of *Lactobacillus*, and a species of *Bifidobacterium*) (Douglas, 2018).



(Jang and Kikuchi, 2020)

Additionally, the gut microbiota is rarely directly touched with intestinal epithelial cells due to their unique placement. Most of the time, bacteria that live in the gut are found in the lumen of the endoperitrophic space, a chitinous barrier that lines the middle of the gut.

Interaction of insect and their related microbiota

Insect-microbiota interactions are quite diverse, Insects rely on symbiotic bacteria for a variety of essential activities. Symbiotic bacteria can be critical for host survival and growth. They can help break down food, provide energy, make vitamins, and even help shape the body's natural defenses. Microbial symbionts have been proven to have many consequences on insect health and behavior. Certain insects have specialized organs that can only house a few symbiont species, while others have a far more diverse and variable flora in their guts and other internal organs. Numerous associations are developed with a sole or a few species of microbiota. They might require establishing specialized insect organs and cells (i.e., subsequent midgut crypts, mycangia, and microbiome) to house definite obligate symbionts.

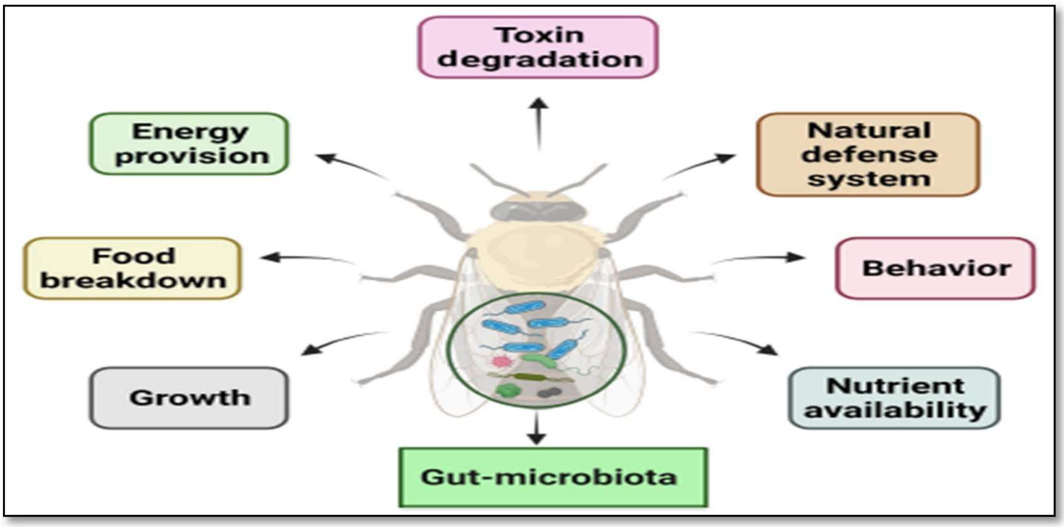


Fig- Interaction of insect and their related microbiota (Siddiqui *et al.,*2022)

In these partnerships, the genetic integral of biochemical processes essential for the persistence of both interrelating groups is frequently observed. Some insect species are more involved in symbiotic associations with bacteria than others. Among the insects, three taxonomic groups are regularly involved. These groups include *Blattaria*, Coleoptera, Homoptera, and Hymenoptera. The subgroup contains symbiotic organisms closely related to significant human diseases, such as *Francisella tulariensis*, *Coxiella burnetii*, and several Enterobacteriaceae.

Impacts of gut microbiota on the activity of pesticides

The insect-associated microbial community is dynamic and responsive to various stressors. The related microbiota, like the insect, is subject to natural selection pressure, and its composition can be influenced by variables such as dietary changes, food scarcity, and exposure to toxic substances (Akami *et al.,* 2022). The microbiota of hosts exposed to pesticides as a source of selection pressure may also assist the host in metabolizing these substances. It may act as a source of variation, resulting in the host's reduced susceptibility to pesticides.

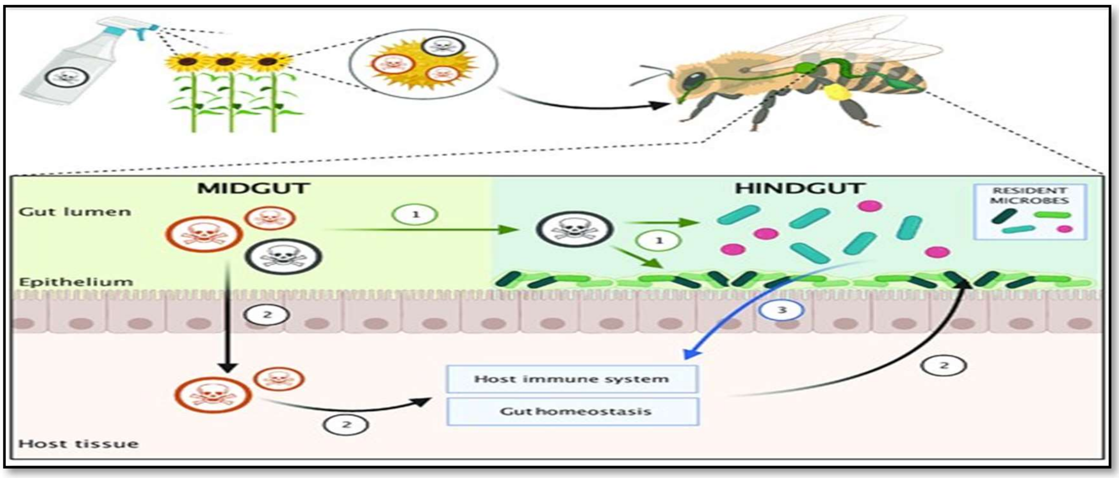


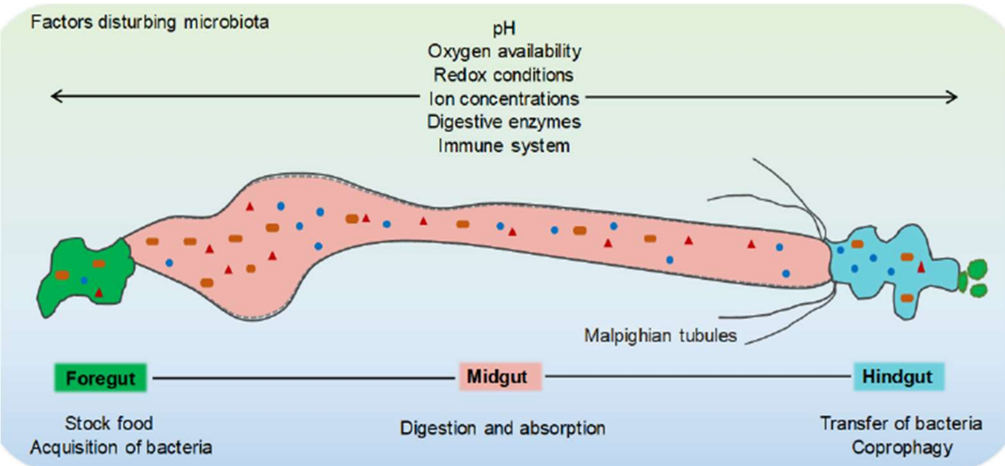
Fig- Interaction between pesticides, bee gut microbiota and bee host

Impacts of gut microbiota on the activity of pesticides is significant, it can be explained through following mechanisms.

- **Pesticide degradation:** Gut microbiota can possess enzymes that break down or metabolize certain pesticides, reducing their toxic effects on the insect host.
- **Detoxification:** Gut microbes may detoxify pesticides by modifying their chemical structure, making them less harmful to the insect.
- **Enzyme inhibition:** Gut microbes can produce enzymes that inhibit the activity of pesticides, reducing their effectiveness as pest control agents.
- **Altered gut permeability:** Gut microbiota can influence the permeability of the insect gut lining, affecting the absorption and distribution of pesticides within the insect's body.
- **Competition for binding sites:** Gut microbiota may compete with pesticides for binding sites in the insect gut. If microbes bind to the target sites of the pesticide, it can reduce the pesticide's ability to interact with its intended target and decrease its effectiveness.

Factors influencing composition of the insect gut microbiota

Factors influencing composition of the gut microbiota of insects include insect development, physiochemical conditions in different gut compartments, available sources for bacteria acquisition, and capability to transfer bacteria to progeny.

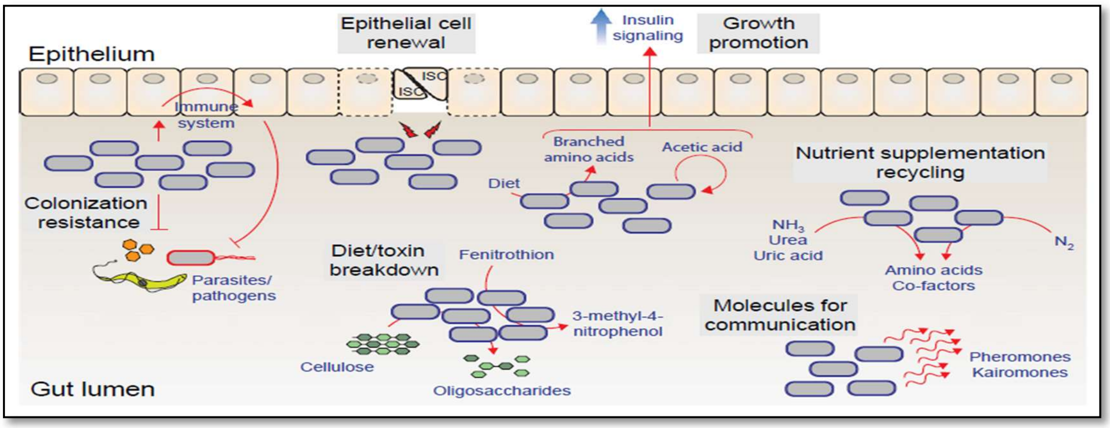


(Engel & Moran,2013)

Functions of insect gut bacteria

- ✓ **Colonization resistance** against pathogens or parasites has been described for the bumble bee, *Bombus terrestris*, the desert locust, *Schistocerca gregaria*, and various mosquito species.
- ✓ In *Drosophila melanogaster*, the commensal gut microbiota has been shown to be involved in **intestinal cell renewal** and promotion of systemic growth.
- ✓ A prime example for **diet breakdown** is the degradation of cellulose by the characteristic gut microbiota in the hindgut of termites.

- ✓ Gut bacteria have also been shown to degrade toxins ingested with the diet. The insecticide fenitrothion is hydrolyzed into 3-methyl-4-nitrophenol by the *Burkholderia* gut symbiont of the stinkbug *Riptortuspedestris*.

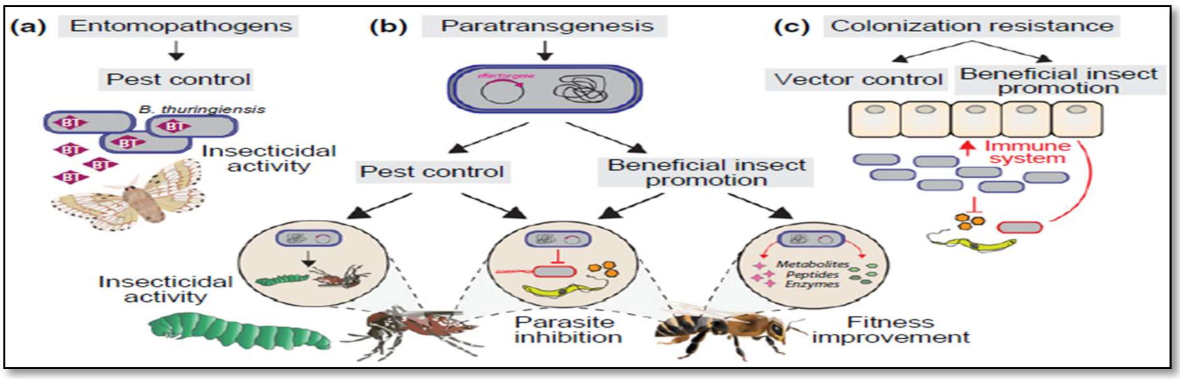


(Engel & Moran,2013)

- ✓ **Nutrient supplementation**, such as the synthesis of vitamins and essential amino acids or the fixation of nitrogen, has been shown for gut symbionts of blood-feeding kissing bugs, stinkbugs, and termites, respectively.
- ✓ Certain gut bacteria of termites can also **recycle nitrogenous waste products** excreted by the host by converting them into high-value nutrients. Similar functions might also be carried out by gut bacteria of ants and cockroaches.
- ✓ Insects, gut bacteria produce **molecules involved in intraspecific and interspecific communication**, such as pheromones and kairomones.

Practical applications of insect gut microorganisms

Harnessing human gut bacteria to supplement and balance diets or to sustain gut homeostasis and treat diseases has been in the focus of the food industry and medicine for a long time. Likewise, gut bacteria of insects might be utilized to manage insect species of human concern. This is particularly promising for the control of so-called pest species, that is, insects with ecological characteristics that negatively affect human health, economic interests, or environmental quality.



Different applications of gut bacteria for the management of insects

(Engel & Moran,2013)

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a) Insecticidal potential of entomopathogenic gut bacteria can be used to control pest species.

b) In paratransgenesis, bacteria are used as vehicles to express molecules in the gut, which negatively or positively affect health of the host or suppress parasite colonization. These approaches could be applied for the management of pest species and beneficial insects.

c) Alternatively, gut bacteria that naturally inhibit parasite colonization could be disseminated in insect populations, for example, to prevent the spread of human disease via insect vectors or to protect beneficial insects from parasitic diseases.

Pesticides	Gut microbiota	Insect pests	References
Benzoylurea	*Enterococcus mundtii*	*Spodoptera frugiperda*	de Almeida et al., 2017
	Microbacterium arborescens		
	Staphylococcus sciuri subsp. *sciuri*		
Carbamate	*Pseudomonas melophthora*	*Rhagoletis pomonella*	Boush and Matsumura, 1967
Methoprene	*Clostridium* spp.	*Aedes* spp. and *Anopheles* spp.	Receveur et al., 2018; Giambò et al., 202
	Lysinibacillus spp.		
	Staphylococcus spp.		
Neonicotinoid	*Acetobacter* spp.	*Drosophila melanogaster*	Chmiel et al., 2019
	Lactobacillus spp.		
	Lactobacillus plantarum		Giambò et al., 2021
	Arsenphonus spp.	*Nilaparvata lugens*	Pang et al., 2018
Organochloride	*Pseudomonas melophthora*	*Rhagoletis pomonella*	Boush and Matsumura, 1967
Organophosphate	*Microbacterium* sp.	*Anopheles stephensi*	Soltani et al., 2017
	Exiguobacterium sp.		
	Aeromonas spp.		
	Pseudomonas spp.		
	Citrobacter spp.	*Bactrocera dorsalis*	Cheng et al., 2017; Guo et al., 2017
	Actinobacteria spp.	*Bombyx mori*	Chen et al., 2020; Giambò et al., 2021
	Staphylococcus spp.		
	Enterococcus spp.		
	Lachnospiracease spp.		Li et al., 2020; Giambò et al., 2021

Fig- List of insect gut microbiota involved in pesticide degradation

Mechanism of microbial degradation of pesticides

Degradation by microbes

- ✓ Some pesticides are readily degraded by microbes
- ✓ They use organic chemicals in pesticides for growth and reproduction
- ✓ e.g.: *Flavobacterium*, *Pseudomonas*, *Rhodococcus*

Pesticide transformation reactions

- ✓ Microbes transform pesticides into non toxic forms
- ✓ Reduce pesticide accumulation in environment
  - Oxidative
  - Reductive
  - Hydrolytic and synthetic

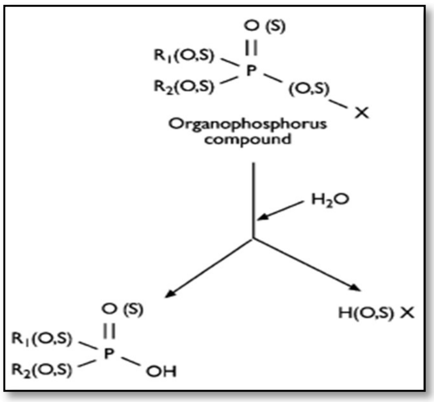
Oxidation reactions

- ✓ Monooxygenase, dioxygenase, laccase
- ✓ Major oxidation reactions
  - Hydroxylation
  - Dealkylation
  - Epoxidation
  - Sulfoxidation

2) Reduction reactions
3) Hydrolytic reactions
4) Synthetic reactions

General pathway for biodegradation of organophosphate compounds

Most organophosphorus compounds are ester or thiol derivatives of phosphoric, phosphonic or phosphoramidic acid. R1 and R2 are mainly the aryl or alkyl group, which can be directly attached to a phosphorus atom (phosphinates) or via oxygen (phosphates) or a sulphur atom (phosphothioates). In some cases, R1 is directly bonded with phosphorus and R2 with an oxygen or sulfur atom (phosphonates or thion phosphonates, respectively).



At least one of these two groups is attached with un-, mono- or di-substituted amino groups in phosphoramidates. The X group can be diverse and may belong to a wide range of aliphatic, aromatic or heterocyclic groups. The X group is also known as a leaving group because on hydrolysis of the ester bond it is released from phosphorus.

Microorganisms	Compound
Bacillus, Staphylococcus	Endosulfan
Enterobacter	Chlorpyrifos
Pseudomonas putida, Acinetobacter sp., Arthrobacter sp.	Ridomil MZ 68 MG, Fitoraz WP 76, Decis 2.5 EC, malation
Acenetobactor sp., Pseudomonas sp., Enterobacter sp. and Photobacterium sp.	chlorpyrifos and methyl parathion

Conclusion and Future Perspective

Microbes are known to degrade a wide variety of allelochemicals and pesticides, providing numerous opportunities for insects to develop detoxifying symbiotic relationships. The gut microbiota plays various roles in the host's physiology, including immunological modulation and toxin degradations. Arguably, the microbiota evolves more rapidly than their host insects, resulting in rapid pest adaptation to pesticides through the employment of mutualistic microbes. Additionally, insects can swiftly obtain novel metabolic activities and colonize new ecological niches through symbiotic interactions with microbiota that previously have fully developed well-tuned metabolic pathways. As results of the ever-dynamic climatic conditions and human populations, it is imperative that novel insect pest management strategies are implemented to synergize the existing ones. Exploring symbiotic microorganisms as a means of managing their associated hosts could be one way to meet this need. Currently, sterile insect technology, introduction of natural enemies such as parasitoids or predators, application of entomopathogenic fungi or bacteria, etc., are some of the most commonly used integrated pest management techniques.

Detoxifying microbiota that can be isolated could be used in bioremediation or to treat pesticide poisoning. Detoxifying microbiota that can be isolated could be used in bioremediation or to treat pesticide poisoning. The multifaceted roles of gut microbiota in host's physiology, modulation of immunity and toxin degradation open a new arena to exploit the potential of these microbes for sustainable pesticide degradation.

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Chapter- 16

Revolutionizing Horticulture Systems: by Artificial Intelligence and Machine Knowledge

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Abstract

Since horticulture produces food (fruit and vegetables) and raw materials (vegetables) which is essential to the survival of human civilization. Horticulture has undergone a technological revolution with machine Knowledge (MK) and artificial intelligence (AI) being two major contributors. An overview of the horticultural industry is given in this article along with a focus on the value of technical improvements in horticulture. It looks at how horticultural systems have changed as a result of AI and MK. The need for technological breakthroughs has grown in response to issues like population expansion, climate change and scarce resources. Increased production, effective resource management, disease and pest control and supply chain optimization all demonstrate the value of technology in horticulture. Technologies like AI and ML have become extremely useful in horticulture. They make it possible to monitor crops and soil, apply horticultural robots and predictive analytics and use horticultural drones. The horticultural industry could benefit from increased productivity, decreased resource waste, sustainability and the ability to make data-driven decisions appreciations to the integration of AI and ML.

Keywords: Artificial intelligence (AI), fruit, horticulture, Machine knowledge (MK) and vegetables.

Introduction

The horticulture assumes a crucial part in giving food and natural substances to support human development. Horticulture has been transformed over time by technological advancements, resulting in increased productivity, effectiveness and sustainability. One of the key drivers of this change is the joining of Man-made reasoning (computer based intelligence) and Machine Knowledge (MK) procedures in horticultural practices. Here, we will give an overview of the horticultural industry like talk about how important technological advancements in horticulture are and look at how artificial intelligence and machine knowledge are changing horticulture systems. A subfield of computer science known as artificial intelligence (AI) focuses on developing intelligent machines that are able to carry out tasks that typically call for human intelligence.

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Artificial intelligence frameworks intend to imitate human mental capacities for example, getting the fall of, thinking, critical thinking and making decisions. A subset of artificial intelligence (AI) known as machine knowledge (MK) focuses on the creation of models and algorithms that allow computers to learn from data and make predictions or decisions without being explicitly programmed. The most important concepts in ML and AI are:

1. **Algorithms:** ML calculations are numerical techniques that empower machines to learn designs from information. They give the rationale and rules to information handling, highlight extraction, design acknowledgment, and expectation. Various kinds of calculations are utilized contingent upon the front and center concern.
2. **Controlled Learning:** The MK model learns from characterized data in managed learning where input data are linked to known output labels. The model sums up from this named information to make expectations on new concealed information.
3. **Data:** AI and ML are built on top of data. It can be in the form of text, images, audio, video or structured data among other things. The accuracy and performance of AI and ML models are greatly influenced by the quantity and quality of data.
4. **Invalid Learning:** Unaided learning includes preparing the model on unlabeled information where the information doesn't have comparing produce names. The model independently examines the data to discover relationships, structures and patterns.
5. **Support Learning:** Support learning is an area of ML where a specialist figures out how to cooperate with a climate to expand a prize sign. The specialist advances by experimentation getting criticism as remunerations or retributions in light of its activities.
6. **Training:** In order to learn relationships and patterns ML models are trained on labeled or unlabeled data. Preparing includes taking care of the model with input information and relating result or target values. The model then at that point changes its interior boundaries to limit the distinction among anticipated and genuine qualities.

Types of Machine Knowledge Algorithms Commonly Used in Horticulture

1. **Gaussian Processes:** Gaussian cycles are probabilistic models that can be utilized for decline and vulnerability assessment. They are helpful in anticipating crop yield, water stress and soil properties.
2. **K-Nearest Neighbors (KNN):** KNN is a simple algorithm that categorizes objects based on their similarity to close examples. It is beneficial for farm duties such as crop disease classification and weed identification.
3. **Neural Networks:** Neural networks are computational models encouraged by the human brain. They consist of interconnected nodes likes neurons systematized in layers. Deep knowledge a division of neural networks has been successful in image analysis tasks like plant disease identification, yield prediction and weed detection.

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4. **Random Plantations:** Irregular plantations consolidate different choice trees to make a more powerful and precise model. They are suitable for crop yield estimation and plant disease detection because they can handle large and complex datasets.
5. **Support Vector Machines (SVM):** SVM is a strong calculation utilized for order and regression tasks. It isolates information into various classes by seeing as an ideal hyperplane in a high-layered space. Crop classification and weed detection are two examples of applications for SVMs.

AI and ML Applications in Crop Production

1. **Crop yield forecast and optimization:** Simulated intelligence and ML procedures assume a significant part in foreseeing and streamlining crop yields. By investigating verifiable and constant information including weather conditions, soil conditions and harvest development stages calculations can produce exact forecasts of harvest yields. These prescient models consider different elements that impact crop efficiency like temperature, precipitation, daylight, supplement accessibility and irritation invasions. By understanding these connections farmers can settle on educated choices in regards to the application regarding composts, pesticides and different contributions to boost yields.
2. **Crop quality valuation and sorting:** Simulated intelligence and ML advancements add to surveying and evaluating crop quality, guaranteeing consistency and proficiency in the agrarian production network. Images of harvested crops can be graded using parameters like size, color, shape and defects by computer vision algorithms *via* robotizing this interaction computer based intelligence frameworks dispose of human subjectivity and increment the speed and exactness of yield quality appraisal.
3. **Disease and pest recognition and control:** Early recognition and control of illnesses and irritations are pivotal for forestalling huge crop misfortunes. Man-made intelligence and ML advances help farmers in distinguishing and dealing with these dangers productively. Artificial Intelligence (AI) is able to recognize the visual signs of diseases or nuisance pervasions by examining pictures of leaves, stems, or natural products. ML models that have been trained on a lot of data can accurately identify specific pathogens or pests and suggest the best treatments.
4. **Harvesting and post-harvest handling:** The harvesting and post-harvest handling processes are being transformed by AI and ML technologies which are increasing productivity and decreasing losses. Mechanical frameworks furnished with artificial intelligence vision calculations can independently recognize and gather mature harvests with accuracy and speed. These frameworks can survey factors like tone, size and readiness to decide the ideal time for gather, limiting yield misfortunes and guaranteeing steady harvest quality.
5. **Site-specific crop management and Precision farming:** Through techniques for site-specific crop management and precision farming AI and machine Knowledge (MK) are transforming crop production. These advancements empower farmers to streamline their horticultural practices by gathering and investigating tremendous measures of information about their fields.

Man-made intelligence and MK calculations can handle information acquired from different sources like satellite symbolism, drones, weather conditions stations, soil sensors and yield sensors. By incorporating this information farmers can acquire bits of knowledge into the inconstancy of their fields including soil creation, dampness levels and supplement content.

6. **Water and Irrigation management:** Proficient water sustenance is fundamental for reasonable harvest creation especially in water-scant areas. Man-made intelligence and ML applications help in streamlining water system practices and rationing water assets. Artificial intelligence (AI) algorithms are able to generate precise irrigation schedules that are personalized to specific field conditions by analyzing data from soil moisture sensors, weather forecasts and crop water requirements. These timetables guarantee that harvests get sufficient water without over the top use, forestalling water pressure and enhancing water utilization proficiency.
7. **Weed revealing and management:** Controlling weeds is an essential part of growing crops because they compete with crops for sunlight, nutrients and water. Weed management and detection are made easier and more accurate by AI and MK applications. Utilizing PC vision methods, simulated intelligence calculations can examine pictures of fields caught by robots or cameras mounted on farming hardware. Farmers can precisely target weed-infested areas because these algorithms can distinguish between crops and weeds.

AI and MK in Horticultural Robotics and Automation

1. **Role of AI and ML in Horticultural Robotics:** Artificial intelligence (AI) and machine knowledge (MK) play crucial roles in agricultural robotics in enhancing farming operations' automation, precision and effectiveness. These innovations empower robots and independent frameworks to see and collaborate with the farming climate, settle on informed choices and perform errands with negligible human mediation. Simulated intelligence and MK calculations engage horticultural robots to investigate immense measures of information gathered from sensors, cameras and different sources empowering them to distinguish and answer complex examples and changes in the field. These advances can handle information connected with soil conditions, weather conditions, crop development and irritation invasions among others to make constant choices and enhance cultivating rehearses. Farmers can improve crop yields, cut costs, reduce resource waste, and make data-driven decisions for sustainable and effective farming by incorporating AI and MK capabilities into agricultural robotics.
2. **Applications of Robotic in Horticulture Field Operations:**

a. **Harvesting:** Harvesting is a work serious and time-delicate responsibility. Robots with AI and MK capabilities are able to precisely identify ripeness indicators like color, size, and texture allowing them to harvest crops selectively at the appropriate time. This declines misuse and moves along proficiency in the collecting system.

b. **Planting:** Computer based intelligence and MK calculations can break down soil and climate information to decide ideal establishing designs, seed dispersing and establishing profundity.

Robots furnished with accuracy establishing components can independently sow seeds with high precision, guaranteeing consistency and boosting crop yield.

c. **Spraying:** Horticultural robots outfitted with man-made intelligence and MK abilities can distinguish and separate among crops and weeds. They can use this information to apply pesticides or herbicides only where they are absolutely necessary and thereby minimizing chemical use and impact on the environment.

d. **Weeding:** Weeds can be identified and categorized in real time using AI and machine knowledge algorithms. Robots outfitted with weed recognition frameworks can definitively target and eliminate weeds, lessening the requirement for herbicides and physical work.

Challenges and Limitations

1. **Adoption Challenges and Technical Barriers:** The productive execution of artificial intelligence and MK in horticulture additionally faces a few specialized obstructions and reception challenges. One of the essential specialized obstructions is the absence of computational assets in numerous farming settings, especially in remote or immature districts. Algorithms for AI and machine knowledge often require a lot of storage and computing power which may not be readily available in these areas.
2. **Data Availability and Quality:** One of the significant difficulties in the use of Man-made reasoning (man-made intelligence) and Machine Knowledge (MK) in horticulture is the quality and accessibility of information. Weather conditions, soil composition, the prevalence of pests and diseases and crop management practices are just a few of the many variables and factors that influence crop growth in Horticulture. In request to construct exact and dependable artificial intelligence and MK models a huge volumes of excellent information are required.
3. **Data Privacy and Ethical Considerations in AI and MK in Horticulture:** The organization of artificial intelligence and MK in horticulture raises moral contemplations and information protection concerns. Sensitive data in horticulture include personal information about farmers, farm management practices and exclusive horticulture knowledge. Gathering and breaking down such information utilizing artificial intelligence and MK advancements can bring up issues about information possession, assent and protection. There is a need to lay out vigorous structures and guidelines to safeguard farmers' information and also guarantee its dependable and moral use. Information sharing arrangements and approaches ought to be carried out to characterize the expectations of partners engaged with information assortment, capacity and investigation. Protections should be set up to predict unapproved access, information breaks or abuse of farmer's information.

Conclusion:

The joining of computerized reasoning and AI advancements in horticulture has arisen as an extraordinary power and reforming the way cultivating and related practices are directed. The advancements in AI and MK have empowered farmers and rural partners to pursue more educated choices, streamline asset portion, increment efficiency and advance supportable practices. One of the critical advantages of computer based intelligence and MK in horticulture is the capacity to accumulate, process and investigate huge measures of information from different sources for example weather conditions, soil conditions and crop wellbeing and market patterns. This data driven approach enables farmers to pursue ongoing choices and go to proactive lengths to moderate dangers, amplify yields and limit natural effect. By utilizing man-made intelligence and MK calculations farmers can precisely foresee atmospheric conditions, recognize infection flare-ups, advance water system plans and productively oversee crop turn techniques. Besides, AI and MK play had a significant impact in accuracy farming permitting farmers to fit their ways to deal with explicit region of their fields. Using sensors, robots and satellite symbolism these advances can give point by point data on soil quality, dampness levels and supplement lacks empowering farmers to apply composts and pesticides with accuracy, diminishing waste and expenses. This designated approach not just improves crop wellbeing yet additionally diminishes the natural impression related with conventional agrarian practices. Farm automation and machinery are also included in the AI and MK integration. Keen robots, furthermore, independent vehicles outfitted with computer based intelligence calculations can play a scope of undertakings from cultivating and planting to gathering and arranging crops. These progressions in robotization not just smooth out work concentrated processes yet in addition increment productivity and diminish the reliance on physical work. Focusing on higher-value tasks like data analysis, making decisions and coming up with new ideas can ultimately result in increased profitability and sustainability for farmers.

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Chapter- 17

Effect on Growth and Yield of Summer Squash (*Cucurbita pepo* L.) from Different Cultivars and Spacings in Punjab's Heavy Soils

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The current investigation entitles "Effect on growth and yield of summer squash (*Cucurbita pepo* L.) from different cultivars and spacings in Punjab's heavy soils" was planned and carried out in *kharif* season of 2021-2022 at Campus for Research and Advanced Studies, Dhablan, P.G. Department of Agriculture, G.S.S.D.G.S. Khalsa College, Patiala. A field experiment was laid out in Factorial Randomized Block Design (FRBD) comprising of twelve treatments and three replications. Results showed that cultivar C_1 (Punjab Chappan Kadoo-1) recorded significantly higher plant height (46.05 cm), plant spread (42.25 cm), number of leaves plant^{-1} (27.61), yield parameters such as number of fruits plant^{-1} (6.58), fruit weight (74.55 g), fruit diameter (6.31 cm) and total fruit yield (236.41 q ha^{-1}) whereas highest fruit yield (263.91 q ha^{-1}) was obtained from spacing level S_3 (45 × 45 cm). However, treatment combination of C_1 (Punjab Chappan Kadoo^{-1}) with plant spacing level of S_3 (45 × 45 cm) significantly influence the growth, yield and economics parameters of summer squash as compared to all treatments.

Keywords – Summer squash, Cultivars and Spacing.

Introduction

Cucurbits having family Cucurbitaceae constitute the major group of summer vegetables grown in tropical and subtropical regions of the world. These squashes are eaten in a variety of ways including in deserts, salads and pickles whereas summer squash locally known as 'chappankaddoo' or 'valyatikaddoo'. Summer squash is regarded as one of the most widely consumed vegetable crops in Uttar Pradesh, Punjab and Haryana in India. The chromosome number of summer squash is 2n=40. Summer squash is grown commercially in many countries like USA, China, Europe and Japan etc. The cultivated area of summer squash in open field conditions was 2483 hectares in 2020 and production was 44672 tons, while the cultivated area of greenhouses was 151 hectares and the production was 12231 tons (NHB, 2021).Although it is a delicate, annual, warm season crop, summer squash can endure slightly colder temperatures than the majority of other cucurbits. As a result, it can grow in both hot and low temperatures. The ideal temperature for its growth is between 24° and 27°C. The ideal soil temperature for its seed germination is between 21°C to 30°C (Molinar *et al*. 1999), while germination is inhibited at temperature below 15°C. Germination typically takes 7-10 days, depending on the temperature of the soil. When the temperature is higher than 35°C, the yield has negatively impacted. Low nighttime temperatures, long days and abundant light encourage the development of female flowers. Otherwise, the formation of male flowers is encouraged by lengthy, hot days. It favors well drained soil and the soil pH should be 6.0-7.0.

Vitamin A, riboflavin, vitamin B6, niacin, folic acid and vitamin C are all abundant in the fruit of this plant. Fruits with yellow to pink flesh are higher in vitamin A than those with green flesh. Summer squash plants are annual herbs with thick bristles that are monoecious, spreading or bushy. The petioles of the leaves, which are typically covered with prickly hairs are deeply lobed. The plant develops fruit sets and stems with docked internodes in quick succession. The firm, highly wrinkled, five angled peduncle supports the green, unheroic or white colored fruits with white or pale-colored flesh. Fruits come in a variety of shapes including flat, twisted, spherical, disk shaped, long, short, blunt and phased. The fruits which are plucked when they are Physiologically immature, range varies from dark green, green, yellow, white and variegated (Sarhan *et al.* 2011).

Summer squash is a directly sown crop. In northern plains of India, it's generally sown in February-March but in areas of frost free sown from October to January. Summer squash needs about 2-3 kg seed for sowing in a hectare area. Spacing depends on whether the variety is spreading or bush type and varies from 45 × 45 cm between plants. Summer squash is a quick growing and short duration crop thus it takes 45 to 60 days from flowering to harvest. Summer squash is very soft rind vegetable therefore it's handling, transportation and storage requires proper attention and quick disposal up to consumer end (Nunes *et al*., 2003).

Plant spacing and varied genotypes are important factors in agricultural production, and it is generally known that plant spacing significantly affects crop development and yield. Because the plants utilize light, nutrients, and water in a coordinated manner, optimal plant spacing may enable the farmer to increase yields per unit area. To increase production, it is vital to choose high producing cultivars because the cultivars exhibit wavering in plant density due to variances in morphology and phenology.

Material and methods

The current investigation entitles "Effect on growth and yield of summer squash (*Cucurbita pepo* L.) from different cultivars and spacings in Punjab's heavy soils" was planned and carried out in *kharif* season of 2021-2022 at Campus for Research and Advanced Studies, Dhablan, P.G. Department of Agriculture, G.S.S.D.G.S. Khalsa College, Patiala which is situated at a height of around 249 m above mean sea level and about 30°-20° North latitude and 76°-28° East longitude. It is located in Punjab State, Northwest India, in the South-Eastern direction. During growing season (February to June), the weekly minimum and maximum temperature ranged between 10.22 to 44.4°C respectively. The average relative humidity ranged from 52 to 100 % and total annual rainfall during the season was 30.01 mm. Three replications of the experiment were done using a Factorial Randomized Block Design (FRBD). A total of 12 treatments were performed i.e., T_1- Punjab Chappan Kadoo- 1 (C_1) + 45 × 15 cm (S_1), T_2 - Punjab Chappan Kadoo- 1 (C_1) + 45 × 30 cm (S_2), T_3 - Punjab Chappan Kadoo- 1(C_1) + 45× 45 cm (S_3), T_4 - Punjab Chappan Kadoo- 1(C_1) + 45 × 60 cm (S_4), T_5 - Pratap (C_2) + 45 × 15 cm (S_1), T_6 - Pratap (C_2) + 45 × 30 cm (S_2), T_7 - Pratap (C_2) + 45 × 45 cm (S_3), T_8 - Pratap

(C_2) + 45 × 60 cm (S_4), T_9 - Desi (C_3) + 45 × 15 cm (S_1), T_{10} - Desi (C_3) + 45 × 30 cm (S_2), T_{11} - Desi (C_3) + 45 × 45 cm (S_3) and T_{12} - Desi (C_3) + 45 × 60 cm (S_4). During the duration of the experiment, observations were made on the following parameters : plant height (cm), plant spread (cm), number of leaves plant^{-1}, number of fruits plant^{-1}, fruit weight (g), fruit diameter (cm) and total fruit yield (q ha^{-1}). For statistical studies, the average of data from the sampled plant of each treatment was used to generate reliable results.

Results and discussion

The findings of the present study indicated that different types of cultivars and spacing has a positive effect on the vegetative growth parameters of summer squash viz., plant height (cm), plant spread (cm) and number of leaves plant^{-1}. In comparison to other cultivars, (C_1) Punjab Chappan Kadoo^{-1} had obtained highest plant height (46.05 cm), number of leaves plant^{-1} (27.61) and plant spread (42.25 cm) while (C_3) Desi had the lowest plant height (39.53 cm), number of leaves plant^{-1} (20.61) and plant spread (37.01 cm).The variations in growth parameters may be brought about by the different varying capacities for absorbing nutrients and varying levels of photosynthetic efficiency both of which may be related to their underlying genetic makeup. These findings closely match those of Al-Rawahi *et al.* (2011) in case of cucumber and Andino and Mostenbocker (2004) in case of watermelon.

Table 1: **Effect on growth parameters of summer squash from different cultivars and spacings.**

Treatments / Cultivars	Plant height (cm)	Number of leaves plant^{-1}	Plant spread (cm)
Punjab Chappan Kadoo^{-1} (C_1)	46.05	27.61	42.25
Pratap (C_2)	41.74	24.68	38.71
Desi (C_3)	39.53	20.61	37.01
S.E (m)±	0.64	0.59	0.58
CD (5%)	1.32	1.22	1.20
Spacing (cm)			
45 × 15 cm (S_1)	38.35	21.86	31.24
45 × 30 cm (S_2)	41.47	23.14	38.11
45 × 45 cm (S_3)	44.11	25.24	42.39
45 × 60 cm (S_4)	45.64	26.65	43.78
S.E m±	0.74	0.68	0.67
CD (P=0.05)	1.53	1.41	1.39

Among different spacing treatments, plant height (45.64 cm), number of leaves plant^{-1} (26.65 cm) and plant spread (43.78 cm) was found to be superior in spacing level (S_4) 45 × 60 cm whereas plant height (38.35 cm), number of leaves plant^{-1} (21.86 cm) and plant spread (31.24 cm) were lowest in spacing level (S_1) 45 × 15 cm. Due to close competition for nutrients and aeration, closer spacing had poor results although the plant obtained more sunshine, aeration and nutrients as well. Similar conclusions had been made in broccoli and other vegetables by Tejaswini *et al.* (2018).

Table 2. Effect on yield parameters of summer squash from different cultivars and spacings

Treatments / Cultivars	Number of fruits plant^{-1}	Fruit weight (g)	Fruit Diameter (cm)	Fruit yield (q ha^{-1})
Punjab Chappan Kadoo-1 (C_1)	6.58	74.55	6.31	236.81
Pratap (C_2)	5.84	66.02	4.68	191.64
Desi (C_3)	3.51	58.91	2.78	153.36
S.E (m)±	0.36	0.80	0.54	6.30
CD (5%)	0.74	1.66	1.11	13.07
Spacing (cm)				
45 × 15 cm (S_1)	2.13	51.32	2.82	161.92
45 × 30 cm (S_2)	4.15	56.23	3.56	172.94
45 × 45 cm (S_3)	6.26	75.21	5.33	263.91
45 × 60 cm (S_4)	7.11	77.12	6.61	178.71
S.E m±	0.41	0.93	0.62	7.28
CD (P=0.05)	0.85	1.91	1.28	15.10

Different cultivars showed significant results for various yield parameters. When compared to other cultivars, (C_1) Punjab Chappan Kadoo- 1 had the highest values for the number of fruits plant^{-1} (6.58), fruit weight (74.55 g), fruit diameter (6.31 cm) and total fruit yield (236.81 q ha^{-1}). Fruit weight (58.91 g), number of fruits plant^{-1} (3.51) and total fruit yield (153.36 q ha^{-1}) was found to be lowest in (C_3) Desi. The significant yield variations between the cultivars may be brought on by genetic factors. As a result, variation in yield parameters may be related to genetic differences across kinds, which result in higher yield. Similar observations were recorded by Keerthika *et al.* (2016) and Ashwini (2014) in snake gourd.

Among all spacing treatments (S_3) 45 × 45 cm recorded highest results for fruit yield (263.91 q ha^{-1}) whereas number of fruits plant^{-1} (7.11), fruit weight (77.12 g) and fruit diameter (6.61 cm) were recorded maximum in (S_4) 45 × 60 cm. Plant spacing level (S_1) 45 × 15 cm showed poor performance for all yield parameters. This was possibly due to increase in plant number per unit area, which might contribute to the production of more yields per unit area and leading to higher yield. Optimum plant densities produced maximum fruit yield as compared to wider spacing. Similar results are in agreement with Ban *et al.* (2006) in melon and Watanabe *et al.* (2003) in watermelon.

Conclusion

From the above results, it is evident that cultivar Punjab Chappan Kadoo-1 (C_1) demonstrate superior in terms of growth and yield characteristics as compared to other two cultivars. Plant spacing 45 × 45 cm (S_3) is regarded as the most productive for growing summer squash and produced the highest yield (q ha^{-1}). In the treatment combinations, cultivar Punjab Chappan Kadoo^{-1} (C_1) with plant spacing 45 × 45 cm (S_3) exhibited better growth parameters and gave higher yield as according to the observations made above.

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Chapter- 18

Sugarcane – Source Sink Relationship on Yield

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Abstract

Sugarcane, a vital global cash crop, contributes about 70% of the world's sugar production and is a key source of biofuel. In India, it holds strategic importance, being the second-largest sugar producer globally after Brazil. Sugarcane is a versatile resource, providing not only sugar but also fiber, ethanol, fertilizer, and other sustainable byproducts. The sugar industry in India, second only to textiles, plays a significant role in employment generation. The unique source-sink system of sugarcane distinguishes it from other plants. It stores high concentrations of sucrose in parenchyma tissue rather than insoluble polysaccharides like starch. Understanding the regulation of this source-sink system is crucial for optimizing crop yield. Uttar Pradesh leads in sugarcane cultivation with a yield of around 370,500 tons and productivity of 80.5 tonnes/hectare. Abiotic factors, such as water deficit and mineral deficiencies, profoundly affect sugarcane yield. Drought alters plant architecture and reduces photosynthetic production, impacting carbon flow to sinks. Mineral deficiencies, including nitrogen, phosphorus, magnesium, and potassium, influence carbon partitioning and phloem loading. Soil pollutants like cadmium and air pollutants like sulfur dioxide can disrupt sieve tube function, affecting long-distance sugar transport. Biotic stress, induced by microbes, viruses, and aphids, further challenges sugarcane productivity. Mutualistic and pathogenic interactions disrupt the source-sink balance, altering sugar transport and partitioning. The study of these factors provides insights into optimizing sugarcane cultivation practices for sustainable and high-yielding production. Understanding how environmental factors impact the intricate source-sink dynamics in sugarcane is crucial for developing strategies to enhance crop resilience and productivity in the face of global challenges.

Keywords: Sugarcane, source, sink, abiotic, biotic stresses

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Introduction

About 70% of the sugar produced globally comes from sugarcane, one of the oldest and most significant cash crops in the world. It also serves as a major source of biofuel. India's primary source of sugar is sugarcane, which is also a significant cash crop. After Brazil, India was the world's second-largest producer of sugar in 2018–19. In addition to producing sugar, sugarcane also produces fiber, ethanol, fertilizer, and a wide range of other ecologically sustainable byproducts. This makes sugarcane a renewable, natural agricultural resource. Its juice is utilized to make jaggery, brown sugar and white sugar.

One of the primary crops used to generate foreign exchange is sugarcane. Molasses and bagasse are the two primary byproducts of the sugarcane industry. The primary use of bagasse is as fuel. Moreover, it is employed in manufacturing paper, plastics, furfural, and boards made of compressed fiber. Distilleries employ molasses to make butyl alcohol, citric acid, ethyl alcohol, and other products. A promising organic fertilizer is sugarcane press mud. Cattle can benefit from cane green tops as a source of feed. In India, the sugar industry is second only to the textile sector in terms of importance and employs a significant number of people in employment opportunities. While beet sugar is produced in cooler places cane sugar is produced in warmer places. The average person consumes 24 kilograms of sugar yearly (33 kg in industrialized nations). Although it is mostly seen as dangerous, it also offers a lot of health advantages and may help treat depression, diabetes, and low blood pressure.

With a yield of around 370500 ('000) tons and a productivity of about 80.5 tonne/hectare, sugarcane is grown on 4603 ('000) hectares in India (2019–20, India Stat). When it comes to productivity and output, Uttar Pradesh is the state that cultivates the most sugar cane.

Sink System in Sugarcane

An integrated system of photosynthetic carbon sources and non-photosynthetic carbon-consuming sinks, such as growth and respiration, may be used to characterize all higher plants. Two factors make sugarcane a special source-sink system: (i) it stores assimilate at very high concentrations in the form of sucrose, an osmotically active solute, whereas most plants store insoluble polysaccharides like starch; and (ii) storage takes place in the parenchyma tissue of the stalk (culm), not in the terminal sink organs. The sugar beet (Beta vulgaris L.), which stores sucrose in its roots, is one of the several plants that can hold large amounts of sugar.

The rate at which sucrose capitalizes up in sugarcane may be restricted by several physiological mechanisms including Rates of photosynthesis in leaves and the distribution of carbon into pools apart from culm storage, Phloem loading in leaves and unloading in culms, and developmental restrictions, such as maturation time and length. Thus, Cell metabolism, including membrane transfer into storage vacuoles, partitioning, turnover, and remobilization of sucrose.

Source Sink Regulation

Sugarcane, a member of the Poaceae family, stands out among C4 plants for its highly efficient conversion of solar energy into chemical energy. Carbon partitioning is a crucial process in allocating the chemical energy derived from photosynthesis. In C4 plants like sugarcane, photosynthesis and carbon assimilation occur in both the chloroplasts of leaf mesophyll cells and bundle sheath cells. During photosynthesis in source cells, fixed carbon transforms into sugars or their derivatives, which are then transported to distant sink cells through the phloem. In sink tissues, sugars face the choice between storage and consumption.

Approximately 35–40% of carbohydrates are absorbed by living cells to provide energy for various processes, including cell growth, expansion, division, differentiation, nutrient absorption, and maintenance throughout plant development. These living cells contain a variety of simple sugars, amino acids, organic acids, and other metabolic intermediates. The remaining sugars can either be incorporated into polymers such as cellulose, hemicelluloses, and lignin, or stored in vacuoles. Polymers may contribute to structural biomass or be remobilized, similar to starch in plastids.

Source-Sink System in Sugarcane

Sucrose, generated in photosynthetic leaves, undergoes transportation in the phloem to reach stem parenchyma cells. Within these cells, the transfer can occur either through symplastic routes or apoplastic pathways. The movement of sucrose to storage parenchyma can proceed bidirectionally without alteration, although during apoplastic transfer, cell wall invertase may catalyze the hydrolysis of sucrose into hexoses. Transporters facilitate the entry of both sucrose and hexoses into parenchyma cells. Additionally, cells can produce hexoses from sucrose through the activity of vacuolar acid or neutral invertases in the cytoplasm. Sucrose finds storage in both vacuoles and the cell wall space, with the equilibrium influenced by factors such as transporters and the release of sucrose to the apoplast. Maintaining a balance between UDP-Glu (UDP-glucose), a precursor for cell wall biosynthesis, and internal sucrose supply is crucial for competing with carbon sinks. Key processes in this dynamic include the reversible reactions of sucrose synthase (SuSy), sucrose-P-synthase (SPS), and sucrose-P-phosphatase (SPP).

Factors affecting Crop Yield: Abiotic Factors

Effect of Water Deficit

Among the various abiotic factors that significantly impact crop growth and production is water deficiency. Unfavorable circumstances are imposed on a plant's roots (sink) and leaves (source) by drought. Plant architecture changes as a result of reduced shoot development and continued root growth in modest water deficits. Drought reduces the amount of newly developing organs in monocots, such as grasses. This technique of avoiding water stress may result in a decline in global photosynthetic production, which in turn may affect the flow of carbon to various sink organs. The concentrations of the primary organic nutrients—sugars and amino acids—that flow through the sieve tubes alter when there is a water deficit. The analysis of collected alfalfa phloem fluid revealed a notable rise in total amino acid concentrations and sugar contents as the leaf water potential dropped from -0.4 to -2.0 MPa. In sink organs, osmotic stress favored sucrose biosynthesis over starch biosynthesis by inducing sucrose-phosphate synthase (SPS) and inhibiting ADP glucose pyrophosphorylase. Examples of the detrimental effects of drought on sink development have been documented in potato tubers. Yang *et al*. (2004) found a correlation between the buildup of starch in grains and the breakdown of certain storage carbohydrates in stems, such as fructans and starch. Studies on the impacts of water shortage at various stages of development have also been conducted. Senescence can be induced and reserve mobilization can be enhanced by drought stress.

Effects of Mineral Deficiency

Through their roots, plants take up mineral nutrients necessary for growth and development. Therefore, one essential adaptation trait that enables plants to adjust to a changing environment is the plasticity of the architecture of their root systems. According to Marschner *et al.* (1996), nutrient deficiencies may have an indirect effect on photo-assimilate partitioning by lowering sink demand or directly affecting phloem loading and transport.

Response to nitrate limitation

A nitrogen deficiency causes the roots to retain more carbon, which raises the root/shoot ratio and causes a buildup of carbohydrates in the leaves. A reduction in electron transfer and a drop in RubisCO quantity and activity, which lower photosynthesis, provide some indications as to how nitrogen deficit affects the shoot's metabolism of carbohydrates and raises the biomass ratio of the roots compared to the shoots.

Response to phosphorus limitation

After nitrogen, phosphorus is the second most scarce mineral ingredient for crop productivity. Reduced carbon absorption and decreased photosynthesis are the immediate results of low phosphorus in leaf mesophyll cells, as P availability in the chloroplast is affected.

Response to magnesium and potassium deficiency:

According to Camak and Yazici (2010), magnesium affects several metabolic processes and reactions, including the production of chlorophyll, photosynthetic carbon dioxide fixation, and photo-assimilate phloem loading and partitioning. The export of sucrose to the roots is affected by magnesium deficit, which also inhibits leaf development more than root growth. It is believed that magnesium shortage affects phloem sucrose loading by lowering the availability of Mg-ATP. The main cation in the phloem is K^+. As a result, variations in its concentrations can significantly impact phloem functioning. There is no development or increase in the sugar content of the roots in K-deficient plants despite the high sugar concentration seen in their leaves.

Effects of NaCl

Effects of NaCl: One of the main factors limiting plant development and production is salt stress, which is often caused by irrigation with low-quality water. Because a decreased soil water potential surrounding the roots is the main symptom of both salt stress and drought stress, there are numerous similarities between the two conditions. This initial stress is compounded by sodium toxicity, which is transported into the plant by the transpiration stream. The plant's internal Na^+ recirculation system is thought to involve potassium channels. To reduce the quantity of Na^+ in leaves, Na^+ can be loaded into the leaf phloem and sent to the roots for excretion (Berthomieu *et al*., 2003). However, this flow may only be somewhat more than the xylem flux (Davenport *et al*., 2007). Regarding the effects of salt stress on sucrose translocation into the phloem, not much is known. Salt stress inhibits photosynthesis and frequently results in stunted development, which is more significant in leaves than in roots.

Effect of Light

Phloem loading is directly impacted by light through photosynthesis, which produces sucrose and uses energy from light. The structure of the loading zone itself is impacted by light as well, though. The response to the change in light levels (and consequently the adaptation to increased photosynthesis) varied depending on the loading mode (apoplastic or symplastic). For example, in apoplastic species like peas, there was an increase in cell wall invasions in the companion cells surrounding the SE (Amiard *et al*., 2005). This suggested a larger exchange surface that enabled a greater loading of sucrose in the phloem. As a result, starch accumulated

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in the leaves of symplastic loaders like pumpkin, where plasmodesmata frequencies did not rise.

Effect of Low Temperature

Different cell types, including intermediate cells, parenchyma transfer cells, and SEs, can be impacted by low temperatures in different ways when it comes to phloem sugar transport. Insufficient tocopherol, or vitamin E, causes greater callose deposition in the vascular tissues of monocot and dicot plant species, which hinders photo assimilate export from source leaves (Hofius *et al*., 2004). Phloem loading in low temperatures has also been reported to have a similar effect. Phloem movement in dicots temporarily ceases across the cooled zone when brief segments of stems or petioles are steadily exposed to cold temperatures. Even if tissues are kept at low temperatures, phloem movement can resume, therefore this cessation is limited and temporary.

Effects of CO_2

The increased atmospheric CO_2 directly affects plant photosynthesis because, in C_3 plants, the active site of RuBisCO has a low affinity for CO_2, which limits the photosynthetic reaction at the current CO_2 concentration (Woodward, 2002). Consequently, a rise in CO_2 should promote the rates of photosynthetic energy conversion, the synthesis of carbohydrates, and the development and transport of phloem. According to Makino and Mae (1999), the majority of plants growing under high CO_2 show greater accumulation of carbohydrates in their leaves, with species-specific differences in biomass partitioning between source and sink organs.

Effects of Some Soil and Air Pollutants

Effects of Cadmium

Earth's soils include heavy metals, metalloid arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg), among other contaminants. According to Vollenweider *et al*. (2006), the degeneration of sieve tubes and companion cells in those trees was proportionate to the increase in Cd concentrations given at the root level. As a result, there was a reduction in long-distance transport and a decrease in leaf size and biomass.

Effects of Sulfur Dioxide

During the latter part of the 20th century, sulfur dioxide (SO2) was a significant air pollution and was thought to be the primary reason for the collapse of forests in central Europe (Kurczyńska, 1997). 0.035 parts per million of SO2 in the air can create up to 35 parts per million of SO2 in an aqueous solution due to SO2's high solubility in water (Puckett *et al*., 1973). In leaf tissues, the pollutant can build up and interfere with physiological processes including transpiration, respiration, and photosynthesis. In addition to decreased photosynthesis, restricted phloem loading also affects photo-assimilate translocation in bean leaves and castor bean cotyledons (Lorenc-Plucinska and Ziegler, 1987). In a more recent anatomical investigation, it was demonstrated that SO_2 reduced the number of phloem cells; nevertheless, it was challenging to distinguish between differences in phloem mother cell division and a general decrease in cambial activity.

Effects of Biotic Stress

Microbes such as bacteria, viruses, and fungi, as well as herbivores and occasionally other plants that function as parasites, must be dealt with by plants as they grow. Those organisms, in any case, have the potential to impact the phloem transport of sugars since they develop at the expense of the sugars produced by plants.

Mutualistic and Pathogenic Microbes

Based on their lifestyles, microorganisms may be classified into two groups: pathogenic (biotrophic to necrotrophic; Newton *et al.,* 2010) and mutualistic (e.g., mycorrhiza). Microorganisms have developed complex ways to evade, inhibit, or overcome plant defenses and to siphon resources, particularly sugars, from the host plant for their development, even if their routes of colonization are different. For instance, intricate interfaces called arbuscules and haustoria, which move nutrients to and from the plant, allow mutualistic microbes and biotrophic pathogens to proliferate (Voegele and Mendgen, 2011; Smith and Smith, 2012). On the other hand, to feed on macerating tissues, necrotrophic bacteria emit poisons and generate hydrolytic enzymes that destroy host cells (Van Kan, 2006).

Microbes can colonize source or sink organs. Both mutualistic and pathogenic interactions disrupt the source-sink balance because they depend on the host plants providing sugar to the heterotrophic invading agent. The general view is that colonized source organs undergo a source-to-sink transition that alters the plant's overall mechanism for sugar transport and partitioning. Plant-biotrophic fungal interactions are frequently used as models in pathosystem studies to examine pathogen-induced changes in carbon partitioning. For this reason, we only briefly discuss a few unique characteristics of other pathosystems and instead concentrate specifically on plant-biotrophic fungal interactions here.

Rust and powdery mildew are examples of biotrophic fungi that produce haustoria to build a long-term feeding relationship with their host's live cells. These are permeable cell-wall structures that preserve the host cell protoplast while forming an apoplastic interface that allows the fungus to receive released host nutrients. According to Hall and Williams (2000) and Voegele and Mendgen (2011), autoradiography experiments employing radiolabeled chemicals provide indirect evidence for the major function of haustoria in the transfer of sugar and amino acids from host to biotrophic pathogens. The fungal carbon requirement in diseased tissues generates a second significant sink that competes with host sinks. A combined experimental modeling strategy has been recently used to investigate the competitiveness between plant and fungal sinks.

Viruses

Plant pathogens are infected by viruses through movement proteins (MPs) encoded in the viruses. These MPs change the plasmodesmata's exclusion size, indicating that viruses can take advantage of the PD-mediated cell-to-cell trafficking of photo assimilates. During viral infection, there can be a direct impact on signaling and the distribution of carbohydrates. These alterations imply that virus-infected leaves serve as sinks. However, depending on how viruses make use of the host transport system, viral MPs can have different impacts on the distribution of carbohydrates. According to Biemelt and Sonnewald (2006), in certain instances, the cause may not be the PD size exclusion limit, but rather induced callose deposition at the PD level, which obstructs symplastic sucrose transport.

Aphids

Aphids are "experts" in probing the phloem and manipulating the plant tissues for their benefit. They are the vectors of several plant viruses (Brault *et al*., 2010; Dedryver *et al.,* 2010). They create minute punctures in tissues with the use of fine stylets, and after a brief period, they examine the physicochemical characteristics of the microenvironment around the stylet tip. According to Hewer *et al*. (2010), aphids' capacity to detect high sucrose concentrations and pH is connected to their capacity to locate sieve tubes, as demonstrated using empirical systems.

Conclusion

Sugarcane plays a vital role globally as a significant cash crop, a source of biofuel, and a renewable agricultural resource. The intricate source-sink system in sugarcane, characterized by sucrose storage in stalk parenchyma cells, is essential for its high productivity. Various abiotic factors, such as water deficit, mineral deficiencies, salt stress, temperature fluctuations, atmospheric CO_2 levels, and pollutants, significantly impact sugarcane yield. Additionally, biotic stresses from microbes and pests, including viruses and aphids, further contribute to challenges in maintaining the delicate balance of the source-sink system in sugarcane.

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Chapter- 19

Role of Tri-trophic Interactions in Biological Control of Insect Pests

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Abstract

An agro-ecosystem consists of at least three trophic levels: plants, herbivores and their enemies. The members of alternate trophic levels usually act in mutualistic manner. Natural enemies of herbivores benefit the plants by reducing the abundance of herbivores. Plants may benefit the enemies of herbivores by making them more accessible to the trophic level above. Therefore, plants defend themselves either by producing chemicals, such as toxins, or digestibility reducers, or through physical defense by trichomes or toughness, or by a combination of the two, as with glandular trichomes or resins (intrinsic defense of the plants) and by benefitting natural enemies of the herbivores (extrinsic defense of the plants). Almost every mechanism of the intrinsic defense of a plant has an effect on the trophic system and this may impact positively or negatively upon the third trophic level as well as on those factors involved with extrinsic defense. The intrinsic and extrinsic defenses of plants reduce the colonisation rate of the herbivores. The conflict between intrinsic and extrinsic defenses affects the evolution of plant allelochemistry. The plants have three options: (1) they become highly attractive to beneficial insects, thus reducing the herbivore population, or (2) they become poisonous to herbivores, it may harm third trophic level (extrinsic defense), or (3) they achieve some compromise which exploits both protective mechanisms. The toxic substances of plant tissues that repel, retard growth, reduce vigour, or kill susceptible herbivores may poison bioagents or cause physiological/metabolic changes in herbivores, which reduce its value as a food source for the entomophages. Combination of these two approaches in insect pest management has been argued because understanding of multi-trophic interactions is essential in evaluating the roles of natural enemies in population dynamics of herbivores. The influence of transgenic plants on the natural enemies of herbivores is also reviewed.

Keywords: Agro-ecosystem, Entomophagous, Bioagents, Allelo-chemistry, Natural enemies, Toxic substance.

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Introduction:

An agro-ecosystem typically comprises a minimum of three trophic levels, involving plants, herbivores, and their predators. Interactions among these levels often take on a mutualistic nature. The natural enemies of herbivores play a crucial role in benefiting plants by curbing herbivore abundance. In return, plants can facilitate the accessibility of herbivores to their predators in the trophic level above. To safeguard themselves, plants employ various defense mechanisms, including the production of chemicals like toxins or digestibility reducers, as well as physical defenses like trichomes or toughness. Some plants utilize a combination of intrinsic defenses (such as glandular trichomes or resins) and extrinsic defenses (benefiting natural enemies of herbivores). These defensive strategies impact the trophic system, influencing both positively and negatively the third trophic level and factors associated with extrinsic defense. Intrinsic and extrinsic plant defenses collectively contribute to reducing herbivore colonization rates. The interplay between these defenses shapes the evolution of plant allelochemistry. Plants face choices: they can attract beneficial insects to reduce herbivore populations, become toxic to herbivores (potentially impacting the third trophic level), or strike a compromise utilizing both protective mechanisms. The toxic substances that repel herbivores may also affect bioagents or alter herbivore physiology, diminishing their value as food for entomophages. Integrating both approaches in insect pest management is advocated, underscoring the importance of understanding multi-trophic interactions in evaluating the roles of natural enemies in herbivore population dynamics. The impact of transgenic plants on the natural enemies of herbivores is also explored in this context.

1. Tri-trophic interaction:-

Ecological interaction between three trophic levels on each other: plant, herbivore and natural enemies. HIPVs (Herbivore-induced plant volatiles) play important role in tritrophic interaction. (Turling and Erb, 2018). Plants recruit natural enemies to protect them from herbivores through herbivore-induced plants volatiles. Natural enemies have to utilize different infochemicals to locate the appropriate host plants and host prey in their whole life span. Example of tritrophic interactions: 1. Daphnia are herbivores on a unicellular algae and they are prey of damsel flies. 2. Hover flies sip nectar from flowers and in turn are eaten by spider 3. Mustard aphids (*Lipaphis erysimi*) are herbivores on a mustard plant and they are prey of syrphid flies larva.

Interaction among host plants, insect pests and their natural enemies (tritrophic interaction) leads to effective defense and attack at each level. (Fig. 1). On this basis, two types of plant resistance of plant defense have been recognised (Price, 1986).

1. **Intrinsic resistance**
2. **Extrinsic resistance**

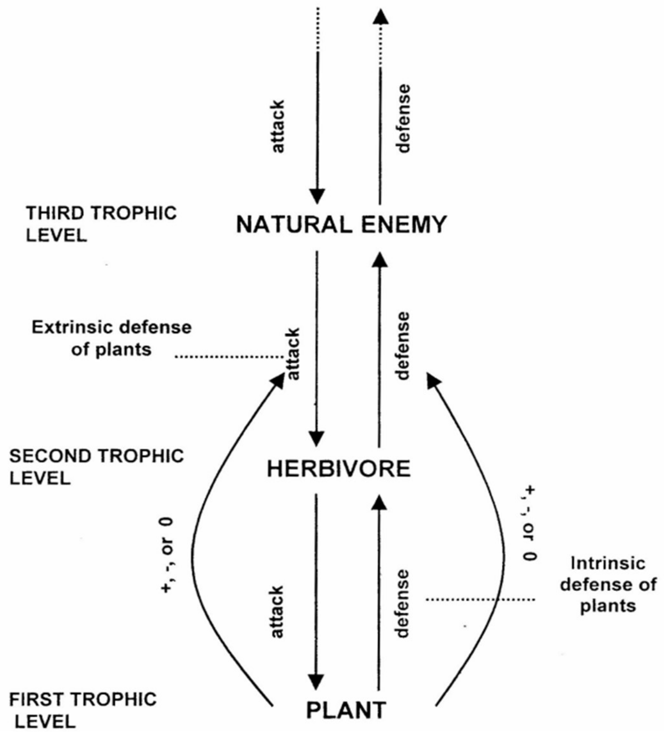


Fig. 1: Relationship between intrinsic and extrinsic defenses of plants. +, - and 0 indicate positive, negative or no effects. (Price, 1986).

1. Intrinsic resistance/defense:-

Here the plant alone produces defense through physical means (trichomes or toughness) or through production of chemicals (toxins or digestibility reducers) or both (glandular trichomes or resins). It is also known as direct defense. The physical, morphological and chemical characteristics of plants interact with the parasitoids and predators of insects by influencing their host seeking ability and affecting the efficacy with which they locate and utilize hosts.

Mechanical defenses:-

Plant have many external structural defenses that discourage herbivory. Some defensive compounds are produced internally but are released onto the plant's surface such as resins, lignins, silica and wax cover the epidermis of terrestrial plants and alter the texture of the plant tissue. A plant's leaves and stem may be covered with sharp prickles, spines, thorns or trichomes-hairs on the leaf often with barbs, sometimes containing irritants or poisons, e.g. glandular trichomes on tomato leaves also provide protection against adults of white fly (*Bemisia tabaci*). (Kisha, 1984). Epicuticular waxes form films and crystals that cover the cuticle of most vascular plants, e.g. oviposition of *Pieris brassicae* on *Arabidopsis thaliana* induce changes in the wax composition, increasing the amount of fatty acid tetratriacontanoic acid, arresting the egg parasitoid and while decreasing the amount of tetracosanoic acid increasing the egg parasitoid (*Trichogramma brassicae*). These changes lead to attraction of egg parasitoid. (Blenn *et al.* 2012), e.g. the thorns on the stem of this raspberry plant, serve as a mechanical defense against herbivory (Japanese beetle, woodboring beetle).

Physical defenses:-

Leaf toughness slow the larval development of pierid butterfly and thus exposing them to natural enemies for longer period. Leaf toughness delay the development of herbivores, e.g. the searching behaviour of *Trioxy indicus* (parasitoid of aphids) is influenced by pubescence (soft body or short hairs on stems) of host plant.

Chemical defenses:-

Plants have evolved many secondary metabolites involved in plant defense, which are collectively known as anti-herbivory compounds. Secondary metabolites known as bioactive specialized compounds, are used to protect the plant against herbivores. It can be classified into various sub-groups such as alkaloids, benzoxazinoids, glucosinolates. Alkaloids are derived from various amino acids. Over 3000 known alkaloids exist, examples include nicotine, caffeine, morphine, colchicine and quinine. Alkaloids found in the *Leguminosae* spp., *Liliaceae* spp. and *Solanaceae* spp. [*Solanum demissum* (nightshade potato) containing the alkaloid demissine is resistant to *Leptinotarsa decemlineata* (Colorado beetle) and *Empoasca fabae* (leafhopper)].

Benzoxazinoids are found in *Gramineae* family (maize, rye and wheat) produces the defense-related bioactive specialized compounds such as 2,4-dihydroxy-1,4-benzoxazine-3-one-glucoside (DIBOA-Glc) and dihydroxy-7-methoxy-1,4-benzoxazine-3-one-glucoside (DIMBOA-Glc, from indole-3-glycerol phosphate. Benzoxazinoids are secondary defence metabolites, e.g. DIMBOA has been shown resistance to *Ostrinia nubilalis* (first-brood European corn borer). Glucosinolates (GSL) are sulphur and nitrogen containing compounds found extensively in Brassicaceae. Glucosinolates are activated in much the same way as cyanogenic glucosides and the products can cause gastroenteritis, salivation, diarrhea, and irritation of the mouth. Flea beetle (*Phyllotreta cruciferae)* feeds preferably on older cotyledons of *Sinapis alba* (white mustard), due to the lower levels of GSL. Some other examples of chemical defense such as Monarch butterfly sequesters cardiac glycosides from milk weed plants so that it becomes distasteful to predatory birds. Honeydew as a contact kairomone for aphid parasitoid. (Budenberg, 1990). The honeydew excreted by the aphids also serve as food for the parasitoids (Singh *et al.,* 1996).

2. Extrinsic resistance/defense:-

Here the natural enemies (third trophic level) of insect trophic level) benefit the host plants (first trophic level) by reducing the pest abundance. When the natural enemies of insect pests benefit the host plants by reducing the pest population. Major components and pathways involved in extrinsic plant defense. Indirect plant defense can be both constitutive and inducible. Constitutive defense includes extrafloral nectar, food bodies and domatia. Inducible defenses is initiated with the production of various elicitors, including enzymes, fatty-acid-amino-acid conjugates, sulfooxy fatty acids, fragments of cell walls, peptides and ester. Elicitors trigger the activation of hormone pathways results in the synthesis of a broad spectrum of volatiles such as terpenes, volatile, indoles, aldehydes, ketones, esters, alcohol and nitrogenous compounds. These volatiles can be used as cue to attract natural enemies including predators, parasites and omnivores, resulting in the suppression of pest population.

Two mechanisms are involved in extrinsic resistance first one is herbivore- induced plant volatiles (HIPVs) and second one is extra-floral nectar.

Herbivore-induced plant volatiles (HIPVs):-

HIPVs can mediate indirect defenses by attracting foraging carnivorous predators and parasitoids. More than 1000 volatile organic compounds (VOCs), primarily consisting of 6-carbon aldehydes, alcohols, esters and various terpenoides are released from plant flowers, vegetative parts or roots. VOCs are used to attract pollinators and predators or repel herbivores, e.g. roots VOCs: *Z. mays* roots attacked by Western corn rootworm insect larvae release the sesquiterpene (E) - β-caryophyllene as well as small amounts of alpha-humulene and caryophyllene oxide which attract its natural enemy.

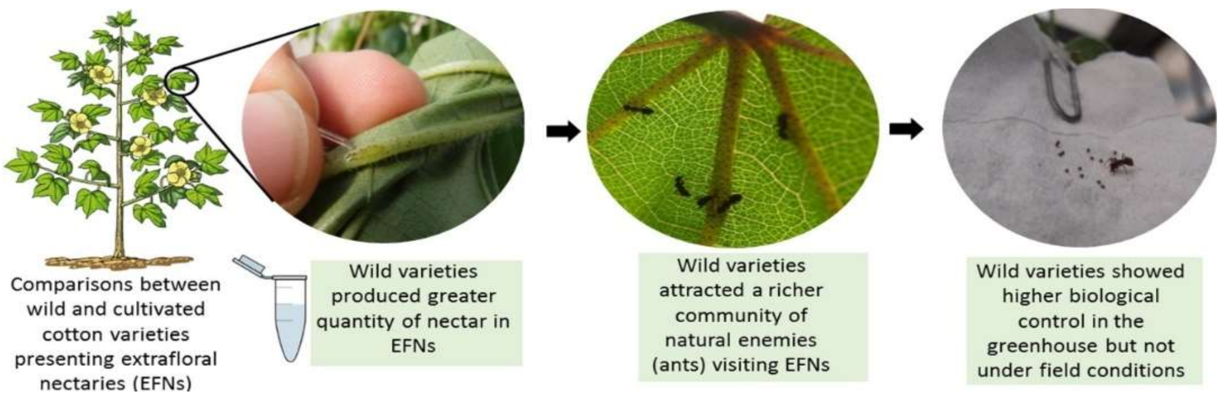
Herbivore-induced plant volatiles

HIPVs	Plant	Herbivore	Natural enemies
β- caryophyllene β- ocimene	Tobacco	*H. virescens*	*Cardiochiles nigriceps*
(E)-β-farnesene (E)-caryophyllene (E)-□-bergarmortene	Maize	*S. litoralis*	*Cotesia marginiventris*
(E)-4,8-dimethyl-1,3,7-nonatriene	Maize	*M. separata*	*Exorista japonica* *Cotesia kariyai*
Linalool Alpha-pinene 1-hexanol	Maize	*M. separata*	*Exorista japonica* *Campoletis chlorideae*

Source:- Rodrigues *et al*., (2015)

Extra-floral nectar (EFNs):-

Extrafloral nectar, used to attract pollinators. EFN is secreted on leaves and shoots to attract predators and parasitoids. Plant pollen and nectar increase the life spans and fecundities of many parasitoids and predators, fostering greater herbivores mortality.



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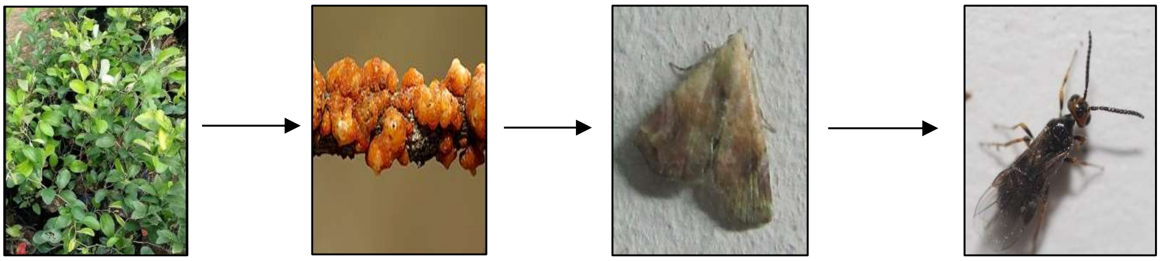
Family	Species	Herbivore	Trait enhanced
Bibnoniaceae	*Catalpa bignonioides* (Indian bean)	*Ceratomia catalpa* (Catalpa sphinx)	Sugar content in EFN
Malvaceae	*Gossypium herbaceum* (Cotton)	*Spodoptera littoralis*	EFN volume
Euphorbiaceae	*Ricinus communis* (Castor)	*Spodoptera littoralis*	EFN volume
Euphorbiaceae	*Triadica sebifera* (Chinese tallow tree)	*Garirtha inexacta and Grammodes geometrica*	Secretion of toal solids

Source:- Martin, (2015)

Multitrophic interaction:-

The multitrophic interaction is an ecological interaction between two or more trophic levels and formation of complex food web. Example of tetratrophic interactions: E.g. 1.



Ber Lac insect *Eublemma amabilis* (Predator) *Bracon greeni*

Fig. 2: Carnivores of third trophic level harmful for plants by reducing the abundance of herbivores and the carnivores of fourth trophic level benefit the herbivores by reducing their over predation.

E.g. 2. Tetra-trophic interaction under cabbage ecosystem: [e.g., cabbage butterfly-braconid parasitoids/sphecid predators-hyperparasitoid (*chalcidoid* wasp) - spiders-spider wasps] are linked into a food web by the same spiders feeding on both braconid wasps and predators of aphids (ladybird beetles, anthocorid bug, syrphid flies).

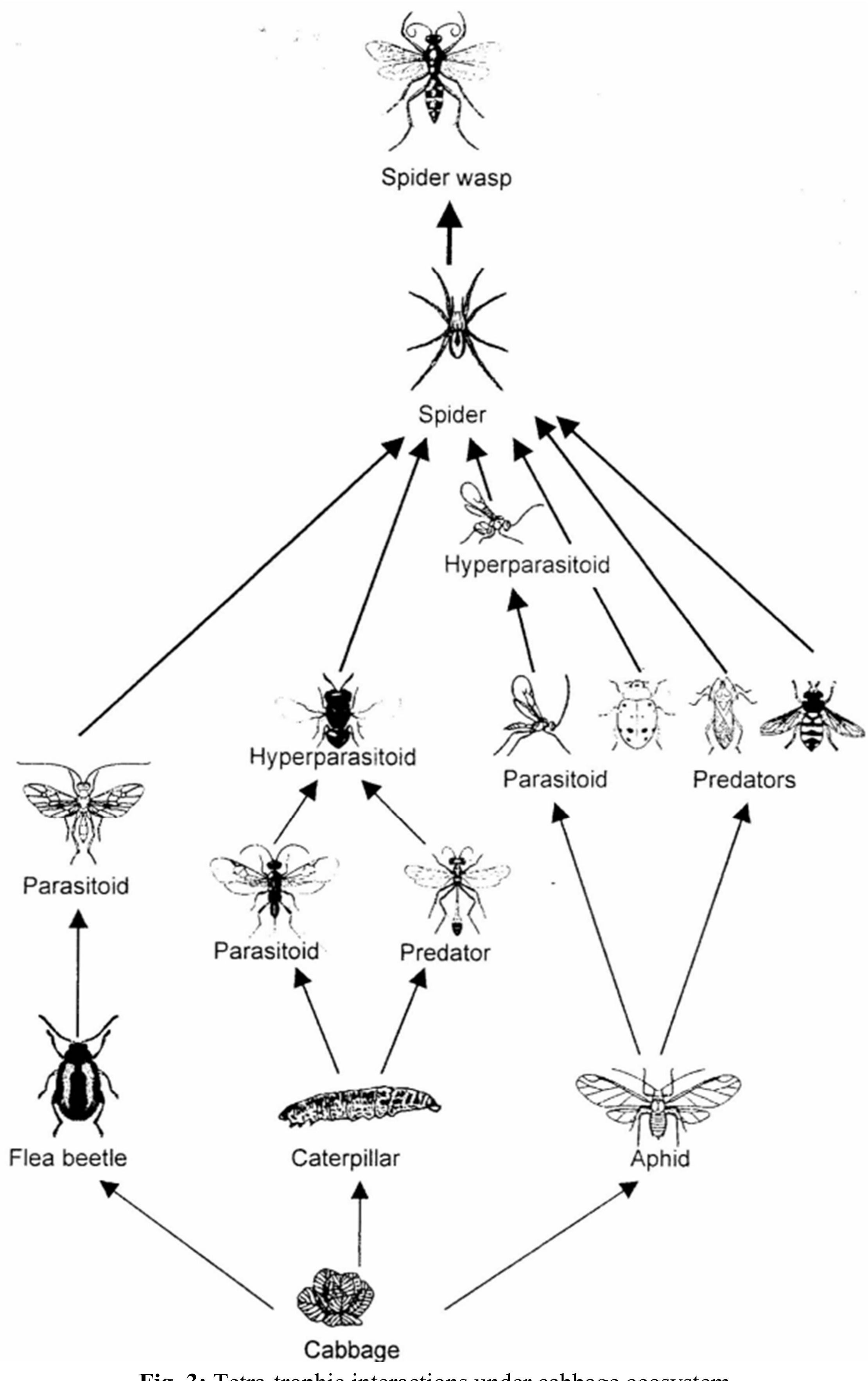


Fig. 3: Tetra-trophic interactions under cabbage ecosystem

E.g. 3. Carnivores of third trophic level benefit the plants by reducing the abundance of herbivores and the carnivores of fourth trophic level benefit the herbivores by reducing their over predation/parasitism.

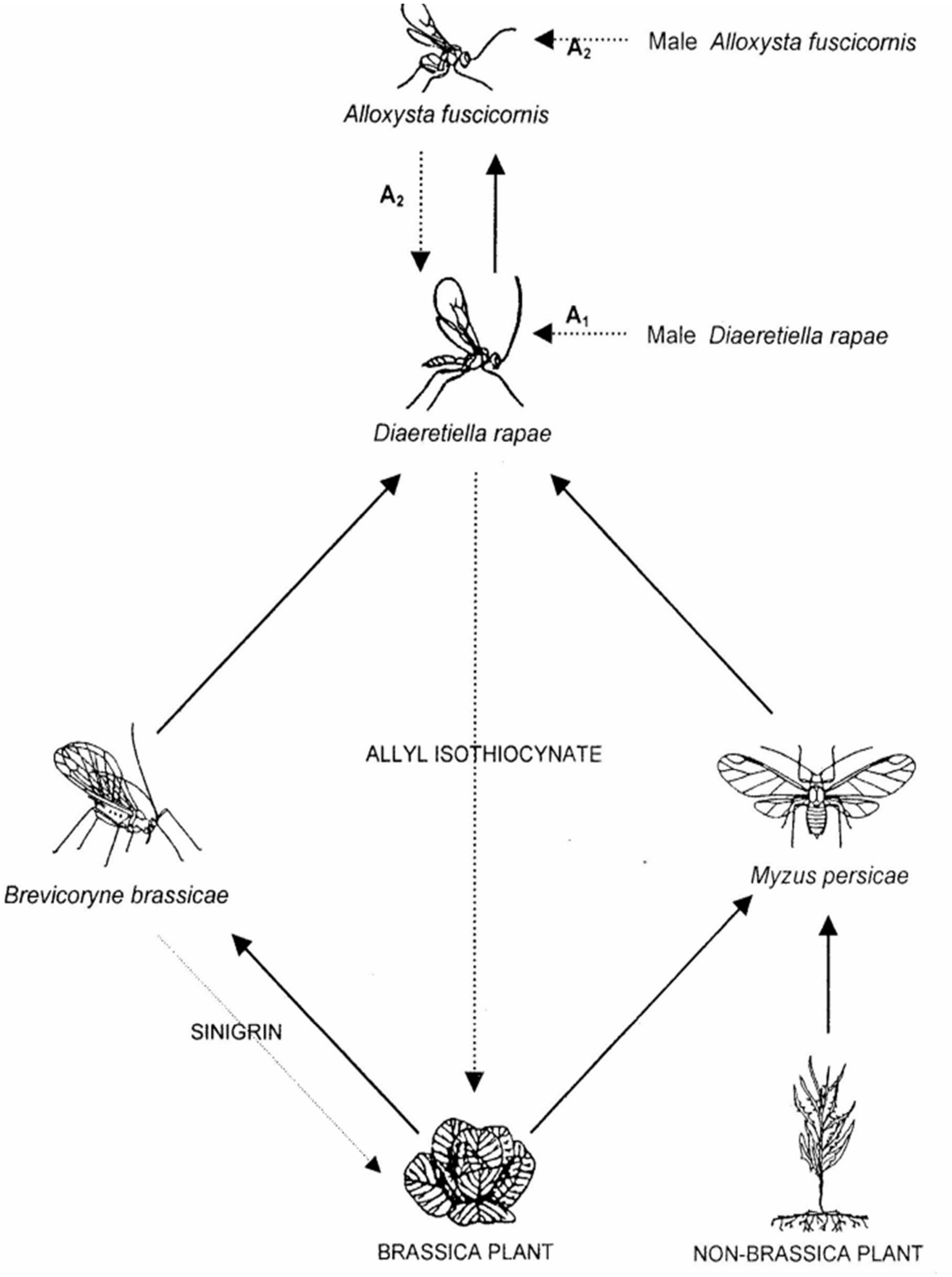


Fig. 4: Food web on Brassica plant and non-Brassica plant

Effects of Bt transgenes on herbivores insect – parasitoid interaction

Transgenic plants, do not affect the natural enemies and non-targets as given example:
1. Effects of Bt maize on aphid parasitoid *Aphidius rhopalosiphi.* There was no effect found that neither the foraging efficacy nor the oviposition behavior of *Aphidius rhopalosiphi* was influenced by a Bt-toxin expression in maize compared to control plants.

2. Effect of Bt canola on aphid parasitoid *Diaeretiella rapae.* There was no effect found on *Diaeretiella rapae* by Bt canola and the expression of the Bt-toxin Cry-1Ac did not impact the development of the parasitoid. (Von and Steinbrecher, 2004).

Advantages of tritrophic interactions:-

Humans can take advantage of tritrophic interactions in the biological control of insect pests. Tri-trophic interactions join pollination and seed dispersal as vital biological functions, which plants perform via cooperation with animals. Natural enemies: predators, pathogens and parasitoids that influence plant-feeding behaviour of harmful insects also encourage sustainable crop production.

Constraints in applying tritrophic interaction for IPM:-

Experimental studies of tritrophic interactions in field settings are difficult. Tri-trophic interactions studies are time consuming. Expensive to conduct. Farmers are not aware of the benefits of tritrophic interactions.

Conclusion:-

The available information demonstrates that there is a need for understanding the biology and ecology of each trophic level in detail before we can understand the interaction between plant resistance and biological control. Therefore, the food plant should be considered as an important factor for the success of any bioagent. To increase the success rate of control, one should not only seek to find a more efficient parasitoid or to try to breed host plant cultivars that are qualitatively poorer to the herbivore insects but also to try to breed food plant cultivars that are qualitatively better for the parasitoids or other natural enemies. The host plants play a major role in tritrophic interactions and can be exploited for increasing the influence of natural enemies. Tri-trophic interaction is a powerful tool for suppressing herbivore populations. Continued efforts to utilize these methods in IPM are essential for environmentally and economically sustainable global crop production. Plant volatiles play a critical role as signals in tritrophic level interactions, e.g. lady bird beetle *Coccinella septumpunctata* make extensive use of volatile chemical cues in locating habitats, plants and prey.

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Chapter- 20

Genetic Engineering and other Approaches in Improving the Nutritional Quality of Food Crops

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Abstract

Advances in genetic engineering offer powerful solutions to combat global micronutrient deficiency, termed 'Hidden Hunger.' Strategies encompass diverse approaches, including agronomic interventions, microbiological techniques, breeding, and genetic modification, to enhance crop nutrition significantly. These methods aim to fortify staple foods with essential nutrients like vitamins A, C, E, folate, amino acids, and minerals. Efforts in biotechnological interventions have resulted in the creation of high-nutrient crops such as vitamin A-enriched Golden Rice, showcasing promising solutions to address widespread nutritional deficiencies. Despite challenges like public skepticism and regulatory constraints, these innovations focus on reducing allergens, enhancing nutritional content, and fortifying staple foods, crucial for global food security. Collaborative efforts between governments, industries, and nonprofit organizations are crucial for inclusive progress in combating malnutrition and fostering agricultural resilience. While genetically modified (GM) crops face skepticism, their proliferation across the globe indicates potential, engaging millions of farmers and spanning numerous nations, particularly benefiting developing countries. Prioritizing research to enhance nutritional values in 'orphan crops' remains essential, overlooked despite their significance to impoverished communities worldwide. Transparency and sustainable endeavors in biotechnological advancements hold the key to revolutionizing agriculture and alleviating food insecurities among vulnerable populations.

Introduction:

Micronutrient deficiency, known as 'Hidden Hunger,' poses a profound global challenge, particularly prevalent among poor communities. Its impact ranges from decreased productivity in adults to severe outcomes, notably premature death, especially affecting women and children. For human metabolic needs, approximately 49 essential nutrients are required, and the absence of these vital minerals and vitamins results in significant health complications. Efforts to combat hidden hunger encompass diverse strategies such as fortifying staple foods, promoting varied diets, implementing supplementation programs, and fostering nutrition education. This multifaceted approach is crucial for addressing the intricate nature of malnutrition and ensuring access to necessary nutrients for vulnerable populations worldwide.(Bhullar and Gruissem, 2013)**.** Genetic engineering has accelerated the enhancement of crops and animals. Plant genome engineering, also known as genetic modification or genetic engineering, is a powerful tool that enables scientists to make targeted modifications to the genetic makeup of plants. These types of crops are called the designer crops and are specifically designed to meet certain specified requirements of consumers or industries. The designer crops have a specified protein, fat or starch quality; nutritional features like vitamin content, elimination of an antinutritional factor, colour, flavour, taste, keeping quality etc., Fortification refers to the deliberate addition of vitamins, minerals, and trace elements to food, aimed at boosting its nutritional value with minimal risk to public health. The advent of commercially available genetically modified (GM) crops in 1996 catalyzed a remarkable 100-fold increase in cultivation across 28 countries (James, 2014). Over 2000 studies have systematically investigated the safety of GM crops, consistently affirming their equivalence to conventionally bred crops in terms of food and environmental safety. Despite this consensus, persistent skepticism surrounds this technology (James, 2014). Concerns among the public are amplified when discussing the introduction of 'foreign' genes from distantly related organisms, despite evolving evidence challenging the perception of this process as 'unnatural' (Abdallah *et al*., 2015). Regulatory requisites contribute to significant delays in product launches, while skepticism and stringent governmental policies confine transgenesis to a select few crops like cotton, soybean, and corn. Further, this chapter discusses the approaches and researches on nutritional improvement in crops.

Approaches to improve the nutritional quality of crops:

Nutritional enrichment of food crops could be achieved by several ways. Some of the potential ways are:

Agronomic interventions: Various agronomic interventions, including foliar fertilization, seed priming, soil application of fertilizers, and seed coating, effectively boost the mineral concentration in cereal and pulse crops (Rengel *et al*.,1999). These methods enhance the solubility and movement of elements in the soil, ensuring their accessibility to plants (White and Broadley, 2009). Disadvantage of this approach is the economic impact and cost of the fertilizers. For effective translocation of nutrients to the edible tissues, nutrients are preferable applied through foliar application rather than applying to roots. Foliar application gives maximum economic yield. Most common and effective method for nutrients application is through soil application, whereas under certain conditions, foliar application is preferable (Singh *et al*., 2016)

Microbiological interventions: The use of microbiological approaches, such as the application of plant growth-promoting rhizobacteria (PGPR) and mycorrhizal fungi, can enhance nutrient uptake by plants and improve the concentration of mineral elements in the grain (Rana *et al*., 2012). The utilization of PGPR and other biological agents remains limited. Microorganisms and plants releasing phytosiderophores strategically in specific windows of time and space offer an efficient means for plants to absorb iron and other micronutrients from the rhizosphere. Studying rhizosphere ecology can equip us with insights and tools to devise strategies that improve plant nutrition and health while reducing dependence on chemical inputs. Thus, applying PGPR for biofortification of crops can be seen as a potential additional measure that, when combined with the use of breeding approaches, can enhance micronutrient concentrations in addition to increasing yield and soil fertility.

Breeding approaches: Breeding strategies utilizing natural genetic diversity and modern molecular tools such as DNA markers and marker-assisted selection (MAS) are crucial in developing nutrient-rich varieties (Welch and Graham, 2005). Breeding methods involve uncovering genetic variations that influence inheritable mineral traits and testing their stability across diverse conditions. The goal is to breed for heightened mineral content in edible tissues without compromising yields or other quality aspects. This approach offers several advantages, particularly in sustainability, yet no high-mineral varieties developed this way have entered the market due to extended development periods, especially when incorporating traits from wild relatives (Singh *et al*., 2016). Molecular biology tools like QTL maps and MAS aid breeders in swiftly identifying high-mineral varieties, but they must navigate soil differences (such as pH and organic composition) that can impact mineral uptake and accumulation.

Genetic modification: Transgenic technologies have successfully improved crop nutrition, notably seen in crops like golden rice. These methods allow the introduction of new genetic elements or foreign genes into crops, essentially turning plants into specialized factories for producing desired compounds. However, the success of this approach depends on how the nutritional compound is synthesized—whether by the plant itself (de novo) or accessed externally. Adjusting organic molecules like amino acids, fatty acids, and vitamins produced by the plant may require metabolic engineering to enhance nutritional value. The predominant technologies utilized include Zinc-Finger Nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs), and the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 System. Other genetic engineering (GE) methods employed in agriculture encompass meganucleases and Oligonucleotide-Directed Mutagenesis (ODM). Furthermore, while RNA interference (RNAi) does not belong to genome editing technologies, it enhances crop traits by regulating gene expression without directly editing the genome (Patel *et al*., 2023)

Nutritionally improved crops:

Vitamin A rich crops: Carotenoids, lipid-soluble antioxidants, are naturally occurring pigments found across various organisms, spanning bacteria, fungi, algae, and higher plants (Maoka, 2020). These compounds, classified into xanthophylls and carotenes, play crucial functional roles and their synthesis in plants begins with the conversion of GGPP into phytoene, followed by subsequent stages leading to the formation of lycopene, β-carotene, and astaxanthin (Wan *et al*., 2021).

The quest to combat childhood blindness spurred efforts to enhance provitamin A levels in rice, traditionally lacking in the endosperm. Recent advancements have enabled the

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bioengineering of carotenoids in various higher plants like potato, tomato, soybean, rice, maize, carrot, and lettuce (Fang *et al*., 2019). Strategies such as introducing phytoene synthase and lycopene β-cyclase in rice grains led to the creation of Golden Rice, rich in β-carotene (Ye *et al*., 2000). Similar methods were applied to develop a "golden" potato with enhanced β-carotene content (Diretto *et al*., 2007). Biotechnologists also have created golden mustard, also rich in provitamin A, hinting at the potential extension of this technology to benefit other crop varieties.Recently, gene editing techniques focused on carotenoid cleavage dioxygenase *CCD4* in bananas have impeded further β-carotene metabolism, significantly boosting β-carotene accumulation (Awasthi *et al*., 2022), opening new avenues for enhancing β-carotene levels. CGIAR conducted research into developing genetically modified cassava capable of accumulating increased beta-carotene levels in the roots, achieved by introducing *nptII, crtB,* and *DXS* genes. The utilization of a *PSY* transgene resulted in heightened carotenoid concentration in these transgenic cassava varieties (Telengech *et al*., 2014). Bioengineered cassava, enriched with heightened β-carotene levels, was administered to healthy volunteers in porridge form, showcasing increased concentrations of β-carotene and retinyl palmitate TRL in their plasma, hinting at the potential to combat vitamin A deficiency.

Folate rich crops: Metabolic engineering for folate biofortification in crops presents a sustainable solution for combating folate deficiency, especially among impoverished rural populations in remote areas (Blancquaert *et al*., 2014). Efforts have been directed toward crops like tomato, rice, lettuce, white corn, and potato. There are two primary approaches: first, the overexpression of GTP cyclohydrolase I (*GTPCHI*), a key enzyme in the pterin branch of folate biosynthesis; second, the simultaneous overexpression of *GTPCHI* and amidodeoxychorismate synthase (*ADCS*). In *Arabidopsis,* elevating *GTPCHI* expression resulted in a 2-8.5-fold increase in folate levels (Hossain *et al*., 2004). Co-expressing *GTPCHI* and *ADCS* proved successful in enhancing folate content in tomato fruit (Diaz de la Garza *et al*., 2007) and seeds (Storozhenko *et al*., 2007). This approach led to remarkable increases, with folate levels reaching up to 25-fold in tomatoes and 100-fold in rice compared to wild-type levels.

Vitamin E rich crops: Vitamin E, encompassing tocopherols and tocotrienols, is vital for lipid antioxidant functions and animal reproduction. While α-tocopherol is biologically potent, γ- and δ-tocopherols are excellent antioxidants, crucial for cooking oil stability (Traber, 2007). To date, numerous QTL and association mapping studies have investigated vitamin E levels in major crops such as rapeseed, maize, soybean, rice, barley, and tomato. In soybean, the overexpression of *VTE4* led to an impressive increase in α-tocopherol content, elevating it by up to 11 times (Vinutha *et al*., 2015). Yusuf *et al*. (2007) demonstrated a sixfold increase in α-tocopherol levels by introducing Arabidopsis *VTE4* into *B. juncea* plants which prevented oil peroxidation and enhanced the oil quality. Notably, transgenic expression of the *VTE1* gene led to rice and tobacco plants exhibiting improved tolerance to salt and drought stress, respectively, while concurrently enhancing tocopherol content (Liu *et al*., 2008). These findings suggest the potential for biotechnological methods to breed vitamin E-enriched crops resilient to environmental stresses.

Amino acid rich crops: Certain amino acids crucial for humans and animals must be acquired through diet since they cannot be produced internally. Lysine (Lys), tryptophan (Trp), and methionine (Met), essential amino acids, are especially important for biofortification due to their limited presence in cereals (Lys and Trp) and legumes (Met). Over the last decade, a handful of transgenic approaches have focused on modifying plant protein composition to engineer metabolic pathways for essential amino acids, aiming to increase specific amino acid content to enhance nutritional value (Kumar *et al*., 2020). Transgenic wheat and rice were created by introducing lysine-rich pea legumin protein into their endosperm (Stoger *et al*., 2001). Another success involved engineering a seed storage protein from *Amaranthus hypochondriacus*, rich in all essential amino acids, into cereals. In soybean, where the embryo dominates the seed, the introduction of a bacterial feedback-insensitive *DHDPS* controlled by an embryo-specific promoter resulted in a remarkable 100-fold surge in seed free Lys content (Falco *et al*., 1995). In maize, despite the endosperm being the primary seed portion, expressing the bacterial feedback-insensitive *DHDPS* under an embryo-specific promoter significantly enhanced seed soluble Lys levels, contrasting the expectations (Frizzi *et al*., 2008). To date, maize has been commercially introduced in two transgenic events showcasing modified amino acid traits, utilizing the *cordapA* gene sourced from *Corynebacterium glutamicum*. These events elevate free lysine levels in maize kernels through the embryo-specific expression of the *cordapA* gene, encoding a dihydrodipicolinate synthase (*DHDPS*) enzyme that kick-starts the lysine biosynthesis pathway in plants and bacteria (Azevedo and Lea, 2001).

Nutritionally improved oil crops: Plant oils, derived from various sources including perennial tree species like olive, tea seed, avocado, and macadamia, boast naturally existing high oleic (HO) properties. Chemical or radiation mutagenesis has been utilized to introduce random genetic variations in oilseed crops like safflower, with some early successes in the breeding of high oleic traits (Wilson, 2012). However, the labor-intensive nature of classic breeding prompted the exploration of genetic modification (GM) strategies to meet the increasing demand for HO oils. Techniques like TALEN and CRISPR involving the downregulation of *FAD2* expression, crucial for polyunsaturated fatty acid (PUFA) biosynthesis, have become an attractive approach to reduce PUFA content in plant oils (Wallis *et al*., 2022). Notably, soybean oil commercialized as Plenish®, generated through the downregulation of *FAD2* expression combined with *FAD3* mutation, offers an oxidatively stable oil rich in oleic acid and low in alpha-linolenic acid (ALA) (Brink *et al*., 2014). Similar methods have been employed in the creation of HO oils in canola, Indian mustard, and peanuts, significantly altering their fatty acid compositions (Wen *et al*., 2018). The use of RNA interference (RNAi) techniques targeting genes related to fatty acid biosynthesis, such as *FatB* and *FAD2*, has led to the development of HO soybean oils like Vistive Gold® by Monsanto (Wilkes and Bringe, 2015). Commercialization of transgenic events with modified lipid content from oilseed crops like Argentine canola, safflower, and soybean underscores the advancements in this field (ISAAA database 2019). For instance, the USDA-approved Vistive Gold® soybean exhibits lower linolenic acid content, leading to improved oil stability and reduced trans-fatty acids (Haun *et al*., 2014). Additionally, genetic modifications in *Camelina sativa* have enabled the production of omega-3 fatty acids resembling fish oil, while Argentine Canola has been engineered for enhanced production of the omega-3 fatty acid DHA (Ruiz-Lopez *et al*., 2014).

Vitamin C rich crops: Ascorbate, known as vitamin C, serves as an antioxidant and plays a crucial role as a coenzyme in various enzymatic processes essential for collagen, carnitine, cholesterol, and specific amino acid hormone synthesis. The deficiency of vitamin C leads to scurvy, a condition marked by connective tissue deterioration (Bartholomew, 2002). Enhancing plant ascorbate levels can occur not only by boosting its biosynthesis but also by augmenting the rate of its recycling. For instance, in lettuce, overexpressing L-gulono c-lactone oxidase (*GLOase*) led to a sevenfold increase, accumulating up to 580 nmol/g fresh weight of ascorbate (Jain and Nessler, 2000). Similarly, expressing the same gene in potato tubers resulted in a twofold rise in ascorbate levels (Hemavathi *et al*., 2010). Maize engineered with the rice *dhar* gene from the ascorbate recycling pathway exhibited a sixfold increase in accumulated ascorbate, contributing to the development of multivitamin maize.

Micronutrients enriched crops: Wheat has been a focus in efforts to enhance zinc (Zn) levels, a crucial need in low-income countries where Zn deficiency ranks fifth among prevalent diseases. The deficiency affects many relying on Zn-deficient cereal-based diets, leading to issues like stunted growth, weakened immunity, cognitive impairments, and birth complications. Recent strides in nitrogen (N) management have shown promise in boosting Zn concentrations in wheat grains, leveraging both soil Zn availability and foliar applications (Hefferon, 2015)**.** Goto *et al*. (1999) successfully introduced the soybean ferritin gene into rice, leading to a three-fold increase in iron content in rice seeds. This approach holds promise in mitigating iron deficiency in developing countries, where plant-based diets are prevalent. To address iron deficiency further, there have been substantial efforts to elevate iron content in staple foods like maize and rice. Genetically engineered low-phytate maize containing approximately 35% of wild-type maize's phytic acid demonstrated 50% greater iron absorption, suggesting a potential avenue for preventing iron deficiency.

Anthocyanin rich crops: In 2017, Japan introduced GABA-enriched tomatoes, a successful example achieved by scientists who employed CRISPR/Cas9 technology. They deleted a C-terminal autoinhibitory domain of SlGAD2 and SlGAD3, enhancing glutamate decarboxylase activity and elevating GABA content in tomato fruit. Additionally, the USDA recently sanctioned a transgenic purple tomato, developed through the overexpression of two genes related to anthocyanin synthesis sourced from the snapdragon (Waltz, 2022)

Reduction in Allergens: Targeted gene silencing is a potent tool for modulating biosynthetic pathways to attain desired traits. Phytic acid, an anti-nutrient present in cereals, legumes, and oilseeds, limits mineral nutrient absorption. Efforts to reduce gossypol, a toxin in cottonseed, involved seed-specific inhibition of the Δ-cadinene synthase gene, resulting in transgenic plants with significantly reduced gossypol levels approved by the WHO (Sunilkumar *et al*., 2006). Soya bean (Glycine max) and grass pea (Lathyrus sativus) seeds are significant sources of dietary proteins but contain oxalic acid (OA), an anti-nutrient that can lead to nephrolithiasis by forming calcium oxalate crystals in the kidneys. Moreover, OA is a precursor of β-N-oxalyl-L-α, β-diaminopropionic acid (β-ODAP), a neurotoxin found in grass pea. Oxalate decarboxylase (OXDC), an OA-degrading enzyme from the fungus *Flammulina velutipes*, was purified, offering potential for managing OA levels (Mehta and Datta, 1991). Similar strategies targeted reducing or limiting food allergens in crops. Efforts in rice and soybean used antisense or sense constructs under seed-specific promoters to suppress or silence allergenic proteins. (Herman *et al*., 2003).

Conclusion:

The challenges in modern agriculture, such as limited arable land and increasing food demands, demand innovative solutions for global food security. Biotechnological advancements offer precision and efficiency, potentially surpassing conventional methods in enhancing crop resilience and yield. Site-specific nucleases (SSNs) show promise in boosting agricultural productivity. Despite resistance, genetically modified (GM) crops cover over 300 million acres globally, notably benefiting developing countries and engaging millions of farmers. Meeting the World Health Assembly's 2012 malnutrition targets appears unlikely, leading to new Sustainable Development goals by 2030. Transparency in biotech development, risk assessment, and regulations is crucial for public trust. Research focus on enhancing nutritional value in overlooked crops like sorghum and millet is vital for impoverished communities. Addressing hunger among rural populations necessitates collaboration among governments, industries, and nonprofits. Together, these efforts can transform agricultural landscapes and alleviate food insecurities globally.

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Chapter- 21

Nanofertilizers: A Novel Approach in Agriculture

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Abstract

Inefficiently absorbed traditional fertilizers must be applied in huge amounts. Phosphorus- and nitrogen-based fertilizers have low nutrient uptake efficiency and rapidly convert into chemical forms which plants cannot use. Nanofertilizers exhibit a gradual nutrient release mechanism, potentially enhancing nutrient utilization efficiency while avoiding any associated negative consequences. The aim of designing these nanofertilizers is to gradually release nutrients over a prolonged duration and significantly minimize nutrient loss, thereby ensuring environmental safety.

Keywords: Nanofertilizers, conventional fertilizers, nutrition, formulation

Introduction

Agriculture has always been a source of strength for developing countries. Aside from satisfying people's hunger, food also plays a significant role in a country's economy. The new developing science of nanotechnology aids in the resolution of these food production issues. Nanotechnology is a promising field that integrates basic science secrets and evidence at the tiny level. Fertilizer alone accounts for approximately 50% of agricultural yield in modern agricultural techniques. Increased fertilizer uses and higher doses increase productivity, but it is also the root cause of numerous problems such as pollution of surface and underground water resources, low input use efficiency, soil degradation, multinutrient deficiency in soil, deterioration of food material quality, and toxicity in the food chain. Now a days, nanotechnology produces nanofertilizers for effective nutrient control. Immobilization of nutrients and growth stimulants in nanoscale polymers results in targeted and gradual release of nanofertilizer. Because these nano-based fertilizers have extra infiltration capacity, large surface area, nutrient use efficiency, and stress tolerance ability, and above all, it is very eco-friendly, thus avoiding residues in the environment, nanofertilizer is a successful tool in agriculture for enhancing nutrient supervision (Panpatte *et al*., 2016).

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Role of nanofertilizer in agriculture

Today's agriculture is generally chemically focused, with additional dosages of chemicals used for fertilizer management to maximize output while disregarding natural resources and ecosystems. Over usage of chemical fertilizer also results in waste and nutrient shortage in soils. One of the most effective techniques to deal with this problem is to combine the application of macro- and micronutrient sources with value addition to standard fertilizers. Nanotechnology is utilised to modify and manufacture compounds that are employed to improve soil for increased yield and crop quality and quantity. These materials are known as nanofertilizers. They play a vital role in increasing nutrient efficiency through regulated release of nutrients depending on plant demand and lowering environmental safety costs by decreasing fertilizer overuse. Nanofertilizers are a modified version of traditional fertilizers that are either bulk materials or generated from vegetative or reproductive components of plants utilising various physical, chemical, or biological approaches. Because of their tiny size and enhanced surface-to-volume ratio, nanoparticles have fundamentally different molecular characteristics than bulk materials. Substituting nanofertilizer for traditional fertilizer can be a strong step toward sustainable agriculture.

Characteristics of nanofertilizers

Some of the qualities of nanofertilizers that make them useful and preferable to conventional fertilizers (Singh *et al*., 2017) are as follows:

a) Higher surface area: Nanofertilizers have a high surface area because to their small particle size. Because of this feature, it can be provided in more locations to aid various metabolic processes in plant systems, resulting in the generation of additional photosynthates. Increased surface area increases nanofertilizer reactivity with other compounds and improves nutrient uptake and use efficiency.

b) Small particle size: Nanofertilizers have particle sizes less than 100 nm, which enhances their penetration capacity in plants from applied surfaces such as soil or leaves, hence improving nutrient intake by plants.

c) High solubility: Nanofertilizer is highly soluble in a variety of solvents, including water. This feature of nanofertilizer aids in the solubilization and dispersion of insoluble nutrients in soil, increasing nutritional bioavailability.

d) Easy penetration and regulated release of fertilizers: Because of their high rate of penetration, nanofertilizers play a key role in enhanced nutrient availability to the plant and consequently good seedling growth. The regulated release of nanofertilizers reduces fertilizer toxicity: When compared to bulk Zinc sulphate, nano-ZnO has a higher percentage of peanut seed germination and root growth.

e) Nutrient absorption efficiency: Nanofertilizers boost fertilizer efficiency and soil nutrient uptake ratio in crop production, salting away fertilizer assets. Nanofertilizers also reduce fertilizer leaching.

f) Effective duration of nutrient release: Bulk fertilizers when applied is effective for short-term duration, but by using nanofertilizers, the duration of nutrient release can be increased.

Nanofertilizer a better alternative than conventional fertilizers

The most common methods of applying conventional fertilizers are spraying and broadcasting. The technique of application of conventional fertilizers is primarily determined by the actual final fertilizer concentration in the plants. Conventional fertilizers provide nutrients in a chemical form that plants cannot use; there is also leaching loss, drift, chemical runoff, hydrolysis, evaporation by moisture in soil, and microbial destruction. It is estimated that 40-70% of applied fertilizers' nitrogen, 80-90% of phosphorus, and 50-90% of potassium content are lost in the environment before reaching the plants. All crops suffer output losses if correct fertilizer doses are not applied. Excessive fertilizer application can harm groundwater, promote eutrophication of aquatic ecosystems, and pollute the air (Quereshi *et al*. 2018). Thus, large-scale application of traditional fertilizer may improve crop production but is not long-term sustainable. As a result, smart approaches, such as nanofertilizer, should be developed to optimize the usage of chemical fertilizers. Nutrients are enclosed within nanomaterials or protected with nanofilm and distributed as emulsions in nanofertilizers.

Different types of nanofertilizers

Nanofertilizers serve a key part in fertilizer modification, which helps to increase crop output, which helps to feed the world's growing population. There are various varieties of nanofertilizers, each with its own set of features that contribute significantly to the agricultural revolution (Malik and Kumar, 2014).

a) Phosphate-based Nanofertilizers: Phosphorus is one of the most important nutrients for agricultural plants. Phosphate-based nanoparticles are useful in heavy metal remediation because they form insoluble and stable phosphate compounds, for example, Vivianite (Fe_3 $(PO_4)28H_2O$) particles (about 10 nm) can successfully lead to a decrease in the toxicity characteristic leaching procedure, leach ability, and physiologically based extraction test bio-accessibility of Pb^{2+} in calcareous, neutral, and acidic (Liu and Lal, 2012).

b) Nitrogen-based Nanofertilizers: Nitrogen is the most important ingredient in crop plant biomass production. Conventional fertilizers, such as urea, are the primary source of nitrogen and have particles with a particle size higher than 100 nm. Nanotechnology-based nanofertilizers have proven to be considerably superior to polymer-coated conventional fertilizers; for example, urea-modified hydroxyl-apatite (HA) (Ca_{10} $(PO_4)_6(OH)_2$ nanoparticles are one of the top nitrogen-based nanofertilizers that can also deliver phosphorus (Kottegoda *et al*. 2011).

c) Zinc-based Nanofertilizers: Zinc is one of the most important macronutrients for plants, and its shortage inhibits agricultural crop productivity, particularly in alkaline calcareous soils. Zinc should thus be incorporated in macronutrient fertilizers to improve crop productivity and quality. When conventional zinc fertilizers are applied to the soil, they gradually become less accessible and precipitate (i.e., $ZnCO_3$) or are adsorbed to oxide phases (e.g., Fe and Al oxides). Nanomaterials have proven to be useful in the development of zinc-based nanofertilizers, which are more soluble and diffusible sources of zinc fertilizer and hence beneficial to agriculture.

d) Iron-Based Nanofertilizers: Iron is a necessary metal element that serves as a crucial cofactor for enzymes involved in a variety of physiological processes in plants. The majority of the iron form is insoluble, rendering it unavailable to plants despite its abundance. So, the best strategy to make iron available to plants is to use iron chelate nanofertilizer, which is highly stable and has a delayed release of iron across a wide pH range (i.e., pH 3–11). Another advantage of employing iron-based nanofertilizers is that they contain no ethylene-based compounds, which protects plants against premature ageing and senescence. Iron nanofertilizers also enhance the ferrous iron to ferric iron ratio, which increases chlorophyll synthesis in plants (Tarafdar and Raliya 2013).

e) Silver-Based Nanofertilizers: Silver nanofertilizers are effective at controlling a variety of phytopathogens. Silver nanofertilizers play a vital function in increasing seed germination and root growth of plants even in hydroponic solutions, however extended exposure to silver nanoparticles reduces plant biomass and transpiration. As a result, sufficient precautions should be followed when using silver nanofertilizer to ensure that its good benefits are realized (Jo and Kim, 2019).

There are many other materials based nanofertilizers like titanium based, aluminum based and copper based nanofertilizers that can be used.

Formulation of Nanofertilizers and Their Delivery System

Nanofertilizers are a godsend to agriculture due to their numerous favorable features. They are particularly important in agriculture because of their high solubility, stability, low toxicity, targeted and timely release. However, correct formulations and delivery mechanisms are required for optimal uptake and usage of nanofertilizers by plants. Crop plants can benefit from fertilizers containing chitosan nanoparticles. Mesoporous silica nanoparticles (150 nm) entrap urea, making them an effective nanofertilizer. Nanofertilizer delivery technologies have a significant impact on plant development and output (Wanyika *et al*. 2012).

There are many different methods for nanofertilizers delivery to plants like soil and foliar application. Its main advantages like can be directly sprayed onto leaves, can be used to supply trace elements .

Nanofertilizer uptake, transfer & their fate in plants

The uptake of nanoparticles by plants is regulated by various aspects, including nanoparticle size, plant physiology, and nanomaterial interaction with the environment and plants. The size exclusion limit for nanoparticles moving through the plant is 40-50 nm (Taylor *et al*. 2014). Nanoparticles also interact with other environmental components, influencing the net absorption of nanofertilizers by plants; for example, organic acids such as humic acids boost bioavailability and stability of nanofertilizers. The presence of microorganisms such as bacteria and fungi influence plant nanofertilizer uptake. Nanofertilizers pass through plant tissues in two ways: apoplast and symplast. Apoplastic transport occurs via extracellular gaps, cell walls of neighbouring cells, and xylem arteries where symplastic transport involves plasmodesmata. After reaching the central cylinder, nanoparticles can travel to the aboveground section via the xylem and transpiration stream. However, when nanofertilizers are delivered via irrigation, they must pass through the Casparian strip, which must be done in a symplastic manner via endodermal cells. When nanofertilizers are applied foliarly, nanomaterials must get past the cuticle barrier, either via the lipophilic or hydrophilic pathway. Nanoparticles prefer the stomatal pathway over the cuticular pathway because the size exclusion limit of the stomatal pathway is greater than 10 nm, whereas the cuticular pathway is less than 2 nm. The study of how nanomaterials move through the plant is critical because it provides insight into the best means of applying nanofertilizers; for example, if a nanoparticle prefers to be carried by the xylem, then the best method of applying nanofertilizers is through irrigation as shown in Fig 1. However, foliar spraying is the superior approach if nanofertilizers prefer to pass through the phloem. Some studies show that nanoparticles are absorbed by soil microbes and transferred to the various trophic levels of the food chain, so transporting these nanoparticles to other ecosystems, both terrestrial and marine (Kim *et al*., 2016).

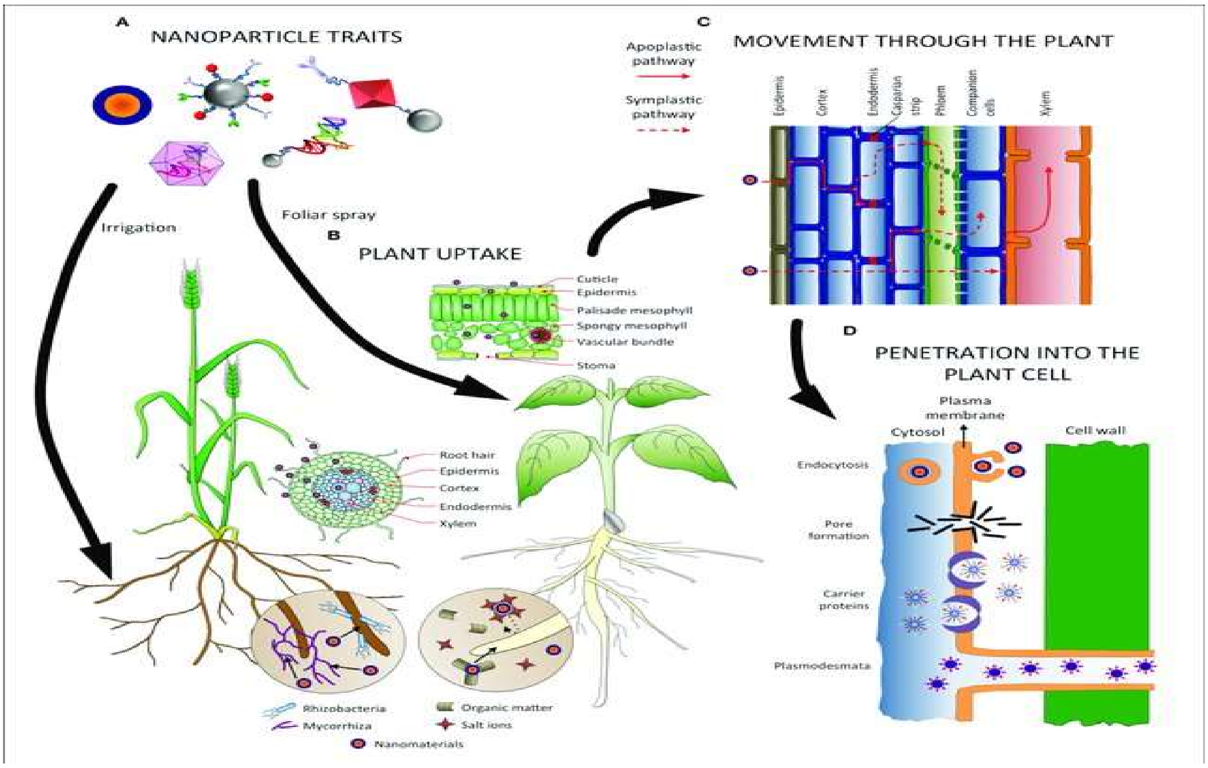

Fig. 1: Uptake, translocation, and biotransformation pathway of various nanoparticles in plant system (Alejandro Pérez-de-Luque, 2017).

Biosafety Concerns Regarding the Use of Nanofertilizers

Nanofertilizers are one of the breakthrough sectors that have benefited the agriculture sector, both economically and in terms of increased crop productivity. However, before the world accepts a new technology, one of the major problems is whether the impact of unknown hazards and health difficulties produced by nanoparticles outweighs its benefits. Before fully using this technology, a proper risk assessment must be performed. Considering the above point, the "nanotoxicology" branch has been formed, with the primary task of assessing nanoelement toxicity as well as advocating safe design and use of nanoparticles. Although there have been no reports of human sickness caused by nanoparticles, previous laboratory investigations have shown that nanoparticles can cause unfavourable biological reactions that can lead to hazardous effects. Nanoparticles, due to their small size, can enter tissues, cells, and organelles and interact with functional biomolecular structures such as DNA, ribosomes, and so on, causing harm to human health. This aura of uncertainty is precisely the feature of nanotechnology that cynics find most frightening. As a result, proper physiochemical investigation and evaluation of the effect of nanoparticle exposure on human health are required (Chaudhry and Castle 2011). Due to the unique and developing nature of nanotechnology, as well as the lack of established procedures and guidelines, no circumstance for analyzing risks and controls can be uniformly applied to determine the outcome. It is difficult to compare different research groups' safety/toxicity ratings.

Benefits of Nanofertilizers

Nanofertilizers serve a critical role in enhancing food crop output in underdeveloped countries. Fertility enhancement and preservation boost agricultural output, quality, and dependability (Veronica *et al*. 2015). Nanofertilizers primarily aid in the improvement of three important agricultural regions, namely:

a) Nutritional value: Various studies have shown that nanofertilizer-treated plants have a 10% increase in protein and sugar content for the majority of agricultural plants.
b) Crop yields increased by up to 20% as a result of the application of Nanofertilizers. Yield parameters include biomass, leaf growth, and the number of fruits and grains produced. In one trial, for example, it was discovered that using nanofertilizers boosts sunflower grain output by 50% and cucumber grain yield by up to 25%.
c) Improved nutrient uptake efficiency: Nanostructured formulations improve fertiliser efficiency and uptake ratio in crop production, saving fertiliser resources.
d) Controlled release: Because the nanofertilizers are encapsulated in a semipermeable membrane coated with resin polymer, waxes, and sulphur, it has both spatial and temporal controls over nutrient release into the soil, increasing the effective duration of fertiliser supply into the soil and lowering the loss rate.
e) In terms of health, the application of nanofertilizers boosted plant tolerance to both biotic and abiotic challenges. Plants have a higher resistance to illness and infection.

Conclusion

The use of various nanofertilizers has a bigger impact on crop output. The use of nanofertilizers will result in lower fertilizer costs and fewer pollution dangers. Nanofertilizer offers a wide range of applications in agriculture and is consequently of major relevance to society. The use of nanofertilizers can improve fertiliser nutrient use efficiency in crop production. Nanofertilizers, as opposed to conventional fertilizers, are more precise, intelligent, effective, quickly manufactured, and less expensive. Though it has many positive applications, it lags behind all other existing approaches in the agricultural sector. People must be taught, and a small number of nanofertilizer sample goods should be delivered to them for testing, which will aid in the application of nanofertilizers. If both the public and business sectors provide equal funding, nanofertilizers can become an innovative approach and further research can be conducted. The usage of nanofertilizers could help feed a burgeoning population. Although the usage of nanofertilizers has few downsides, it may be adopted as an unique technology in the near future. The research and development industry is emphasizing the

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development of nanotechnology and nanofertilizers in order to improve the agriculture sector and benefit farmers.

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Chapter- 22

Genomic Sculptors: Carving Perfection with Base Editing Tools in the Field of Crop Improvement

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Abstract

Genome editing technologies, such as CRISPR-Cas systems, have evolved with the advent of base editing, a precise tool for DNA and RNA modifications. Cytosine base editors (CBEs) and adenine base editors (ABEs) have emerged as powerful contributors to genetic engineering. CBEs, excel in converting C to T with precision, while ABEs, facilitate A to G conversions. These base editors find widespread applications in diverse plant species, impacting traits like herbicide resistance and plant architecture. Challenges include limited editing windows and potential off-target effects, prompting innovations like narrowed editing windows and advanced Cas9 variants. Despite challenges, base editing holds promise for enhancing wild-plant domestication and crop improvement, necessitating further refinements and the integration of strategic sgRNA design and AI-based algorithms to address efficiency gaps and challenges. Overall, base editing stands as a valuable and evolving tool for precise crop modifications in the face of ongoing global changes.

Introduction

Genome editing encompasses various genetic engineering technologies that manipulate living organisms' DNA sequences (Kim, 2016). This versatile tool finds applications in life sciences, biotechnology, agriculture, and clinical and pharmaceutical fields. Early methods like zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) paved the way for programmable genome manipulation but relied on protein–DNA interactions (Yang *et al*., 2019). The CRISPR-Cas system, driven by guide RNA, emerged as a powerful and broadly applicable tool for inducing DNA double-strand breaks (DSBs) (Knott & Doudna, 2018). Repair mechanisms, specifically nonhomologous end joining (NHEJ) and homology-directed repair (HDR), play pivotal roles. NHEJ often leads to gene inactivation, while HDR allows for precise editing, albeit with lower efficiency due to the complexity of homologous recombination (Ceccaldi *et al*., 2016).

Base editing, a recently innovated genetic modification technique, has found success across diverse species by precisely and efficiently inducing targeted changes in DNA and RNA bases (Komor *et al*., 2017). Developed by Alexis Komor and David Liu, it merges APOBEC (apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like) /AID (activation-induced cytidine deaminase) cytidine deaminases with the CRISPR-Cas system to create cytosine base editors (CBEs) (Komor *et al*., 2016). Expanding on this, the coupling of different CRISPR-Cas proteins with various nucleotide deaminases led to the development of a range of base editors (BE), including adenine base editors (ABEs). ABEs, pioneered by Nicole Gaudelli, can convert adenine to guanine (A to G) across cells, animals, plants, and bacteria (Gaudelli *et al*., 2017). Unlike conventional CRISPR/Cas techniques for genome editing, Base Editors (BEs) operate without causing double-strand breaks (DSBs). This unique characteristic limits the generation of insertions or deletions (indels), offering a cleaner and more precise outcome in genome editing. This reduction in on- and off-target indels distinguishes BEs from traditional methods (Komor *et al*., 2017). This discussion explores early studies, the development and applications of base editors, their limitations, and potential future directions.

Cytosine base editors

The pioneering Base Editors (CBEs) were designed to convert specific C to T (or G to A in the opposite strand). Comprising three key elements—cytosine deaminase, modified Cas9, either dCas9 (dead cas9, which do not make DSBs) or nCas9 (nickase cas9, which cuts only one of the strands of DNA), and sgRNA (Single guide RNA) were used to construct the first generation base editors (Komor *et al*., 2016). Cytosine deaminase targets a small window of single-strand DNA in the noncomplementary strand, defined by sgRNA-Cas9-mediated R-loop formation. This activity window typically spans bases 4–8 of the protospacer. When a genomic C is converted to U, the resulting U–G mismatch undergoes either T–A replication or C–G restoration through UDG (Uracil DNA glycosylase) -mediated BER (Base excision Repair) pathway (Molla & Yang, 2016). To increase the efficiency of the base editors Uracil Glycosylase Inhibitors (UGI) were used along with the CBE complex to circumvent the activity of the UDG and to increase the efficiency of the base editors, which constituted the Second generation base editors. Komor and collaborators connected rat cytosine deaminase (rAPOBEC1) to dCas9/nCas9 using an XTEN linker and UGI to the C terminus. Another CBE variation employs sea lamprey cytosine deaminase (PmCDA1) linked to the C terminus of dCas9/nCas9 (Nishida *et al*., 2016). To further improve the efficiency third generation base editors (BE3) were developed, which constituted the nickase cas 9 instead of dead cas9. Nickase cas9 nicks guide RNA complementary strand. BE3 creates a U/G mismatch with an adjacent nick in the complementary strand, serving as a preferred substrate for cellular mismatch repair (MMR). The MMR system, an innate DNA repair mechanism, identifies and removes mismatched bases during DNA replication. It recognizes the U/G mismatch and the accompanying nick generated by BE3, leading to the excision of the complementary strand containing the G of the U/G mismatch. Consequently, subsequent DNA re-synthesis utilizes the remaining U-containing non complementary strand as a template to establish a U/A pair, eventually converting to a T/A pair post DNA replication or repair (Wang *et al*., 2017). By capitalizing on the endogenous MMR system, BE3 facilitates higher frequencies of C-to-T base substitution compared to BE2. Despite integrating one UGI copy into BE3 to prevent UDG-induced abasic site formation, unintended by-products, such as C-to-A or C-to-G conversions from AP sites, were observed in certain instances.

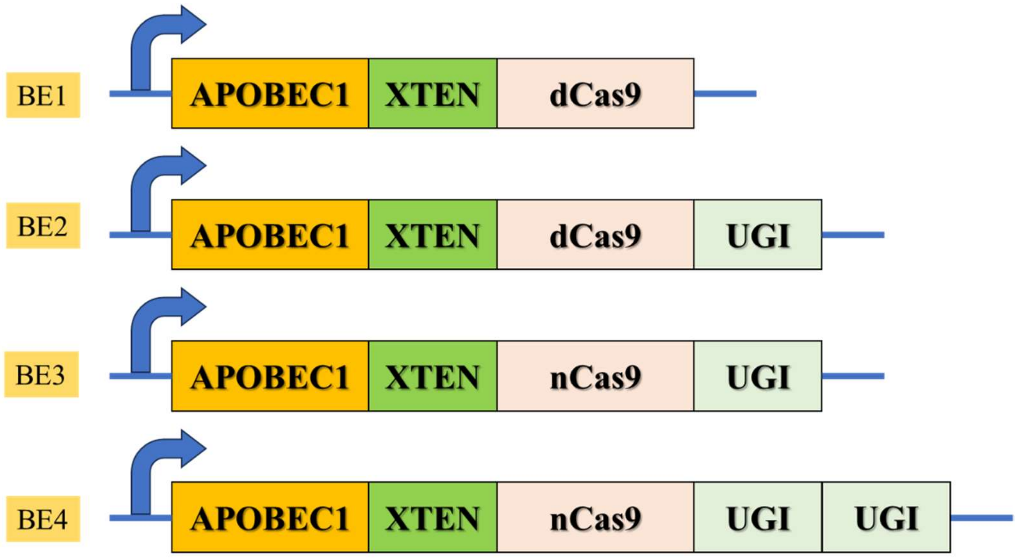

Figure 1: Different generations of Cytosine base editors. BE1 has three components APOBEC1, dCas9 which are connected by a linker XTEN. BE2, BE3, BE4 are the improvements over the BE1 which has UGI, dCas9 replaced by nCas9, additional molecules of UGI, respectively. First generation base editors (BE1); Second generation base editors (BE2); Third generation base editors (BE3); Fourth generation base editors (BE4); APOBEC1 (apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like); dCas9 (dead cas9) or nCas9 (nickase cas9); UGI (Uracil Glycosylase Inhibitors).

These findings implied that U in the editing intermediate (U/G pair) could still be excised by UDG, compromising C-to-T editing efficiency (Azameti & Dauda, 2021). Overcoming challenges associated with BE3 editors, Kim Y. *et al*. (2017) tackled the limitations of requiring an NGG PAM sequence and potential off-target editing. They innovatively addressed these issues by engineering dCas9 variants using the Cas9 homolog from *Staphylococcus aureus* (SaCas9). The resulting SpCas9 variants (VQR-BE3, EQR-BE3, VRER-BE3, and SaKKHBE3) exhibited a notable 2.5-fold increase in editing efficiency. Additionally, they developed editors with modified activity windows to rectify the off-target editing challenges associated with BE3. In the pursuit of enhancing base editing efficiency, the development of BE4 and SaBE4 marked the advent of the fourth-generation base editors. These editors, derived from *Streptococcus pyogenes* Cas9 and *Staphylococcus aureus* Cas9, respectively, featured rAPOBEC1 linked to Cas9D10A at the N terminus, with two UGI molecules fused to the C terminal of Cas9 nickase. To further improve their performance, an advanced version of BE4 and SaBE4 was created by incorporating the DNA end-binding protein Gam, derived from bacteriophage Mu, at the N terminus of the Cas9 nickase. The resulting editors, BE4-GAM and SaBE4-GAM demonstrated increased efficiency in base editing compared to their predecessors (Komor *et al*., 2017). The inclusion of UGI played a pivotal role in minimizing unwanted by-products by obstructing UNG access to the uracil intermediate, thereby preventing base excision repair (BER). Additional improvements were made over these existing base editors regarding narrowing down the editing window, PAM variants, and higher product purity. Swiftly adopted by plant researchers, CBEs have been incorporated into a wide array of plant species. These include Arabidopsis, rice, wheat, maize, tomato, potato, watermelon, cotton, soybean, apple, pears, strawberry, moss, poplar, and rapeseed (Molla *et al*., 2021).

Adenine base editors

Theoretically, inspired by CBEs, the amalgamation of adenine deaminase and nCas9 might yield ABEs, aiming to transform an A-T base pair into a G-C base pair. However, none of the documented naturally existing adenine deaminases exhibit activity on DNA. The advent of ABEs represents a major advancement in genome-editing technologies. Comprising an evolved TadA (tRNA adenine deaminase), Cas9 nickase, and sgRNA. ABEs capitalize on TadA's capability to convert A to inosine (I) in the anticodon loop of tRNAArg, mimicking G in cellular processes. Through genetic engineering in E. coli, Gaudelli *et al*. (2017) created TadA mutants enabling A to I conversion on DNA. ABE construction involves linking mutated TadA with a modified Cas9. The Cas9 nickase induces a single-strand DNA break precisely opposite the A to I conversion, triggering the cell to insert the correct base pair and completing the transition from A/T to G/C. Importantly, this Cas9 variant exclusively nicks DNA, avoiding the typical double-strand break associated with the native enzyme in CRISPR/Cas9 gene editing. To facilitate deamination, TadA operates as a dimer. Scientists devised a heterodimeric protein, by pairing a wild-type TadA non-catalytic monomer with an engineered catalytic monomer (TadA*). ABEs, efficiently converting A to G in mammalian cells with high purity, were generated by combining this heterodimer with nCas9. Unlike uracil excision repair, cellular inosine excision repair displayed weak activity and did not hinder the A•T to G•C conversion. Developing ABEs didn't require an additional glycosylase inhibitor protein. Similar to CBEs, ABEs found rapid application and validation in diverse plant species, such as rice, wheat, *Arabidopsis, Brassica napus, Nicotiana benthamiana*, poplar, and moss (Molla *et al*., 2021).

Applications in agriculture

Cytidine base editors have proven successful in editing a variety of plants, including rice, maize, tomato, wheat, cotton, and watermelon. This "base editing" system involves linking the rat cytidine deaminase enzyme (APOBEC1) to the N terminus of Cas9 (D10A) using an unstructured 16-residue peptide, XTEN, as a linker. The resulting fusion, known as CRISPR/Cas9-xyr5APOBEC1, was integrated into a binary vector controlled by the maize ubiquitin promoter (UBI). In one application, this system induced point mutations in two crucial rice genes, NRT1.1B and SLR1, associated with enhanced nitrogen use efficiency and reduced plant height. The base editing process achieved C to T substitutions at frequencies ranging from 1.4% to 11.5%, with an additional 1.6% to 3.9% of edited plants exhibiting C to G substitutions. The study also observed indel mutations, potentially attributed to Cas9 (D10A) nicking the non-edited strand. Notably, the study did not utilize UGI, despite its recognized potential for enhancing base editing efficiency (Lu & Zhu, 2017).

Rice plants acquired resistance to multiple herbicides through multiplex base editing, as demonstrated by Shimatani *et al.* 2017. Using the Target-Activation-Induced Cytidine Deaminase (Target-AID) system, researchers fused either dCas9 or nCas9 with Petromyzon marinus cytidine deaminase (PmCDA1) and sgRNAs to target the Acetolactate synthase (ALS) gene. This gene, when mutated, confers herbicide resistance, as observed in the C287T mutation resulting in an A96V amino acid substitution and resistance to imazamox (IMZ) in rice. Employing Target-AID-based base editing, researchers introduced a similar point mutation in the ALS gene. Spontaneous resistance mutations occurred at 1.56%, while nCas9OsPmCDA1At transformants exhibited 3.41% IMZ tolerance. Seven out of 14 edited lines displayed the ALS-A96V mutation, with no detected off-target effects.

In a recent experiment, the ALS gene in tomato and potato plants was targeted using a CBE, introducing chlorsulfuron resistance through Agrobacterium-mediated transformation. The specific mutation of Proline-186 in the ALS1 gene of tomato and potato provides resistance to chlorsulfuron. A single sgRNA designed for the SlALS1 gene ensured the Pro186 codon was within the CBE's editing window. Following Agrobacterium-mediated transformation, the first generation yielded 12.9% and 10% edited, transgene-free plants for tomato and potato, respectively. Chlorsulfuron-resistant tomato plants demonstrated an editing efficiency of up to 71%. Co-base-editing the ALS gene with another gene minimized the adverse effects of random T-DNA integration and reduced off-target activity due to the transient expression of the base editor (Veillet *et al*., 2019).

ABEs, akin to CBEs, have demonstrated successful applications in various crops for precise base editing. Adapted from their effective use in mammalian cells, ABEs have been optimized to establish an adenine base-editing system in plants, creating targeted point mutations (Hua *et al*., 2018; Li *et al*., 2018). ABE7-10, known for highly efficient A.T to G.C conversions in mammalian cells, served as a basis for ABE-P1, a modified version utilized for precise editing in rice plants (Hua *et al*., 2018). Evaluating the editing efficiency in rice, ABE-P1 targeted IPA1 (OsSPL14), a key gene influencing plant architecture and grain yield. A designed sgRNA successfully induced T.C substitutions at the OsmiR156 binding site in OsSPL14, with an editing efficiency of 26% observed in 6 out of 23 transgenic lines. Importantly, predicted off-target sites did not exhibit any base-editing events. ABE-P1 demonstrated a broader base-editing window (4-7) in rice compared to ABE7-10 in mammalian cells, emphasizing the specificity and efficiency of ABEs in rice.

In the domain of rice genetics, Yan *et al*. (2018) introduced a fluorescence-tracking A to G base editor known as rBE14. This innovative tool effectively facilitated the conversion of A·T to G·C in key genes, including OsMPK6, OsSERK2, and OsWRKY45, offering a means to introduce DNA variations in rice for further enhancement.

Challenges in base editing

DNA base editors function within a limited editing window, typically spanning several nucleotides (nts). This spatial restriction can sometimes constrain the targetable bases. However, when multiple Cs or As are present within or near this window, unintended base conversions, known as bystander mutations, may occur. To mitigate the frequency of bystander mutations, one effective approach is to narrow the editing window. The width of this window is dictated by the DNA base editor deaminase. Introducing specific mutations in the deaminase can effectively reduce the size of the editing window without significantly affecting deaminase activity (Jeong *et al*., 2020).

Cas nucleases are known for sgRNA-dependent, genome-wide off-target DNA editing, and it is reasonable to expect similar behaviour from both CBEs and ABEs. However, profiling off-target sites for DNA base editors is challenging as they do not generate detectable DSBs. The Kim group pioneered genome-wide profiling for CBE-mediated off-target sites using BE3DUGI, which cleaves the non-edited strand, and a uracil-specific excision reagent (USER). This reagent generates DSBs by cleaving the edited strand, allowing for detection through in vitro nuclease-digested whole-genome sequencing (Digenome-seq). Results revealed that BE3DUGI exhibits less sgRNA-dependent off-target editing compared to conventional Cas9 nucleases (Kim *et al* 2015; Kim *et al* 2017). There have been reports of DNA base editor-mediated deamination of RNA that is independent of sgRNA. Cytidine deaminases, responsible for this process, demonstrate genome-wide deamination effects without relying on sgRNA (Jin *et al*., 2019).

Future prospects

Base editors, transforming plant research, enables precise genome manipulations. Applied extensively, this technology enhances agronomic importance in diverse crops. Despite several improvements, efficiency gaps persist, necessitating further enhancements for comprehensive plant genome manipulation. Base editing offers potential in advancing wild-plant domestication, particularly in crops subjected to selective breeding for desirable features. These traits, influenced by mutations in domestication genes, contribute to the mechanical harvestability and nutritional richness of high-quality food. However, given that key agronomic traits are typically governed by multiple quantitative trait loci, editing individual genes may fall short of inducing the necessary phenotypic changes. Therefore, developing more efficient base editing technologies capable of "stacking" mutated alleles becomes crucial. Further refinements are recommended to optimize the scope and efficiency of base editors. Overcoming challenges like off-target effects and bystander mutation generation is crucial in this process. Employing artificial intelligence-based algorithms for the strategic design of sgRNAs is an additional measure to address these constraints. Despite these challenges, base editing remains a valuable tool for making precise modifications in crops, supporting sustainable production amidst ongoing global changes.

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Chapter- 23

Response of Different Manures and Organic Mulches on Growth and Yield of Garlic (*Allium sativum* L.)

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The current investigation entitles “Response of different manures and organic mulches on growth and yield of garlic (*Allium sativum* L.)” was conducted at Campus for Research and Advanced Studies, Dhablan, P.G. Department of Agriculture, G.S.S.D.G.S. Khalsa College, Patiala, during *Rabi* season 2022-23. The experiment was conducted in split plot design with 12 treatment combination and three replications. It consists of four different manures in main plot and three organic mulches in sub plot. Results showed that M_3 (Rice husk 10 t ha^{-1}) and OM_4 (Vermicompost 2.5 t ha^{-1}) recorded significantly higher growth parameters such as plant height (61.90 and 62.03 cm), number of leaves plant^{-1} (6.50 and 6.13), leaf length (60.21 and 60.04 cm), yield parameters such as length of bulb plant^{-1} (4.23 and 4.14 cm), diameter of bulb (4.51 and 4.71 cm), length of clove bulb^{-1} (3.20 and 3.30 cm) and bulb yield (96.33 and 98.25 q ha^{-1}) However, treatment combination OM_4 (Vermicompost 2.5 t ha^{-1}) and M_3 (Rice husk 10 t ha^{-1}) significantly influence the growth and yield parameters of garlic as compared to all treatments.

Keywords: - Garlic, Organic mulches, Manures, Growth, Yield.

Introduction

Garlic is the second most important bulb crop after onion. Garlic (*Allium sativum* L.) belongs to family Alliaceae and having chromosome number 2n = 16. Plant is herbaceous and annual for the bulb production and biennial for the seed production. The garlic plants usually grow close together and leave enough space for the bulbs to mature. They are easily grown in containers of sufficient depth. Many sub species of garlic are grown in India. Out of which the hard neck garlic and soft neck garlic are the most notable ones. Garlic is native of Central Asia and Southern Europe especially Mediterranean region (Thompson and Kelly 1957). Vegetables plays an important role in human diet by providing nutritious components which are essential constituents of balanced diet. Vegetables are naturally available and cheapest source of nutrients. India is the second largest vegetable producer in the world. It is a rich source of carbohydrates (29%), proteins (6.3%), minerals (0.3%) and essential oils (0.1-0.4%) and also contains appreciable quantities of fat, vitamin C and sulphur. About 142 calories of energy is obtained from 100 g of garlic. Garlic is used in flavorings foods, preparing chutneys, pickles, curry powder, tomato ketchup, etc. Beneficial use of garlic extract has been found against many fungi and bacteria (Pandey 1997). Beside the nutritive value of garlic and its use in various forms, it is included in Indian system of medicines as carminative and gastric stimulant to help in digestion and absorption of food. Allicin present in aqueous extract of garlic reduces blood cholesterol concentration. The garlic extract has nematicide, fungicide, and bactericidal action; therefore, it is used in many Ayurvedic medicines as an ingredient. Garlic oil or its juice is recommended to inhale in cases of pulmonary tuberculosis, rheumatism, sterility, impotency, cough and redness of eyes. The constituents of garlic are divided into main groups Sulphur containing compounds and non-sulphur containing compounds. Most of the medicinal effects of garlic are preferable to a Sulphur compound known as Allicin. Allicin present in aqueous extract of garlic reduces blood cholesterol concentration (Shankaracharya 1974). Organic manure act as a source of energy for micro-organism to perform beneficial functions in soil such as mineralization, nitrogen fixation and enhance soil dehydrogenises and phosphate enzyme activity. Farmyard manure is one of the oldest manure used by the farmers in growing crops because of its easy availability and presence of all the nutrients required by the plants. Organic manure improves soil structure and water holding capacity, resulting in more extensive root development and enhances soil micro flora and fauna activity, which results in availability of micronutrients available to plants (Zeidan 2007). Organic mulches are those natural origin materials which can decompose naturally, like agricultural wastes which are used as mulch, such as bark chips, grass clippings, wheat or paddy straw, plant leaves, compost, rice husk, and sawdust, etc. It decays over time and it increased the water holding capacity of soil. It also provides the soil with nutrients as it breaks down. It helps to improves water use efficiency indirectly. A mulched layer restricts the weed growth. Mulch reduces water loss resulting in more conservation of soil moisture. Mulches help to check weed growth and improve the soil structure and fertility by trapping nutrient rich and wind-borne dust (Geiger *et al.* 1992).

Material and methods

The present investigation entitled "Response of different manures and organic mulches on growth and yield of garlic (*Allium sativum* L.)" was conducted during the *Rabi* season of 2021-2022 at the Campus for Research and Advanced Studies Dhablan, P.G. Department of Agriculture, G.S.S.D.G.S. Khalsa College, Patiala. The soil of the experimental field was clayey having pH 7.5 and organic carbon 0.66. Yamuna Safed - 3 (G-282) is a variety of garlic that was sown at a distance of 15 cm Row to Row and 7.5 cm Plant to Plant as per recommended spacing of crop. The experiment was laid down in Split Plot Design with three replications. A total of 12 treatments were performed i.e., T_1- No mulch (M_1) + No manure (OM_1), T_2 – No mulch (M_1) + FYM (OM_2), T_3 - No mulch (M_1) + Poultry manure (OM_3), T_4 - No mulch (M_1) + Vermicompost (OM_4), T_5 - Paddy straw (M_2) + No manure (OM_1), T_6 - Paddy straw (M_2) + FYM (OM_2), T_7 - Paddy straw (M_2) + Poultry manure (OM_3), T_8 - Paddy straw (M_2) + Vermicompost (OM_4), T_9 - Rice husk (M_3) + No manure (OM_1), T_{10} - Rice husk (M_3) + FYM (OM_2), T_{11} - Rice husk (M_3) + Poultry manure (OM_3), and T_{12} - Rice husk (M_3) + Vermicompost (OM_4). During the duration of the experiment, observations were made on the following parameters : plant height (cm), number of leaves plant^{-1}, leaf length (cm), length of bulb plant^{-1}, diameter of bulb (cm), length of clove bulb^{-1} (cm) and total bulb yield (q ha^{-1}). For statistical studies, the average of datafrom the sampled plant of each treatment was used to generate reliable results.

Results and discussion

The application of different manures and organic mulches significantly improved the vegetative growth parameters of garlic. At the harvest stage, the maximum plant height (62.03 cm) was obtained from OM_4 (Vermicompost) and treatment M_3 (Rice husk) (61.90 cm) which was at par with treatment OM_3 (Poultry manure) (61.02 cm) and M_2 (Paddy straw) (60.99 cm). Lowest results were shown in treatment OM_1 (No manure) (59.11 cm) and M_1 (No mulch) (58.80 cm). This may be due to the reason that vermicompost is rich in nutrients and beneficial microorganisms that can improve soil health. Vermicompost promotes microbial population, which ultimately promotes plant growth. Rice husk can help to conserve soil moisture and suppress weed growth, which can all contribute to healthier plant. Same results were obtained by Suthar (2009) and Rashid and Islam (2019).

Table 1: Response of different manures and organic mulches on growth parameters.

Treatments	Plant height (cm)	Number of leaves $plant^{-1}$	Leaf length (cm)
Different manures			
OM_1 (No manure)	59.11	3.71	57.85
OM_2 (FYM)	60.10	5.38	59.07
OM_3 (Poultry manure)	61.02	5.61	59.57
OM_4 (Vermicompost)	62.03	6.13	60.04
SEm±	0.38	0.39	0.50
CD%	**1.09**	**1.13**	**1.45**
Organic mulches			
M_1 (No mulch)	58.80	3.61	57.77
M_2 (Paddy straw)	60.99	5.52	59.42
M_3 (Rice husk)	61.90	6.50	60.21
SEm±	0.38	0.47	0.51
CD%	**1.05**	**1.31**	**1.43**

At harvesting stage, the significantly maximum number of leaves (6.13) was obtained from OM_4 (Vermicompost) and treatment M_3 (Rice husk) (6.50) followed by treatment OM_3 (Poultry manure) (5.61) and M_2 (Paddy straw) (5.52). Lowest results were shown in treatment OM_1 (No manure) (3.71) and M_1 (No mulch) (3.61). Vermicompost provides the required nutrients which are essential for photosynthesis and overall plant vitality. Rice husk mulch can act as a temperature regulator and protect the garlic plants from extreme level of heat or cold. This favorable microclimate can support leaf development and overall plant health. Similar result was observed in garlic by Arguello *et al.* (2006) and Rashid and Islam (2019).

The maximum leaf length at harvesting stage (60.04 cm) was obtained from OM_4 (Vermicompost) and treatment M_3 (Rice husk) (60.21 cm) which was at par with treatment, OM_3 (Poultry manure) (59.57 cm), OM_2 (FYM) (59.07 cm) and M_2 (Paddy straw) (59.42 cm). Lowest results were shown in treatment OM_1 (No manure) (57.85 cm) and M_1 (No mulch) (57.77 cm). Vermicompost contains several beneficial microorganisms and they helps in plant growth. It improves moisture availability and consistent soil moisture helps in leaf elongation and development. Rice husk decomposes over time and adds several organic nutrients to the soil. This decomposition process releases essential nutrients, like NPK and they are beneficial for leaf length. This result is supported by the findings of Islam *et al.* (2007) and Abbas *et al.* (2006).

Table 2: Response of different manures and organic mulches on yield parameters.

Treatments	Length of bulb plant^{-1}	Diameter of bulb (cm)	Length of clove bulb^{-1} (cm)	Bulb yield (q ha^{-1})
Different manures				
OM_1 (No manure)	2.97	3.09	2.39	67.45
OM_2 (FYM)	3.42	3.77	3.07	80.41
OM_3 (Poultry manure)	3.79	4.13	3.15	86.52
OM_4 (Vermicompost)	4.14	4.71	3.30	98.25
SEm±	0.14	0.14	0.23	0.47
CD%	**0.41**	**0.42**	**0.67**	**1.37**
Organic mulches				
M_1 (No mulch)	2.95	3.24	2.60	63.77
M_2 (Paddy straw)	3.56	4.02	3.12	89.37
M_3 (Rice husk)	4.23	4.51	3.20	96.33
SEm±	0.18	0.12	0.26	0.27
CD%	**0.49**	**0.33**	**0.73**	**0.75**

In different manures significantly maximum length of bulb plant^{-1} (4.14 cm) were recorded with treatment OM_4 (vermicompost) and lowest length of bulb was found in OM_1 (no manure) (2.97 cm). In the case of mulching maximum length of bulb plant^{-1} (4.23 cm) were recorded with treatment M_1 (Rice husk) which was statistically higher over rest of treatments. Lowest length of bulb was obtained from M_1 (no manure) (2.95 cm). This is due to the reason that when vermicompost is added to plant development. These benefits can contribute to increased bulb size and length of bulb. Rice husk mulch helps to create suitable conditions for garlic bulb formation by maintaining soil moisture, regulating temperature and suppressing weed growth. Similar result was recorded with finding of Gopakkali and Sharanappa (2014) and Rahman *et al*. (2013).

In manures OM_4 (vermicompost) (4.71 cm) show statistically maximum diameter of bulb and lowest bulb diameter (3.09 cm) are found in OM_1 (no manure). On another hand in organic mulches significantly maximum bulb diameter (4.51 cm) was attained with the application of M_1 (rice husk) and lowest in M_1 (no mulch) (3.24 cm). The reason behind this result is that vermicompost helps to preventing excessive drying out and provide a favorable environment. It also encourages the proliferation of beneficial soil organisms that aid in nutrient cycling and root development and rice husk plays important role in bulb formation because silica present in rice husk is not to contribute to plant strength and disease resistance. It may help to improve the structural integrity of garlic plants and potentially increase bulb size. Similar, results were also observed by Kaswan *et al.* (2013), Rahman *et al*. (2013) and Baten *et al*. (1995).

Application of different manures has significant affect on length of clove bulb^{-1}. Maximum clove length (3.30 cm) was observed with the treatment OM_4 (vermicompost) lowest in treatment OM_4 (no manure) (2.39 cm). Organic mulches also has significant effect on clove length. Maximum length of clove (3.20 cm) was found with the application of M_3 (rice husk) where lowest length of clove (2.60 cm) is recorded with the application of the M_1 (no mulch). This might be due to the organic matter present in vermicompost also improves soil structure, providing best aeration and root penetration, which can contribute to bulb development and ultimately affects the clove length. Rice husk provide the suitable soil conditions can indirectly support the overall growth and length of garlic cloves. These results are in conformity with the findings of Sachin *et al.* (2017), Gowda (2007) and Anisuzzaman *et al.,* (2009).

In organic mulches the maximum bulb yield ha^{-1} was observed in treatment OM_4 (vermicompost) (98.25 q ha^{-1}) and M_3 (rice husk) (96.33 q ha^{-1}) and the lowest yield was observed with treatment OM_1 (no manure) and treatment M_1 (no mulch). This might be due to with increases in growth parameters (plant height, number of leaves and leaf length) and yield parameters (clove length, bulb length and bulb diameter) ultimately increase bulb yield. Vermicompost contains several plant growth promoters, enzymes, beneficial bacteria and mycorrhizae. This result is supported in garlic by the findings of Suthar (2009) and Sachin *et al.,* (2017).

Conclusion

On the basis of results from experimentation, it can be concluded that among different organic mulches, the rice husk results better on growth and yield whereas among different manures, vermicompost @ 2.5 t ha^{-1} performs best. Because rice husk suppresses the weed growth, conserve moisture, regulating soil temperature and due to decomposition provides nutrients ultimately increases garlic growth and yield. Vermicompost improves soil structure, soil texture and provides an ideal growing environment for garlic roots.

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Chapter- 24

A Review of Novel Control Strategies for Ticks and Tick-borne Diseases

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Abstract

Ticks are obligate ectoparasitic arthropods infesting most species of mammals and rank second in the world as vectors of disease. Increasing public health concern over tick-borne infections demands the strategic control of ticks on animals that transmit diseases to human beings. An effective method to control tick populations is through using chemical compounds. The intensive use of acaricides exerts a strong selective pressure for ticks to become resistant to them. Therefore, there is a need for the development of alternative approaches like the use of biological control methods like entomopathogenic fungi, entomopathogenic nematodes, parasitoids, predators, and herbal acaricides. Possibly including the use of animal husbandry practices, manual removal of ticks, release of sterile male hybrids, selection for host resistance, plant extracts, essential oils and vaccination. Integrated tick management consists of the systematic combination of at least two control methods aiming to reduce pressure in the selection of acaricide-resistant individuals. The development of integrated tick control strategies, including vaccines and herbal acaricides, in combination with managing drug resistance and educating owners, should lead to the sustainable control of ticks and tick-borne diseases.

Keywords: Ticks, Tick-borne diseases, Control, Integrated tick management

Introduction

Ticks are obligate haematophagous ectoparasites of enormous medical and veterinary importance and act as vectors of human and animal pathogens causing direct damage to their hosts. An effective method to control tick populations is using chemical compounds. Fipronil, amitraz, carbaryl, pyrethroids (deltamethrin, permethrin, and cypermethrin) and macrocyclic lactones are among the most frequently used acaricides for the control of ticks (WHO, 2006). The control of ticks in certain areas had been solely based on the use of these acaricides on animals and sometimes in the environment. However, several tick species mainly in tropical and subtropical countries have developed resistance to all major classes of compounds like macrocyclic lactones (MLs) due to the high intensity of their use in tick management (Rodriguez-Vivas *et al*., 2006). This has urged the development of new alternative approaches to control tick populations. Integrated tick management (ITM) involves the systematic application of two or more technologies to control tick populations. The main aim of ITM is to achieve tick control in a more sustainable, environmentally compatible and cost-effective manner than achievable with a single, stand-alone technology. The purpose of this study is to present an updated review of novel strategies for the control of ticks and tick-borne pathogens.

The following strategies are recommended to control tick infestation on animals:

Manual removal of ticks

The manual removal of ticks from the body of an animal can significantly reduce tick infestation. This approach can be applied by removing ticks from infested animals using forefingers. The collected ticks were killed by putting them on fire (Muhammad *et al*., 2008). However, its efficacy was based on the number of animals in the herd and personnel availability. Precautions should be carefully followed during ticks' removal. Otherwise, the blood of ticks can spread pathogens.

Host resistance

Host resistance to ticks is associated with reduced egg viability, egg production and a reduced number of ticks feeding to engorgement. Differences in the ability of cattle to become resistant to ticks have long been recognized, as the fact that the ability to acquire resistance is heritable. It has also been shown that *Bos indicus* or their crossbreeds survive more to babesiosis (a tick-borne disease transmitted by *B. bigemina* and *B. bovis*) than *B. taurus* animals.

Sterile male hybrids release

It has been shown that *Rhipicephalus annulatus* × *Rhipicephalus microplus* matings produce fertile females and sterile males. Backcrossing of the fertile progeny female also produces fertile females and sterile males through three to six generations. Problems with this method of control include the cost of production of hybrids (Rodriguez-Vivas *et al*., 2018).

Biological control

It is defined broadly as the use of live organisms to reduce the populations of pest organisms. It involves the use of control agents such as predators, pathogens and competitors to reduce the abundance of the vector. Nowadays people are increasingly interested in sustainable, 'biological or natural' methods as alternatives to chemicals for treatment of their animals against arthropods and to control ABDs (Athropod-Borne Diseases).

Fungi

The ability of entomopathogenic fungi to penetrate the cuticle of arthropods, the ability of a strain to kill several stages of them and the relatively specific virulence of a single strain to one or a small group of pests make them good candidates as biocontrol agents. *Metarhizium anisopliae* and *Baeuveria bassiana* exhibited the strongest anti-tick pathogenicity. *Metarhizium anisopliae, Lecanicillium lecanii* and *Baeuveria bassiana,* have shown potential efficacy in the control of various developmental stages (egg, larva, nymph, adult) of *R. microplus* (Ojeda-Chi *et al*., 2011).

Entomopathogenic nematodes

Entomopathogenic nematodes have been used for biological control of certain insects. EPN of the families Heterorhabditidae and Steinernematidae are known to be obligatory parasites of insects. Tick mortality is due to the rapid proliferation of the nematode symbiotic bacteria within the ticks since nematodes do not go through their natural cycle, and infective juveniles die immediately after entry. Infective juveniles of *Steinernema riobravus* and *S. feltiae* appeared to be the most effective in killing ticks (30-100%) mailnly *Amblyomma americanum, Dermacenter variabilis and Rhipicephalus sanguineus.*

Parasitoids

Tiny stingless wasps that lay eggs in the body of ticks. When the bees hatch, they kill the ticks. Parasitoids mostly used in the biological control of insect pests of plants belong to the order Hymenoptera. The most widespread species for biological control of ticks is *Ixodiphagus hookeri*. Nymphal ticks were parasitized while engorging on vertebrates and parasitoid egg development was found to be associated with ingestion of blood by its host tick.

Predators

Tick bio-suppressors such as ants, beetles and bird species are predators that feed rarely on ticks. Oxpeckers: *Buphagus africanus* and *Buphagus erythrorhynchus* birds are known to feed specifically on ectoparasites, especially ticks. Oxpeckers are visual predators, first removing the engorged females, then searching large body areas and eating the smaller tick stages. Oxpeckers seem well suited to play an important role in integrated pest management programmes for controlling ticks.

Herbal acaricides

Usage of herbal acaricides has been a traditional practice in India since ancient times for the control of ectoparasitic infestation. These substances are widely available and have the properties of rapid degradation, immuno-stimulatory activity, causing low mammalian toxicity, overall eco-friendly and environmentally beneficial. These compounds act by inhibiting growth, development and reproduction in various ways to control the population of ticks, mites, flies, fleas and lice of veterinary significance.

Herbal acaricides like pyrethrum, chemically it is a mixture of several esters called pyrethrins which are extracted from the flower of *Chrysanthenum cinerariaefolium* and Neem, Azadirachtin is the most biologically active principle found in the neem (*Azadirachta indica*). It is structurally similar to the insect hormones known as "ecdysones" which are responsible for metamorphosis in insects leading to anti-feedant effects and widely used for the control of ticks. Sardá-Ribeiro *et al*. (2008) evaluated hexane extract from the aerial parts of *Calea serrata* to control larvae and adults of *R. microplus and R. sanguineus*, showing 100% mortality in the larvae of both tick species and a reduction in oviposition of 11–14%.

Some natural plant oils (lemon, eucalyptus, chamomile and lavender) have high levels of repellents against ticks although they have a short residual effect because they are usually relatively volatile. Rosado-Aguilar *et al*. (2010) studied the acaricidal activity of methanolic extracts from stems and leaves of *Petiveria alliacea* against larvae and engorged females of *R. microplus* which showed 100% mortality of larvae. Certain studies identified secondary metabolites (terpenes, stilbenes, alcohols, acids, sulfurated compounds and aldehydes) of essential oils and plant extracts, associated with acaricidal effects against the genera *Amblyomma, Rhipicephalus, Hyalomma, Dermacentor* and *Argas* (Cetin *et al.,* 2010).

Vaccination

Tick antigens are usually classified as either exposed or concealed antigens. Concealed antigens from gut epithelium are not exposed to the host immune system during tick feeding, and repeated vaccinations are required to maintain high levels of antibodies. Exposed antigens are they come into contact with the host immune system naturally during tick feeding (i.e., antigens from the cuticle and salivary gland and its secretions) and animals are continually exposed to this class of antigen during infestation (Manjunathachar *et al*., 2014).

Tick introduces different saliva proteins into the host, which serve as antigens for the host to develop an acquired tick resistance. Activation of the immune system by antigens in tick saliva is likely to create an unfavorable environment for transmitted pathogens and hence tick rejection might take place before transmission can occur.

Bm86, an 89 kDa protein from the midgut of engorged female cattle tick *R. microplus* is the first and the only commercialized anti-tick vaccine. Bm86 is expressed in every life stage from eggs to engorged adult tick and it was commercialized in Australia as TickGARD® (Willadsen, 2004). Another commercial vaccine containing a recombinant Bm86 antigen (Gavac®) was released in Argentina, Colombia and Mexico. These vaccines cause leakage of gut content into the hemocoel of ticks, reducing the number of females engorging, their fecundity and larval production. It has been shown that these vaccines reduce tick numbers up to 74% and reduce tick fertility, combining the overall efficacy of up to 91%.

Genetic manipulation of endosymbionts

Tick endosymbionts located in the tick ovaries and from this organ, they can access the eggs. Some of the dominant taxa include *Rickettsia* or an unknown genus of the family *Enterobacteriaceae, Coxiella*, and *Francisella*-like endosymbiont (FLE). Some of these bacteria might provide co-factors and vitamin B (e.g., *Francisella* and *Coxiella*), amino acids and heme (e.g., *Francisella*), or *de novo*-synthesized folate (e.g., *Rickettsia*) to the ticks. Recently *Francisella* endosymbionts were shown to complement the nutritional deficiency of vitamin B in the blood meal of *Ornithodoros moubata*. Antibiotic-based elimination of *Francisella* endosymbiont from tick offspring produced anomalies in tick development and hampered nymph growth and moulting to adults (Duron *et al*., 2018). Thus, transgenerational microbial inheritance in ticks includes bacteria that are indispensable for tick development. The endosymbiont population of arthropod vectors could be exploited in different ways *viz*., as a chemotherapeutic target, vaccine target for the control of vectors. Expression of molecules with antiparasitic activity by genetically transformed symbiotic bacteria of disease-transmitting arthropods may serve as a powerful approach to control certain arthropod-borne diseases.

Chemotherapeutic approach

Targeting the endosymbionts of tick species through antibiotics would be easy to apply against the blood feeding parasites like ticks, as antibiotics or antibacterial can be systemically administered to the animals so as to reach the endosymbionts inside the tick, especially in the gut region. This approach exploits the endosymbionts of arthropods vectors as a chemotherapeutic target with the aim to disturb the symbiosis.

Immunological approach

Immunization of animals with the whole killed endosymbionts or recombinant antigens of the endosymbionts would render them immune to tick vectors. Instead of targeting the vector antigens, the endosymbionts could be targeted to disturb the symbiotic relationship between the vector and the symbiont. Following ingestion of the blood from immunized animals, these antibodies together with complement, will destroy the symbionts inside the vector, leading either to death or to disruption of normal gut physiology of the tick and reduce growth and egg-laying ability.

***Wolbachia* cytoplasmic incompatibility (CI)**

Wolbachia infections in arthropods can manipulate reproduction of their hosts in a variety of ways e.g., induced parthenogenesis, male killing, and cytoplasmic incompatibility (CI). It is the phenomenon in which mating between *Wolbachia* infected male insect and female insect of the same species without *Wolbachia* infection (Unidirectional CI) and mating between insects of the same species with different *Wolbachia* strain infection (Bidirectional CI), result in embryonic mortality. Reciprocal mating (infected female x uninfected male) and mating between infected individuals are fully compatible.

Cytoplasmic incompatibility is explained by modification. It is the process in which *Wolbachia* modifies the sperm of the infected male during spermatogenesis by an unknown process. The modified mature sperm is devoid of *Wolbachia*. If a modified sperm enters an incompatible egg (uninfected or infected with different strain), a delay in breakdown of nuclear membrane of pronuclei of sperm resulting in mitotic asynchrony and embryonic death.

Paratransgenesis

Genetic transformation of symbiotic bacteria of the arthropod vector is to alter the vector's ability to transmit pathogen, it is an alternative means of blocking the transmission of Vector-Borne Diseases's (VBD's). The midgut bacteria of arthropod vectors can be engineered to express and secrete effector proteins which block the parasite invasion or kill the parasite in the midgut or hemolymph or reproductive tract. The arthropod vector that harbours the genetically transformed endosymbionts are called as paratransgenic vector. The endosymbionts of arthropod vectors can be cultured and genetically transformed to express the effector gene inside the vector in such a way the gene product kills the pathogen that the vector transmits.

Semiochemicals

Semiochemicals are chemical signal vehicles of host/tick origin that are secreted into the external environment that mediate tick behavior. Semiochemical communication in nature can be divided based on the type of behavior they mediate and not based on the compounds that mediate behavior as shown below:

Kairomones

Kairomones are information bearing compounds or mixtures released by individuals of one species, detected by individuals of other species that benefit the recipient (Sonenshine, 2003). Locating a host for blood meal is one of the most important challenges for ticks. The questing behavior of ixodid ticks enables, the identification and localization of approaching hosts and these are evoked by chemical cues known as kairomones. The most notable host cue is carbon dioxide produced by host respiration which functions predominately as a nonspecific, general excitant and can activate ticks from distances as great as 30 meters. Additional cues that guide ticks to host include water vapour emitted via host respiration and evaporative losses, lactic acid present on skin, mammalian skin lipid, nitrogen containing host excretory-secretory products, ammonia, urea and the volatile fatty acids emanating from ruminants.

Allomones

Allomones are information-bearing compounds emitted by individuals of one species that affect the behaviour of individuals of a different species for the benefit of the emitter (Sonenshine, 2003). This group evokes behavioural or physiological changes in a receiving organism that adaptively favour the emitter e.g., ticks produce hydrocarbons like squalene as defence secretions against ant predators. Other hydrocarbons identified in the tick defense secretion include C20, C24 and a methyl branched C25 alkane.

Pheromones

Pheromones in ticks used for food finding, arrestment, nest building and sex pheromones. Different chemicals serve as pheromones ranging from the high volatile molecules like substituted phenols namely methyl salicylate, o-nitrophenol or 2, 6-DCP to cholesteryl esters as non-volatile contact pheromones (Sonenshine, 2004). Pheromones can be classified as follows:

Semiochemical - tick control methods

According to Sonenshine (2003, 2004, 2006), manufacture of a long-lived control device required the continuous delivery of pheromone source by a slow-release device. Three specific types of pheromones-assisted tick control devices have been developed in recent years namely arrestment's, confusants and attract and kill devices.

Arrestment pheromone impregnated devices

A patented device incorporating purines from the faecal wastes of the prostriate tick, *I. scapularis* into oily droplets released from a pump sprayer was designed for delivery to vegetation. The oily droplets adhered to vegetation where *I. scapularis* quest for hosts. The arrestment pheromone components like guanine and xanthine along with acaricide, permethrin caused the ticks that encounter the droplets to cling to the contaminated surfaces where they acquire a lethal dose of acaricide.

2, 6-DCP as confusants

A confusant exploits the mate searching behaviour of the male by minimizing their ability to locate females as the emitting source. A sex pheromone–pesticide combination was used to confuse mate seeking male, causing them to acquire more pesticide as they wander through the pheromone and pesticide treated fur.

Tick decoys

A device to attract mate seeking males to bead shaped plastic spherules using attractant sex pheromone and mounting sex pheromone. The attracted ticks were killed by using small quantities of toxicant in the plastic spherules. Micro capsules, plastic decoys, or a trap using rubber septum, hollow fibres, capillary filaments, poly ethylene or gelatine capsules or multi-layer tags made of natural or synthetic polymer resins served as the female mimics or the decoys. Any one of these devices was impregnated with 2, 6-DCP and propoxur. These decoys were attached to the hair coat of the tick-infested animals. This resulted in the death of all male ticks that were attached. The female ticks failed to engorge to repletion and most of them died. Engorged female ticks which dropped off the host failed to lay eggs.

Integrated tick management (ITM)

Integrated tick management consists of the systematic combination of two or more technologies to control pest populations that adversely affect the host species while maintaining adequate levels of animal production. This management aims to achieve pest control in a more sustainable, cost-effective manner and environmentally compatible than is achievable with a single, stand-alone technology (Willadsen, 2006). A wide range of molecular tools are becoming available which provide new insights into diagnosis, spatial distribution of ticks, acaricide resistance, satellite imagery, anti-tick vaccines and biological control of ticks. An integrated system employing vaccination with amitraz treatments and Gavac®, under field conditions achieved nearly 100% control of *R. microplus* populations resistant to organophosphates and synthetic pyrethroids (Rodriguez-Vivas *et al*., 2018).

Conclusion

Use of acaricides alone leads to development of acaricidal resistance and also leads to environmental pollution and residues in the food chain. The control of ticks in certain areas has been solely based on indiscriminate use of chemical methods, which has led to the selection of acaricide-resistant tick populations. Veterinarians play a key role and they should educate people about the problems associated with the inadequate use of acaricides. An integrated strategy for the control of ticks is essential and should include chemical and non-chemical methods. Use of vaccines, herbal acaricides, semiochemical-impregnated devices can play an important role in controlling the ticks and tick-borne diseases (TBDs) as they do not have any residual effects. Control strategy should be based on the knowledge of the tick ecology locally, which would improve the efficiency, reduce the risk of acaricide resistance, and environmental pollution.

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Chapter- 25

Fitness Penalties in the Evolution of Fungicide Resistance

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Abstract

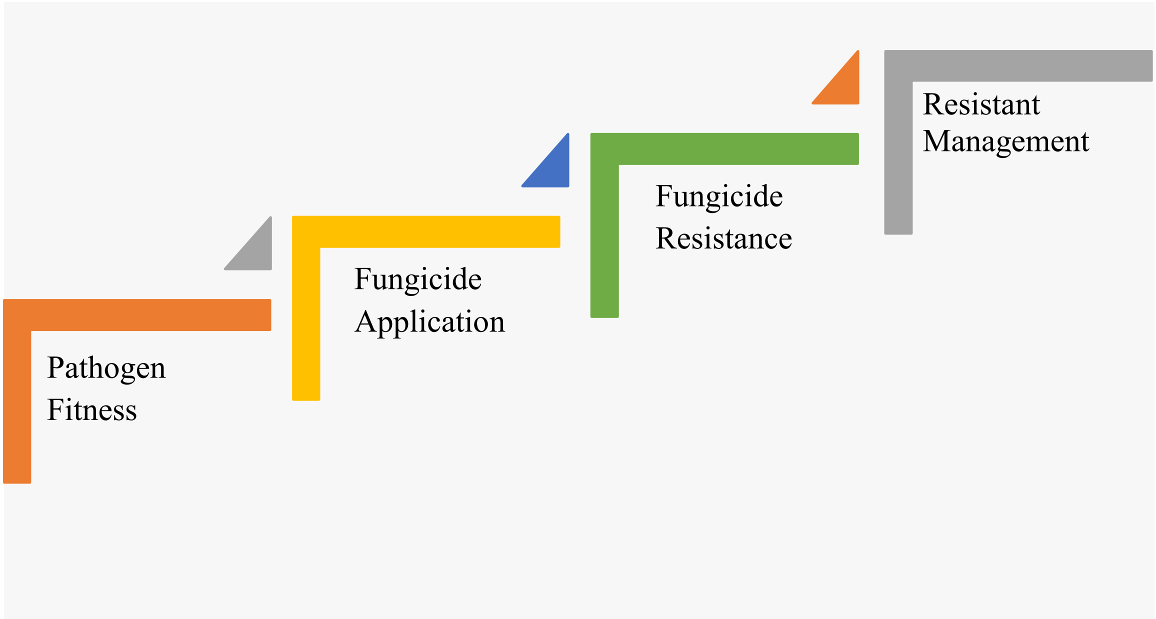
Fungicide resistance is an acquired, heritable reduction in sensitivity of a fungus to a specific antifungal agent or fungicide (FRAC). Fungicides targeting a single biochemical step generally have a low toxicology profile for humans and other non-target organisms. Such fungicides also prone to resistance development by the pathogen. Fungicide resistance can be conferred by various mechanisms including an altered target site, synthesis of an alternative enzyme capable of substituting the target enzyme, mutation of genes encoding fungicide target and a metabolic breakdown of the fungicide. The resistant genotypes may emerge de novo under fungicide selection or be selected from standing genetic variation and may result in effective resistance with a single mutation or a more continuous shift as multiple mutation.

Keywords- Fungicide resistance, Quantitative resistance

Introduction

Our ever-expanding global population relies on successful crop production for food. However, many factors reduce crop yield. These include biotic stress caused by bacteria, fungi, insects, oomycetes, and viruses. Crop disease caused by pathogenic fungi poses a particularly significant risk to food security. Jian *et al*. (2022) estimated that if major fungal and oomycete epidemics simultaneously broke out in the five crops rice, wheat, maize, potato, and soybean, there would be a severe food shortage with enough produce left for only 39% of the global population. Understanding the pathogenesis of fungal plant pathogens is, therefore, of considerable importance.

Battle of Evolution



In order to approach measuring natural selection in such clonal, spatially distributed, non-standard organisms, it is useful to restate the logic that now defines Darwin's concept. Evolution by natural selection has a simple logic that follows if three properties are observed in a population:

- phenotypic trait variation;
- differential fitness of those variants; and
- heritability of those variants.

According to the concept of Survival of fittest, the organism which can survive under variable environmental condition is said to be fit and passed to the future generation.In host pathogen interaction the traits which are responsible for virulence and infection are subjected to rapid evolutionary changes, as a response plants also evolve to have defence mechanisms (Davila *et al*., 2022).

History of Fungicide Resistance

Up to 1970 there were a few sporadic cases of fungicide resistance, which had occurred many years after the fungicide concerned was introduced. With the introduction of the systemic fungicides, the incidence of resistance increased greatly, and the time taken for resistance to emerge was often relatively short, sometimes within two years of first commercial introduction. Many of the fungicides introduced since the late 1960s have been seriously affected, with the notable exceptions of the amine fungicides ('morpholines'), fosetyl-aluminium, anilinopyrimidines, phenylpyrroles and some of the fungicides used to control rice blast disease (e.g. probenazole, isoprothiolane and tricyclazole), which have retained effectiveness over many years of widespread use.

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Pathogen Fitness

Fitness is a common currency in comparative biology. Without data on fitness, hypotheses about the adaptive significance of phenotypes or basic mechanisms of evolution, for example natural selection, remain speculative. Experiments with fungi can address questions specific to fungi or questions with a broader significance. Fungi can challenge the generality of fundamental evolutionary principles, yet there are no standard measures of fungal fitness. We argue that focusing on a single aspect of a complex life cycle, or a single measure of fitness (e.g. the number of asexual spores) is appropriate. Choosing which aspect of fitness to measure can be facilitated by an understanding of how fitness measures are correlated. Choices can also be based on the ecology of a species, for example whether a fungus is semelparous and reproduces (Hawkins and Fraaije, 2018).

Factors which attributes to Fungal fitness

- Aggressiveness
- Reproductive capacity
- Dispersion efficiency
- Survival efficiency
- Virulence

Origin of Resistance

Resistance is heritable. It results from one or more changes in the genetic constitution of the pathogen population. There is overwhelming circumstantial evidence that a mutant gene that causes production of a particular resistance mechanism pre-exists in minute amounts in the population. Before fungicide was ever used in the field, such a mutation would confer no advantage to the growth or survival of the organism, and could well cause a slight disadvantage.

Fungicide Application

Diseases are a common occurrence on plants, often having a significant economic impact on yield and quality, thus managing diseases is an essential component of production for most crops. Broadly, there are three main reasons fungicides are used:

(a) To control a disease during the establishment and development of a crop.

(b) To increase productivity of a crop and to reduce blemishes. Diseased food crops may produce less because their leaves, which are needed for photosynthesis, are affected by the disease. Blemishes can affect the edible part of the crop or, in the case of ornamentals, their attractiveness , which both can affect the market value of the crop.

(c) To improve the storage life and quality of harvested plants and produce. Some of the greatest disease losses occur post-harvest.

Fungi often spoil (render unusable) stored fruits, vegetables, tubers, and seeds. A few which infect grains produce toxins (mycotoxins) capable of causing severe illness or even death in humans and animals when consumed. Fungicides have been used to reduce mycotoxin contamination in wheat affected by Fusarium head blight, but most fungicides developed so far have not been sufficiently effective to be useful for managing mycotoxins associated with other diseases.

- Fungicide- derived from Latin words *fungus* and *caedo*(to kill)
- Fungicides contribute to food security by controlling plant pathogenic fungi
- There are more than 150 active ingredients registered as fungicides worldwide

Mode of Action of Fungicides

The Mode of Action describes what process is impacted in the fungi and is denoted with a letter code. The Group describes the target site, the specific site of activity of the fungicide. Fungicides can have the same mode of action but different target sites. For example, both demethylation inhibitors and amines inhibit sterol biosynthesis, but they work on different target sites to do this. All chemicals within a Group are generally considered cross-resistant to each other. The Chemical Group classifies chemicals and the Common Name is synonymous to the active ingredient.

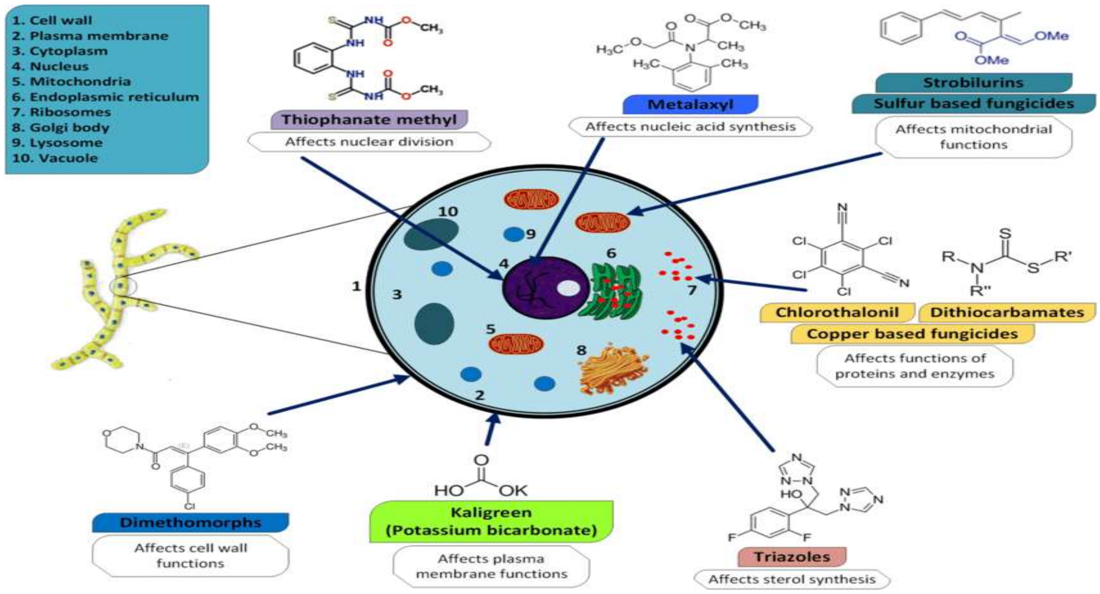
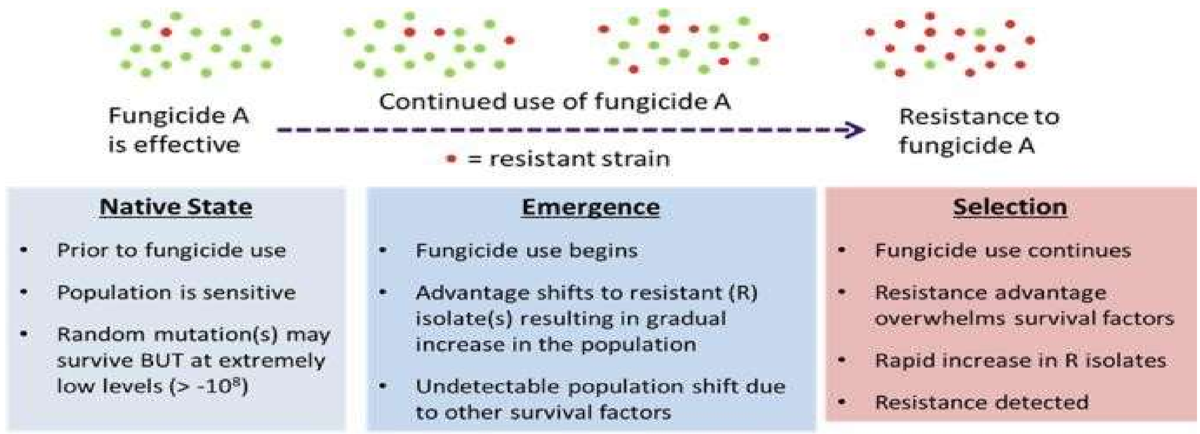


Fig 1. Mode of action of different fungicides

What is Fungicide Resistance?

- **Fungicide Resistance** = a stable, heritable trait that results in a reduction in sensitivity to a fungicide by an individual fungus.
- **Practical resistance** = labeled rates of a fungicide no longer provide commercially acceptable control of a disease.

Occurrence of Resistance



__Qualitative resistance__:

If resistance characterised by a sudden and marked loss of effectiveness, and by the presence of clearcut sensitive and resistant pathogen populations with widely differing responses, is variously referred to as 'qualitative', 'single-step', 'discrete', 'disruptive' or 'discontinuous' resistance . Once developed, it tends to be stable. If the fungicide concerned is withdrawn or used much less, pathogen populations can remain resistant for many years; a well-documented example is the sustained resistance of *Cercospora betae*, the cause of sugar-beet leafspot, to benzimidazole fungicides in Greece. Resistance results from a single mutation in one gene

- When fungicidal resistance results from modification of a single major gene.
- Develops against fungicides which have a single site of action.
- It tends to be stable and persists even after the fungicide is withdrawn (Leach *et al*, 2019).
- Complete loss of disease control
- Resistance cannot be compensated with higher doses or more frequent fungicide application. Ex: Benzimidazole and strobilurin shows this type of Resistance.

Monilinia fruticola and *Podosphera* show qualitative Resistance to Benzimidazole fungicides.

__Quantitative resistance__**:**

In such cases, both a decline in disease control and a decrease in sensitivity of pathogen populations as revealed by monitoring tests, manifest themselves gradually, and are partial and variable in degree. This type of resistance is referred to as 'quantitative', 'multi-step', 'continuous', 'directional' or 'progressive' . It reverts rapidly to a more sensitive condition under circumstances where the fungicide concerned becomes less intensively used and alternative fungicides are applied against the same disease

- Resistance results from mutations in several genes that interact
- When the fungicidal resistance results from modifications of several interacting genes. .
- Higher doses can slow down the development of the fungus (Mikaberidze and Mcdonald,

2015).

- It reverts rapidly to sensitive condition under withdrawal of the concerned fungicide.
- DMI (demthylation inhibitors) show this type of Resistance

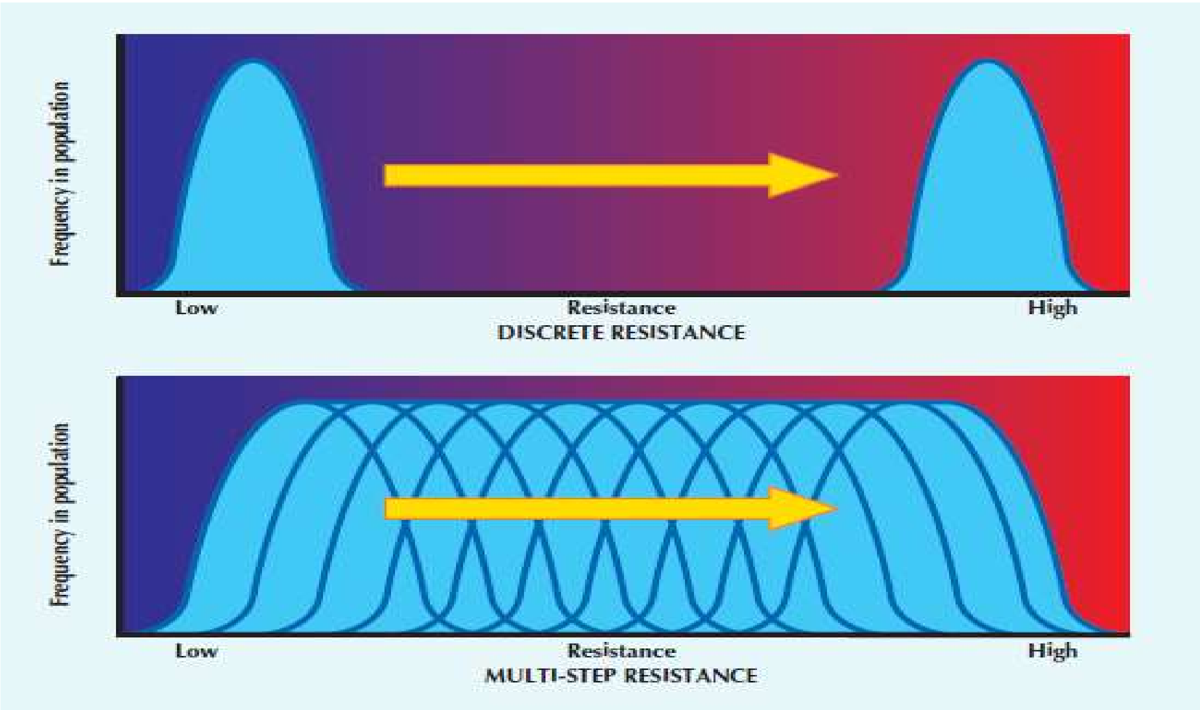


Fig 2. Diagrams showing the bimodal and unimodal distributions of degree of sensitivity which are characteristic of the discrete and multi-step patterns of resistance development.

The first appearance of resistance in a particular fungicide-pathogen combination in one region has almost always been accompanied, or soon followed, by parallel behaviour in other regions where the fungicide is applied at a similar intensity. Whether the fungicide also meets resistance in other of its target pathogens depends on the individual case. Generally, it does occur in other target pathogens that have a comparable rate of multiplication, provided that the fungicide is used in an equally intensive way. It is notable that rust fungi, despite their abundant sporulation and rapid spread, appear to be low-risk, seldom producing resistance problems.

Biochemical Mechanisms of Fungicide Resistance

1) Decreased permeability

- A change in the permeability of the cell membrane is quite often the cause of fungicide resistance especially in specific site inhibitors.
- *Alternaria kikuchiana* resistant strain to **polyoxin B** the uptake is very low as compare to sensitive strain due to reduced activity of dipeptide permease responsible for reduced penetration of fungicide into the cell of resistant

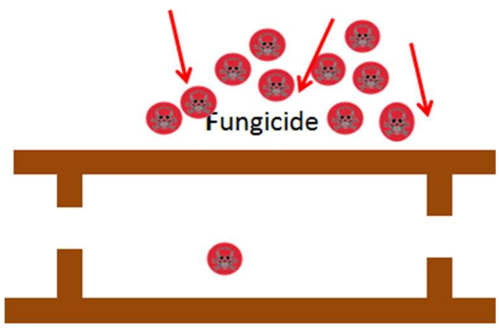


Fig 3. Decreased permeability

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strain (Li *et al.,* 2020).

Reduced uptake of fungicide :

The resistant pathogen simply absorbs the fungicide much more slowly than the susceptible type.

2) Increase of detoxification

- The fungal cell contains a vast array of metabolic machinery for normal cellular processes.
- After the entrance of fungicide into the cell.
- This metabolic machinery may be able to modify the fungicide to a non- toxic form that is no longer harmful to the cell.

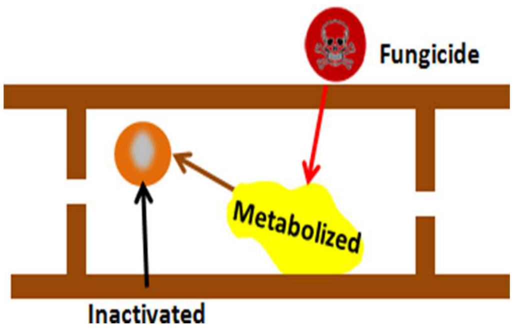


Fig 4. Increase of detoxification

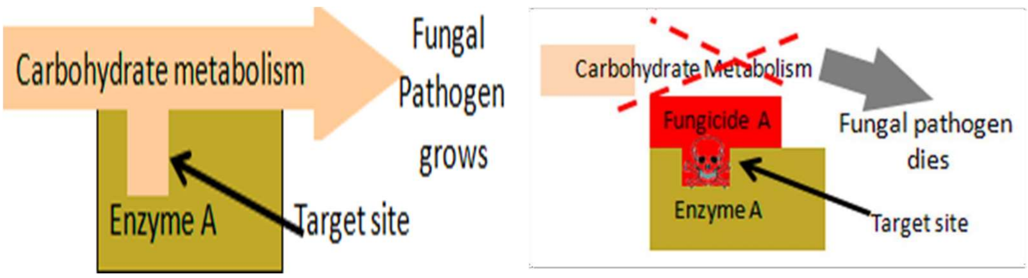
- Ex: *Fusarium oxysporium f.sp. lycopersici* which is resistant to Ascochytin antibiotics is by reducing this into a less fungitoxic derivatives.
- A fungus with the ability to quickly degrade a fungicide can potentially inactivate it before it can reach its site of action.

3) Decrease conversion of fungicide compounds

- Some fungicides are applied as inactive pro-fungicides which require further metabolism by the fungal cell to become the active form.
- If fungal metabolism is altered such that the activation step does not occur the active form of the product is not produced.
- Ex: *Clodosporium cucumerinum* is resistant to **6- Azouracil**, which does not convert it to 6-Azauridine 5-phophate due to the loss of enzyme Uridine phosphorylase.

4) Alteration of the target site

- ✓ When the Fungicide reaches at the site of action without being detoxified ,then the most common way that fungi can become resistant to that fungicide is via a change at the target site.
- ✓ Fungicide binds to the target site where it acts to disrupt a particular biochemical process or function.



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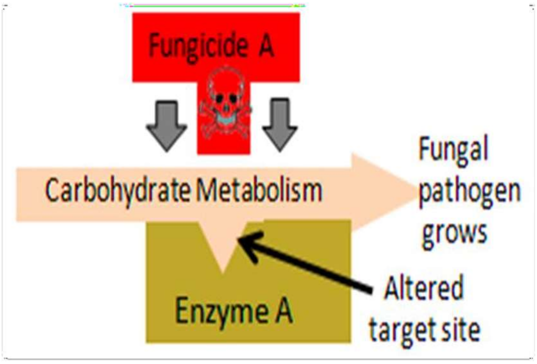


Fig 5. Altered target site

Exclusion or expulsion from the target site:

- ✓ Efflux pumps exclude or expel foreign substances
- ✓ In fungi, the most common efflux pump is ABC (ATP- binding cassette) transporters.
- ✓ Despite these efflux pumps most fungicides can reach effective concentrations inside the cell and inhibit cellular processes.
- ✓ These transporters reduces sensitivity of the pathogen.

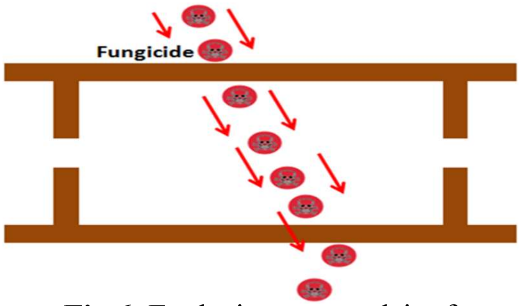


Fig 6. Exclusion or expulsionfrom the target site

<u>Removal:</u> A fungal cell may rapidly export the fungicide before it can reach the target site of action

Detecting Resistance

- Early detection of fungicide resistance in the field is difficult
- Method for diagnosing the level of resistance in a fungal population involves the determination of the resistance factor or RF value
- By comparing the fungicide's lethal effects on a perceived resistant population with a susceptible laboratory colony

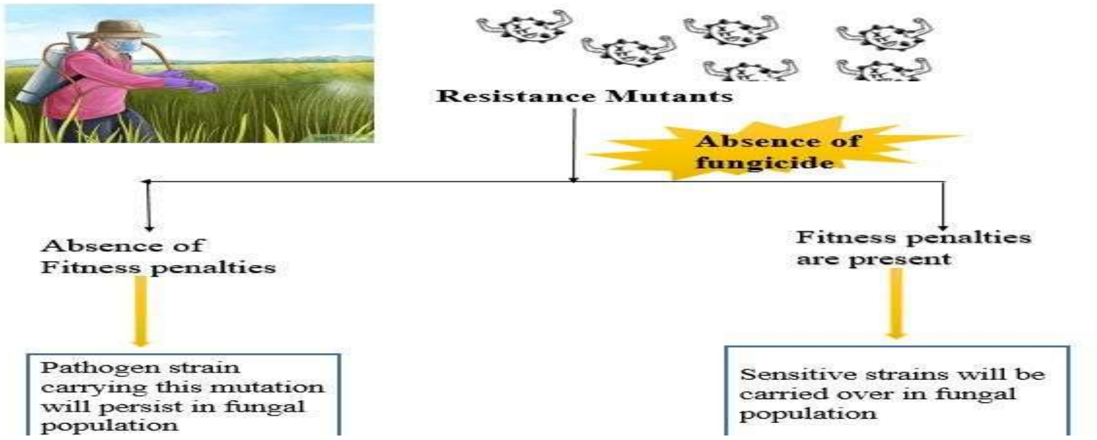
$$RF = \frac{\text{EC50 of Resistant isolates}}{\text{EC50of Sensitive isolates}}$$

- Increased RF values equate to potential resistance. Control is usually lost when the RF>10
- Molecular DNA-based assays may provide a more rapid and reliable assessment of resistance when compared with conventional laboratory methods (Lucas *et al.,* 2015).

How does an understanding of fitness costs affect Resistance management?

The growth rate, r, of the sensitive strain decreases as a function of the fungicide dose, C (solid curves). When resistance is full (left panel), the growth rate of the resistant strain is not affected by the fungicide. Hence, r remains constant versus C with the magnitude that depends on the fitness cost θ (dashed lines). In the absence of a fitness cost (upper dashed line in Fig. 7), resistant mutants have a selective advantage over the sensitive strain as soon as the fungicide is added (i.e., at any C>0). When there is a fitness cost, the sensitive strain is favored by selection at small doses (cf. the range of doses, where the solid curve is above the lower dashed curves in Fig. 7).

Fig 7. Fitness penalties in fungicide resistance



Development of Resistance

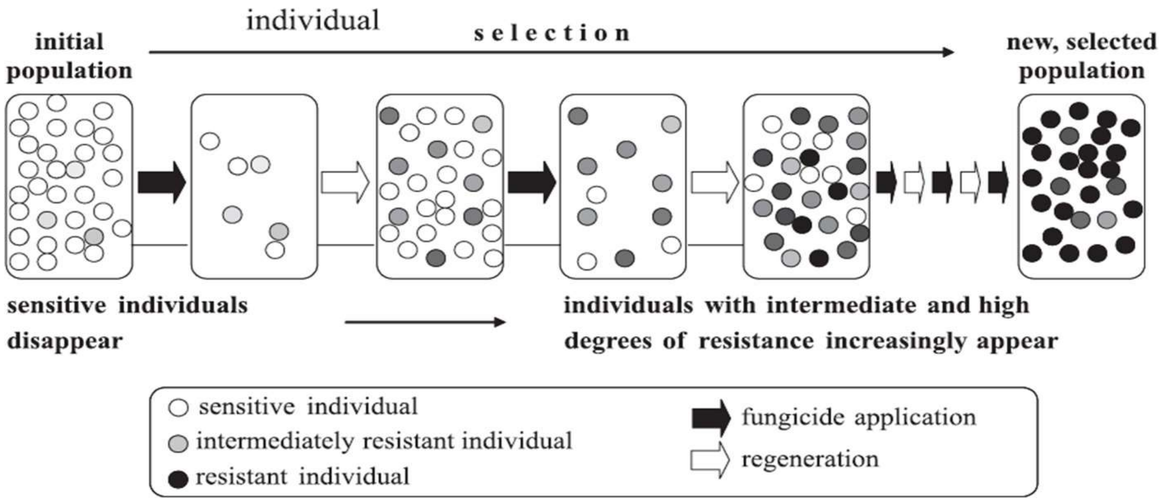


Fig 8. Development of quantitative resistance

Resistance to More than one Fungicide

Cross resistance

- When a pathogen resistant to one fungicide exhibits resistance to other fungicides in the same chemical class, even without exposure.
- Fungus strains which resists benomyl also show resistance to other benzimidazole fungicide such as carbendazim.

Ex : Cross resistance of *Sphaerotheca fuligiana* (Cucurbit PM) to pyrimidines (ethrimol, dimethrimol.

Multiple resistance

- when a pathogen independently develops resistance to fungicides in different chemical classes.

Positive Cross Resistance

- When resistance to one group of fungicides leads to an increase in resistance to another group of fungicides.
- It was observed in *Septoria tritici* between tebuconazole, propiconazole and other DMI fungicides.

Negative Cross Resistance

- When resistance to one group of fungicides leads to an increase in sensitivity to another group of fungicides.
- N-phenyl carbamate shows negative cross resistance with carbendazim.
- This phenomenon is utilized to control *Botrytis* in grapes and other crops.

Fitness Penalties

- Making the resistant strain less fit than the sensitive strain in the absence of a fungicide.
- Fitness costs are connected to mutations that encodes fungicide resistance, will need to be better measured in order to design optimum fungicide deployment strategies.
- In order to determine fitness costs, one needs to measure fitness of both sensitive and resistant pathogen strains in the same, nonselective environment.

Assessing the risk

This is a matter of great importance to the chemical manufacturer who is about to develop a new product. Knowledge of the risk of resistance will help to determine whether the product should be developed and marketed, and, if so, of what nature and how stringent should be the resistance management strategies and how much further monitoring should be done (Malandrakis *et al*., 2021).

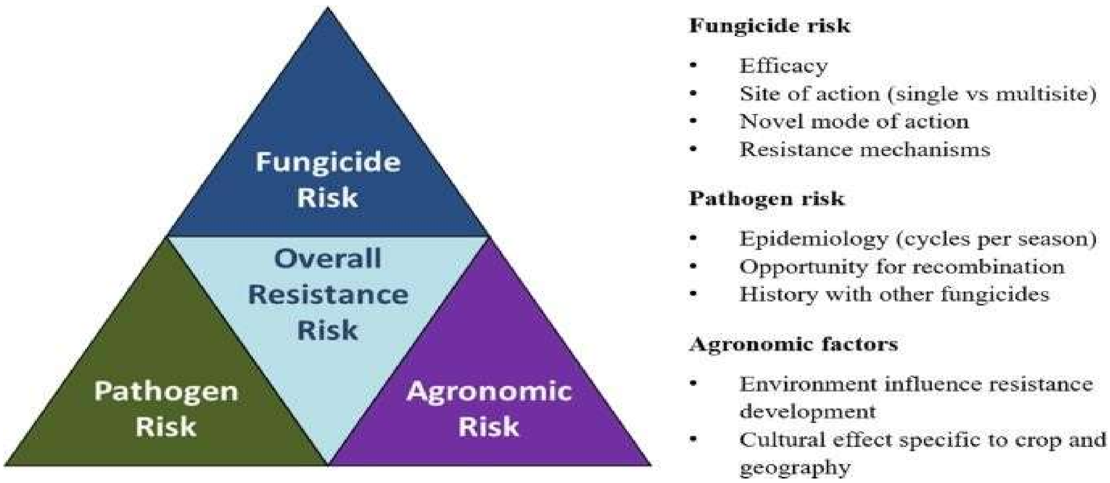


Fig 9. Assessment of fungicide resistance risk

Fungicide resistance work in India

Table 1. Fungicide resistance work in India

Crop	Pathogen	Compound	Reference
Rice	*Magnoporthe grisea*	Edifenphos	Lalithakumari 1987; Annamalai and Lalitha Kumari, 1990)
Potato	*Phytophthora infestans*	Oxadixyl	Thind *et al*., 1995
Grapes	*Uncinula necator*	Triademefon	Singh *et al*., 1993
Grapes	*Plasmopara vititola*	Metalaxyl	Rao and Reddy, 1985
QoI*		NRC (Grapes)	
Potato	*P. infestans*	Metalaxyl	Arora *et al*., 1992
Citrus	*P. parasitica*	Metalaxyl	Thind *et al*., 1999
Cucumbers	*Pseudoperonospora cubensis*	Metalaxyl	Thind *et al*., 2009, 2012

*Fenamidone, Azoxystrobin, Famoxadone, Kresoxim methyl and Pyraclostrobin

After the superannuation of **Dr. T. S. Thind**, PAU in 2012 – not much consistent efforts on Fungicide Resistance in India

Among diseases of horticultural crops the following pose more risk in India:

Grapes (DM, PM); cucurbitaceous vegetables (DM, PM), Potato (Late blight);

Capsicum (PM), Roses (DM, PM), apple (scab)

Conclusion

Knowledge of fitness penalties is crucial for predicting effectiveness and optimizing disease control strategies. Many studies provide initial hints at fitness penalties, but these are often inconsistent between studies. These uncertainties may reflect genuine difference between pathogen species, different fitness components, the use of different growth conditions or intraspecific variation in genetic background. In terms of the development of future crop protection, penalties for resistance are clearly desirable but are not easily incorporated into the discovery process. There is major gap on the knowledge of fitness cost.

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Chapter- 26

Pulsed Electric Field (PEF) Technology in Food Industry

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Introduction

Pulsed Electric Field (PEF) processing is an innovative non-thermal food preservation method that uses high-intensity pulsed electric fields (HIPEF) to extend the shelf life of foods while maintaining their sensory and nutritional quality. This technique involves applying short pulses of high voltage (typically 20 - 80 kV/cm) to food items positioned between two electrodes. PEF treatment is conducted at or slightly above room temperature for less than 1 second, with each pulse lasting less than 5 microseconds to minimize energy loss due to heating and unwanted changes in sensory properties (Quass, 1997). PEF technology is considered superior to traditional heat treatment because it minimizes detrimental alterations in the sensory and physical characteristics of foods. While some studies suggest that PEF preserves the nutritional components of food, a better understanding of its effects on the chemical and nutritional aspects of foods is needed before widespread adoption in food processing. Key considerations in PEF technology include generating high electric field intensities, designing chambers for uniform treatment with minimal temperature increase, and developing electrodes to mitigate the effects of electrolysis. Achieving high field intensities involves storing a substantial amount of energy in a capacitor bank from a DC power supply, which is then discharged as high-voltage pulses. Studies indicate that PEF is an energy-efficient process, particularly when used in continuous systems, compared to traditional thermal pasteurization methods (Qin *et al*., 1995a).

The Historical Development of Pulsed Electric Field (PEF):

In the late 19th century, researchers such as Prochownick, Spaeth, Kruger, Thiele and Wolf explored the bactericidal effects of electric current. However, these early experiments mainly resulted in thermal or electrochemical effects rather than effective microbial inactivation. In the 1920s, the "Electropure" process was introduced in Europe and the USA as one of the first attempts to use electricity for milk pasteurization. It involved the application of continuous 220 V alternating current within a carbon electrode chamber (Moses, 1938).

Although approximately 50 Electropure plants were operational until the 1950s, they gradually declined due to rising energy costs and competition from emerging thermal preservation methods like Ultra-High-Temperature (UHT) processing (Reitler, 1990). Aside from thermal effects, some lethal effects resulting from electrochemical reactions, such as the hydrolysis of chlorine, were observed when applying electrical discharges with a voltage of 3-4 kV (Pareilleux and Sicard, 1970). The use of pulsed discharges of high-voltage electricity across two electrodes for microbial inactivation began to be explored in the 1950s, leading to the development of a process known as electrohydraulic treatment. This method involved submerging electrodes in a liquid medium within a pressure vessel, generating electric arcs through high-voltage pulses, creating transient pressure shock waves and ultraviolet light pulses (Allen and Soike, 1966; Edebo and Selin, 1968). Experiments conducted by Doevenspeck in 1984 demonstrated that pulsed electric fields could be applied to disrupt cells in food materials, leading to further developments in microbial inactivation and wastewater treatment. This work served as the basis for the development of processes like "Elcrack" for disintegrating animal materials and "Elsteril" for decontaminating liquid media, both by Krupp Maschinentechnik GmbH, Germany. The application of electric fields for electroplasmolysis in apple mash was first reported by Flaumenbaum in 1968. This process resulted in increased juice yield and improved product quality compared to traditional heat or enzymatic pre-treatment (McLellan *et al*., 1991). Early patents related to PEF treatment were filed by Krupp in Germany, with electric field strengths reaching up to 30 kV/cm. However, heating caused by high energy dissipation limited the industrial application of these processes. Later patents were applied by Pure pulse Technologies, San Diego, USA, using electric fields ranging from 10 to 25 kV/cm. The effects of microbial inactivation and improvements in fruit juice shelf life were demonstrated by Dunn and Pearlman in 1987. Presently, while approximately 20 research groups worldwide are actively involved in PEF research, there are still no commercially available industrial-scale PEF systems. However, pilot-scale systems for liquid food preservation are in operation at research institutions including Ohio State University (USA), Stork Food and Dairy Systems (The Netherlands), SIK (Sweden) and the Berlin University of Technology (Germany).

Pulsed Electric Field Processing: PEF is a method that uses electric waves with high voltage amplitude. Short electrical impulses (from microseconds to milliseconds each) of high voltage (typically 10–80 kV/cm) are supplied to the product placed between the electrodes in the chamber. It is one of the most appealing technology due to the reasons like: Short treatment time (typically below 1 second), Reduced heating effect, Energy lost during heating food is minimized. For fresh-like characteristics of food, along with high sensorial quality and nutrient content. It is suitable for preserving liquid and semi-liquid foods removing micro-organisms and producing functional constituents. Examples: - milk, fruit juices, soup, egg *etc*.

Fig.1. Schematic representation of pulsed electric field treated strawberry fruits

Principle Of Pulsed Electric Field

- Basic principle is to keeping food below temperatures normally used in thermal processing.
- PEF technology is the application of short pulses of high electric fields with duration of microseconds micro- to milliseconds and intensity in the order of 10-80 kV/cm.
- The processing time is calculated by multiplying the number of pulses times with effective pulse duration.
- The process is based on pulsed electrical currents delivered to a product placed between a set of electrodes; the distance between electrodes is termed as the treatment gap of the PEF chamber
- The process is based on pulsed electrical currents delivered to a product placed between a set of electrodes; the distance between electrodes is termed as the treatment gap of the PEF chamber.

Working f Pulsed Electric Field

- PEF technology is based on a pulsing power delivered to the product placed between a set of electrodes confining the treatment gap of the PEF chamber.
- The equipment consists of a high voltage pulse generator and a treatment chamber with a suitable fluid handling system and necessary monitoring and controlling devices.
- Food product is placed in the treatment chamber, either in a static or continuous design, where two electrodes are connected together with a nonconductive material to avoid electrical flow from one to the other.

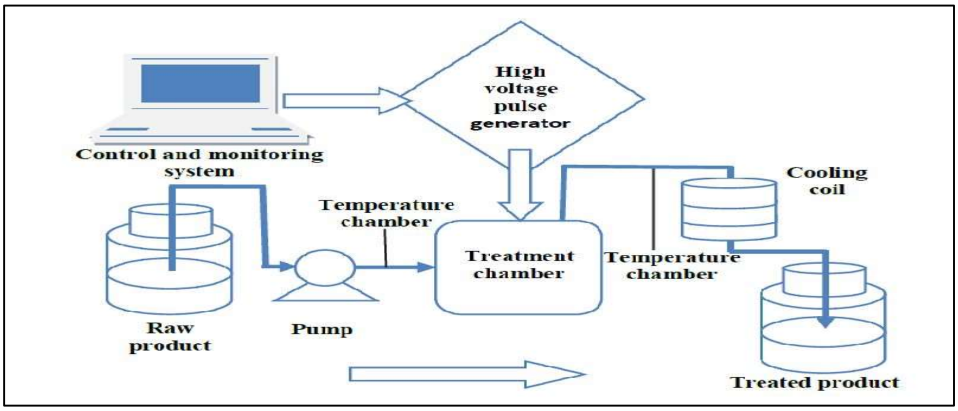

Fig.2. Schematic representation of pulsed electric field process

Mechanisms Involved in PEF

Two mechanisms have been proposed to explain the inactivation of microorganisms using pulsed electric field:

1. Electrical breakdown
2. Electroporation

❖ Electrical Breakdown: A normal microbial membrane has a charge separation across the membrane which leads to a potential difference of around 10 mV. If an external electric field is applied, this increases the potential difference across the cell membrane, which leads to reduction in membrane thickness. When the potential difference across the cell reaches a critical level (normally considered to be around 1 V), pores are formed in the membrane. This leads to an immediate discharge at the membrane pore and consequently, membrane damage as shown in fig.3. The transmembrane potential developed in the direction of an Applied electric field is given by,

$$U(t) = 1.5rE \qquad \text{Equation .... (1)}$$

where, U(t) is the transmembrane potential in the direction of the applied field (V), r is the radius of the cell (μm) and E is the applied electric field strength (kV mm^{-1}).

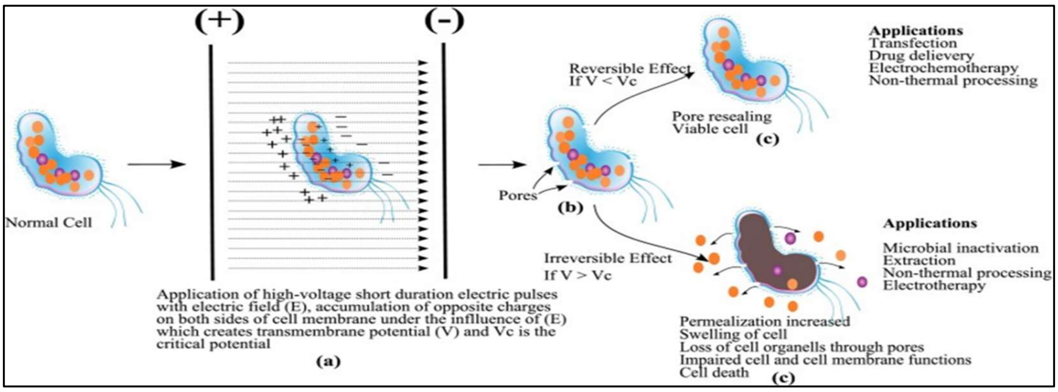

Fig.3. Schematic representation of Electrical breakdown process

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❖ Electroporation: When a microorganism is subjected to a high voltage electric field, the lipid bilayer and proteins of the cell membrane are temporarily destabilized. Changes in the conformation of lipid molecules are induced, existing pores are expanded and structurally stable hydrophobic pores are formed which can conduct current. This leads to localized heating that changes the lipid bilayer from a rigid gel to a liquid crystalline form. Impairing of the semi-permeable nature of the membrane leads to swelling and eventual rupture of the cell is induced as shown in fig.4.

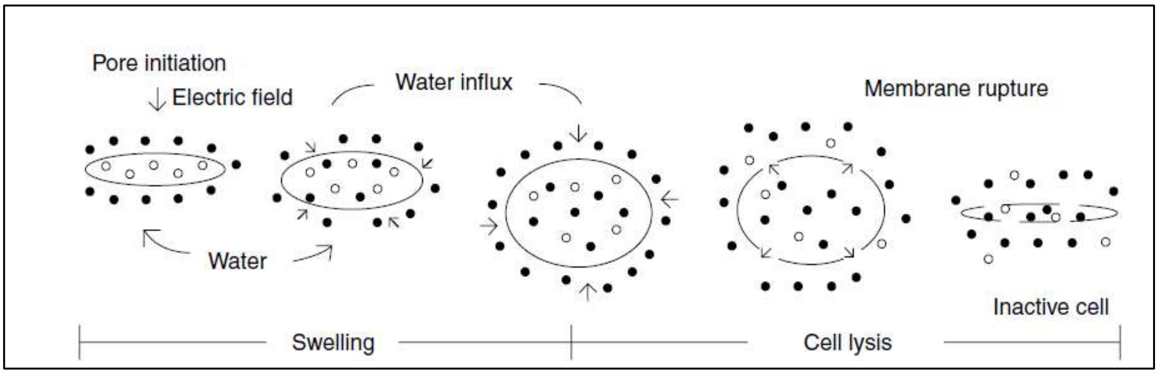

Fig.4: Schematic representation of Electroporation process

Critical factors in inactivation of microorganisms using pulsed electric field: The effectiveness of PEF on microbial inactivation is determined by following factors:

1. Process factors like time, pulse width
2. Product factors like electrical conductivity, composition of products, pH
3. Microbial factors like types of micro-organisms

Advantages of Pulsed Electric Field:

1. Less treatment time.
2. Low treatment temperature.
3. Substitute for conventional heat pasteurization or it can operate at room temperature to retain quality and heat-sensitive vitamins.
4. Increase shelf life and maintain food safety with low processing costs.
5. Minimally processed foods of fresh quality, which have higher nutritional value because of colour and flavour retention.
6. Reduction in microorganisms: 4-6 log
7. It can be used to pasteurize fluids such as juices, milk and soups without using additives.

Disadvantages of Pulsed Electric Field:

1. High capital cost.
2. PEF treatment is effective for the inactivation of vegetative bacteria only.

3. Micro-organisms are destroyed by PEF but spores, with their tough protective coats and dehydrated cells are able to survive.
4. Refrigeration is required to extend shelf-life.
5. Treatment does not inactivate enzymes.
6. PEF is a continuous processing method, which is not suitable for solid food products that are not pumpable.
7. PEF processing is restricted to food products with no air bubbles and with low electrical conductivity.

Applications of Pulsed Electric Field:

1. **Microbial inactivation by PEF:** The applicability of PEF for microbial inactivation of liquid food has been proven by a high number of studies investigating the impact of PEF on vegetative organisms in model as well as real food material.
2. **Processing of apple juice:** Simpson *et al*. (1995), reported that apple juice from concentrate treated with PEF at 50 KV cm^{-1}, 10 pulses and pulse width of 2 µs and maximum processing temperature of 45 °C had a shelf-life of 28 day compared to a shelf-life of 21 day of fresh-squeezed apple juice. There were no physical or chemical changes in ascorbic acid or sugars in the PEF-treated apple juice and a sensory panel found no significant differences between untreated and electric field treated juices.
3. **Processing of orange juice:** Zhang *et al*. (1997), evaluated the shelf-life of reconstituted orange juice treated with an integrated PEF pilot plant system. The PEF system consisted of a series of co-field chambers. Temperatures were maintained near ambient with cooling devices between chambers. Three waveshape pulses were used to compare the effectiveness of the processing conditions. Their results confirmed that the square wave is the most effective pulse shape.
4. **Processing of milk:** Fernandez-Molina *et al*. (1999), studied the shelf-life of raw skim milk (0.2% milk fat), treated with PEF at 40 KV cm^{-1}, 30 pulses and treatment time of 2 µs using exponential decaying pulses. The shelf-life of the milk was 2 weeks stored at 4°C; however, treatment of raw skim milk with 80°C for 6s followed by PEF treatment at 30 kV/cm, 30 pulses and pulse width of 2 µs increased the shelf-life up to 22 days, with a total aerobic plate count of 3.6-log CFU mL^{-1} and no coliform. The processing temperature did not exceed 28°C during PEF treatment of the raw skim milk.

Conclusion:

Pulsed Electric Field (PEF) is a non-thermal cell membrane permeabilization technique with several promising applications. It offers low energy consumption (1–2 kJ/kg for stress induction and 5–10 kJ/kg for plant cell permeabilization) and continuous operability, making it suitable for innovative, cost-effective, and sustainable processing in various industries:

1. Food and Drink Industry: PEF can be used for food preservation, allowing products to be preserved at lower temperatures and for shorter durations. This helps retain the freshness

and nutritional value of food items, offering a competitive edge in markets across the United States, Asia, Europe and Central and South America.

2. Biotechnology: PEF has applications in biotechnology, especially in cell permeabilization for various purposes, potentially reducing the need for harsh chemical treatments and offering more environmentally friendly alternatives.
3. Pharmaceutical Industry: PEF can be explored in pharmaceuticals for cell membrane permeabilization, which may have implications for drug delivery and biopharmaceutical production processes.
4. Innovation Potential: The energy efficiency and waste-free nature of PEF make it a platform for developing novel processing concepts in India and elsewhere, fostering innovation in food technology and related industries.
5. In summary, PEF is a versatile technology with significant potential for enhancing food preservation, biotechnology, pharmaceuticals, and sustainable processing, offering a competitive advantage in growing markets worldwide, including India.

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Chapter- 27

Protein Quality of Millets

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Introduction

Over the last few decades, farm scientists have been trying to increase the production of foodgrains by evolving high-yielding varieties which have in-built attributes like resistance to diseases and pests, increased photosynthetic efficiency and increased harvest index. As a result of these efforts, substantial increases in the yields of major cereal grains like wheat, rice, maize, sorghum and pearl millet have been achieved, and this has resulted in what is popularly described as the 'Green Revolution'. The production of grain legumes, however, has not kept pace either with the increase in population or with the increase in cereal-grain production. The main sources of food continue to be cereals, which provide up to 85% of the total calories and 50-80% of the total protein requirement in the developing countries. The per day consumption of cereals and pulses is lowered due to lower availability of foodgrains and consequently proteins, there has been widespread prevalence of calorie-protein malnutrition.

Animal proteins though nutritionally well balanced, are expensive and beyond the reach of low-income groups. This is because animals are very poor convertors of vegetable protein into animal protein. In commercial production of meat, 7-8 kg of plant proteins in feed produce 1 kg of animal protein. There is a general myth that animal proteins are superior to a combination of vegetable proteins. Adequate use of wholesome combined vegetable proteins can remove the calorie-protein malnutrition. Such a practice will not only bridge the nutritional gap, but would also be economical and conducive to better health because animal foods contain appreciable quantities of cholesterol, while the food from vegetable sources are generally devoid of it. It has also been well established that the elevated levels of serum cholesterol caused by the consumption of diets rich in non-vegetarian food is one of the major factors associated with the susceptibility to coronary heart diseases as a result of atherosclerosis.

In order to effectively control calorie-protein malnutrition, it is essential to know the nutritional requirements as well as nutritive quality of various foodgrains. The main nutritional-deficiency diseases affecting the population of developing countries are anemia due to iron deficiency, goitre due to the lack of iodine, Kwashiorkor due to protein deficiency, and Marasmus due to protein- calorie deficiency. In addition, there is a widespread deficiency of vitamins, especially of group B and vitamin A. Moreover, the deficiency of one or more nutrients affects the utilization of other available nutrients from food. The nutrition problem is further compounded by the presence of various anti- nutritional factors in cereals and pulses leading to decreased utilization of proteins. Fortunately, however, a majority of these factors are destroyed during cooking.

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It is, therefore, essential to know about nutritive value of cereals and grain legumes while planning for nutritionally improved diets. The nutritive value of different food crops in respect of proteins, vitamins, minerals, etc., are presented in the publications of the Indian Council of Medical Research (ICMR). Therefore, the nutritional quality of millets, especially with respect to their protein quality, is presented in this chapter.

Energy and Protein Requirements

For the growth of the body as well as for its maintenance, the essential nutrients required are: proteins, carbohydrates, fats, minerals, vitamins and water. These constituents are supplied mostly by the food we consume. The recommended allowances of these nutrients have been changed from time to time by the national nutritional advisory bodies of many countries in consultation with the WHO and FAO. In 1973, the joint FAO/WHO expert group on energy and protein requirement revised the 1965 recommendation in the light of the newer knowledge gained during the intervening years and the experience of practical application of the recommendations. In the light of these newer developments and some studies on energy and protein requirements carried out, the Committee appointed by the ICMR recommended energy allowance for children and adults depending on their age and activity (Table 1). For moderate work, man and woman, respectively, need 2800 and 2200 K calories per day. In addition, protein allowance of 1-0 g/kg body weight/day for man and woman, 1-8 g/kg/day for infants and 1-2-1-8 g/kg/day for pre-school children have been recommended (Table 2). These protein allowances are for mixed vegetable proteins having net protein utilization (NPU) of 65 % relative to egg.

Table 1. Energy allowance for children and adults

	Age group	Body weight (kg)	Energy allowance (K cal)
Children	4-6 years	18.87	1720
Boys	10-12 years	34.30	2420
Boys	16-18 years	56.50	2820
Boys	10-12 years	36.47	2260
Boys	16-18 years	50.00	2200
Man (moderate work)		55.00	2800
Women (moderate work)		45.00	2200

(ICMR, 1984)

Table 2. Protein allowance for infants, pre-school children and adults

	Age group	Body weight (kg)	Protein allowance* (g/kg/day)
Infants	4-9 months	-	1.80
Pre-school	4-6 years	18.87	1.56
Children			
Boys	10-12 years	30.40	1.24
Girls	10-12 years	36.46	1.17
Man	-	55.00	1.00
Women	-	45.00	1.00

*protein of mixed vegetables origin with NPU 65 relative to egg. **(ICMR, 1984)**

Protein Quality Concept

Dietary proteins provide amino acids for the synthesis of body proteins and are also a source of nitrogen for other biologically important nitrogenous constituents which undergo turn over in the body. Of the 20 amino acids present in proteins, 8 (isoleucine, leucine, lysine, phenylalanine, methionine, threonine, cryptophan and valine; additionally arginine in case of infants) cannot be synthesized by human beings. They have to be provided through food and are therefore called 'essential amino acids'. Deficiency of any one of these reduces the utilization of the remainder. The essential amino acid requirements of infants, pre-school children and adults shown in Table 3. indicate that pre-school children require more essential amino acids than adults.

Table 3. Amino acid requirements (mg/g protein) of infants, pre-school children and adults

Amino acid	Infants	Pre-school children	Adults
lysine	52	55	22
Threonine	44	40	13
Tryptophan	8.5	10	6.5
Total sulphur containing amino acid	29	35	24
Leucine	80	70	25
isoleucine	35	40	18
valine	47	50	18
Total aromatic amino acids	63	60	25

(FAO/WHO, 1973)

Ideal protein requirements per kg of body weight per day:

Infants: 1.85 g

Pre-school children: 0.9 g

Adults: 0.55g

Food protein differ in their nutritional quality depending on their amino acid profile and digestiablity. Cereal grains, in general are deficient in the essential amino acid like lysine and threonine or tryptophane which limit the nutritional quality of cereal grain proteins. On the otherhand, pulses are rich in lysine but are limiting in sulphur containing aminoacids, mainly methionine (table.4). when cereals are taken in combination with pulses, the deficiency of one is made good by the excess in another. Feeding experiments carried out on albino rats have clearly demonstrated this complementary effect.

If one compares the amino acid profile of cerelas and pulses are given in subsequent section with that of the FAO/WHO recommended protein amino acid profile (Table3), it is observed that, as far as adult human population is concerned, the protein present is most cerels grains are nutrietionally balanced. However, the problem is serious when one takes into consideration the requirements of infants and pre-school children. It is for this vulnerable segment of the population that the nutritionists need to pay attention.

Table 4. Essential amino acid profile and protein quality of important cereals and pulses

	Wheat	Rice	Maize	Sorghum	soybean	Chickpeas	Pigeonpea	FAO/WHO provisional pattern, 1973
Isolecuine	3.73	3.87	3.44	4.9	5.0	4.02	4.18	4.0
Leucine	6.58	7.79	11.20	12.6	7.25	7.03	7.21	7.0
Lysine	2.62	3.06	2.84	2.6	6.45	6.84	7.27	5.5
Total sulphur containing amino acid	3.36	4.84	3.86	3.9	2.82	2.39	3.76	6.0
Total aromatic amino acids	8.24	10.07	6.73	7.4	9.38	8.22	12.03	6.0
Threonine	2.89	2.86	2.79	2.4	4.12	2.98	3.36	4.0
valine	4.50	5.40	4.11	5.0	5.18	4.82	5.05	5.0
Tryptophan	1.04	1.20	0.78	1.2	1.60	0.72	0.64	1.0
Protein %	14.4	10.8	12.0	8.75	50.6	27.6	22.6	-
Biological value	66.0	71.2	59.9	62.8	80.0	78.0	62.0	100

Sikka *et. al*. (1978); Chatterjee and Abrol (1975); Sikka and Johari (1970)

Protein quantity, and especially protein quality, plays an important role. The practical significance of protein quality in food may be considered from 2 points of view. One is the direct impact whichis can have in terms of nutrition and health. The second is related to the efficiency of utilization of other nutrients. Dr. bressani, of the institute of nutrition of central America and Panama, has shown that protein quality as a part of good balanced diet of corn (Maize) and bean stimulated the intake of corn and beans, which in turn resulted in more body weight and higher efficiency of protein utilization.

Thus protein quality plays an important role in growth and development. Realizing its importance, attempts have been made by plant breeders to breed cereals and grain- legume varieties that have high protein as well as better nutritional quality.

Protein Quality of Millets

A. Sorghum

Sorghum ranks next only to wheat and rice in production in India. Millions of people in Africa, India, and other Asian countries depend on it as their principal energy source. For these people grain sorghum is also a major source of protein in their diet. An Analysis of the World Sorghum Collection consisting of 522 lines showed the average protein content to be 12.6% with a standard deviation of 1.89. Lysine and leucine contents have been shown to vary from 0.9 to 2.67% and 6-7 to 29.9%, respectively, in protein. Two lines, 'IS 11167' and 'IS 11758', had a lysine content of 3.44 and 3.13% in protein, and a protein content of 15.7 and 17.2%, respectively. The wide genetic variation in protein content and protein quality offers scope for breeding for improvement in nutritional quality.

A comparison of amino acid composition of sorghum protein with FAO/WHO (1973) amino acid profile indicates lysine to be the first limiting amino acid. However, the presence of a high concentration of leucine or imbalance in the leucine: isoleucine ratio in sorghum has been suggested as a possible factor in the development in pellagra in population subsisting principally on sorghum. Further, sorghum grains contain tannins, which affect the digestibility of the proteins.

The protein quality of sorghum depends upon 2 factors:

(a) Presence of a high proportion of prolamine fraction, which has high concentration of glutamic acid, and leucine, and low concentration of lysine.

(b) Presence of tannins (phenolic compounds), which reduce the digestibility of protein. Tannins alter the solubility of protein possibly by binding and forming complexes with the protein.

Biological experiments with low and high-tannin sorghum varieties showed that there was no increase in body weight of rats fed on high-tannin sorghum. When fed on low-tannin sorghum, rats showed better growth and responded well to lysine supplementation (Axtell *et al*., 1975). Lysine is the first limiting amino acid in low-tannin sorghum lines. Among the high-tannin lines, factors other than low level of lysine limited the biological value. Most of the sorghum varieties cultivated in India contain low amounts of tannin.

Increased quantities of insoluble nitrogen observed in the digestive system of rats fed on tannins is due to non-specific binding of proteins to tannins. Besides, inhibition of different enzymes like amylase, cellulase, B-galactosidase and digestive enzymes like trypsin, lipase by tannins has been observed.

Studies on protein fractionation have shown that prolamine and glutelin are the principal protein fractions in sorghum grain. The prolamine fractions of all the varieties has been found to be extremely poor in both lysine and tryptophan and accounts for 28-43% of the total protein present in the different varieties. The albumin and globulin fractions, which constitute only 20% of the total protein, are quite rich in lysine and tryptophan. About half of the total lysine and tryptophan of the grain is derived from the albumin and globulin fractions while glutelin fraction contributes the other half of these amino acids. Protein from low-protein strains of sorghum promoted the growth of chicks and rats faster than that from high protein strains. Further, the amino acid composition of low- protein sorghum was better than that of the high-protein sorghum. Opaque sorghum, 'P- 721', has been shown to have high lysine content (Table 5) mainly due to reduction in the prolamine fraction.

Table 5. Essential amino acid composition of normal and high-lysine sorghum varieties

Amino acid	Normal	High lysine	
	CSH 1	IS 11167	P721
Isolecuine	4.9	3.9	3.9
Leucine	12.6	11.1	12.2
Lysine	2.6	3.2	2.95
phenylamine	4.2	5.0	4.9
Total sulphur containing amino acid	3.9	2.6	3.1
Threonine	2.4	3.4	3.3
Tryptophan	1.2	ND	ND
valine	5.0	5.2	5.1

IARI (1980)

B. Barley

Barley an important rabi cereal crop. It is used both for human consumption as well as animal feed, and very little is used for brewing and malting. The protein content in barley varieties varies from 9.5 to 18.5% with most varieties having a protein content of 10-12%. Like other cereals, barley has storage proteins that are deficient in lysine. The poor nutritive value of barley is mainly due to a large proportion of prolamine fraction called hordein, which is extremely deficient in lysine. Like maize, in barley also several high-lysine genotypes have been identified or induced. These include 'Hiproly', 'Risø 1508', Risø 56', 'Notch 1' and 'Notch 2'. 'Notch 2' barley derived from variety 'NP 113' by EMS treatment besides having high lysine content also possesses higher protein percentage.

Like high-lysine mutants of maize, the high-lysine mutants of barley also give lower yield mainly on account of decreased synthesis of starch. In 'Notch 2' barley, the decreased yield has been ascribed to the presence of a cavity in the grain and also to the reduced activities of enzymes of starch biosynthesis. The high-lysine mutants 'Notch 1' and 'Notch 2' are comparable to 'Hiproly' identified from the World Barley Collection with regard to lysine and essential amino acids. The high protein character of the mutants was stable in different environments. However, because of the shrivelled grains and relatively poor yield, the mutants were not suitable for commercial cultivation. The increased level of lysine and other essential amino acids in the induced mutants 'Notch 1' and 'Notch 2' was a consequence of a reduction in the prolamine fraction and simultaneous increase in albumin fraction. In these strains the improvement in lysine content was substantial compared with the common cultivar 'Jyoti'.

Table 6. Protein quality of barley

Protein value (%)	NP 113	Jyoti
TD	86.6	87.0
BV	75.8	67.5
NPU	65.6	58.7
UP	7.7	7.7

Nutritional feeding trials carried out with normal barley varieties and high- lysine genotypes have shown considerable improvement not only in BV but also in NPU. The TD of most barley varieties (Table 6) was found to be lower due to the presence of higher amount of crude fibre as compared with other cereal grains. The BV of 'Notch 2' barley has been shown to be comparable to that of high lysine 'Opaque 2' maize. The starch-protein adherence trait of the high-lysine lines does not effect BV, TD and NPU.

Although barley genotypes with substantially improved nutritional quality are available, barley varieties combining protein quality and high grain yield have not yet been developed, since in all genotypes increase in lysine is controlled by recessive genes.

C. Pearl millet

Pearl millet (bajra) is an important food crop of India next in production to maize and sorghum. The average protein content of Pearl millet grain is 11-6%. The embryo which constitutes only 2% of the total weight of grains contributes 10% of the total grain protein. Wide variations in the content of protein (8-20%), lysine (1-2 to 3.8% of protein) and tryptophan (0-7-1-7% of protein) have been observed. Further, in contrast to other cereal grains, the prolamine fraction of Pearl millet has high tryptophan concentration. However, in having a high concentration of glutamic acid and proline and a deficiency of lysine it resembles the prolamine of maize. Albumins and globulins contain a higher concentration of basic and sulphur-containing amino acids.

Nitrogen fertilization was found to enhance the prolamine content by about 40%. As a result, the additional protein synthesized was richer in tryptophan but poorer in lysine. Nutritional elevation has shown BV of pearl millet protein compared with normal maize and sorghum (Table 7).

Table 7. Protein quality of pearl millet varieties

Protein value (%)	NHB 5	BM46	BD111
TD	93.8	92.9	93.6
BV	58.2	59.2	59.6
NPU	54.5	55.1	55.8
UP	5.1	5.2	5.3

Being high in fat content (5%), Pearl millet poses problem during storage under high humidity. The higher proportion of unsaturated fatty acids in lipids leads to development of rancidity (off-flavour) due to oxidation of fats as a result the flour cannot be stored for longer one week.

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