AGRICULTURAL MICROBIOLOGY

M.Sc. MICROBIOLOGY

SEMESTER-II, PAPER-IV

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M.Sc. MICROBIOLOGY: AGRICULTURAL MICROBIOLOGY

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FOREWORD

Since its establishment in 1976, Acharya Nagarjuna University has been forging ahead in the path of progress and dynamism, offering a variety of courses and research contributions. I am extremely happy that by gaining 'A+' grade from the NAAC in the year 2024, Acharya Nagarjuna University is offering educational opportunities at the UG, PG levels apart from research degrees to students from over 221 affiliated colleges spread over the two districts of Guntur and Prakasam.

The University has also started the Centre for Distance Education in 2003-04 with the aim of taking higher education to the door step of all the sectors of the society. The centre will be a great help to those who cannot join in colleges, those who cannot afford the exorbitant fees as regular students, and even to housewives desirous of pursuing higher studies. Acharya Nagarjuna University has started offering B.Sc., B.A., B.B.A., and B.Com. courses at the Degree level and M.A., M.Com., M.Sc., M.B.A., and L.L.M., courses at the PG level from the academic year 2003-2004 onwards.

To facilitate easier understanding by students studying through the distance mode, these self-instruction materials have been prepared by eminent and experienced teachers. The lessons have been drafted with great care and expertise in the stipulated time by these teachers. Constructive ideas and scholarly suggestions are welcome from students and teachers involved respectively. Such ideas will be incorporated for the greater efficacy of this distance mode of education. For clarification of doubts and feedback, weekly classes and contact classes will be arranged at the UG and PG levels respectively.

It is my aim that students getting higher education through the Centre for Distance Education should improve their qualification, have better employment opportunities and in turn be part of country's progress. It is my fond desire that in the years to come, the Centre for Distance Education will go from strength to strength in the form of new courses and by catering to larger number of people. My congratulations to all the Directors, Academic Coordinators, Editors and Lesson-writers of the Centre who have helped in these endeavors.

Prof. K. Gangadhara Rao
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M.Sc. MICROBIOLOGY
SEMESTER-II, PAPER-IV
204MB24 - AGRICULTURAL MICROBIOLOGY
SYLLABUS
THEORY

UNIT-I

Rhizosphere - Microbial flora of rhizosphere soil, rhizosphere effect; root exudates; soil fungistasis. Techniques - soil plate, contact slide method, Fluorescence microscopy. Plant growth promoting rhizobacteria.

Phyllosphere microflora and their significance.

UNIT-II

Biofertilizers – Mycorrhiza - Ecto mycorrhizas and Arbuscular mycorhiza, Azatobacter and Azospirillum.

N - fixing cyanobacteria; Legume - Rhizobium association Nitrogenase, Rhizobia complex, cross-inoculation groups; Development, structure and functions of legume root nodules. Phosphate solubilizing microorganisms and their use.

UNIT-III

Concept of Disease in Plants; Symptoms caused by plant pathogenic fungi, bacteria and viruses. Symptomology, etiology, epidemiology and control of following plant diseases:

Late blight of potato, powdery mildew of cucurbits, smut of sorghum, tikka disease of groundnut, blast disease of rice, angular leaf spot of cotton and tobacco mosaic disease.

UNIT-IV

General Principles of Plant Disease Control - Plant quarantine, seed treatment, cultural practices, chemical control, development of disease resistance varieties; Biological control of plant diseases.

Biopesticides - Bacillus thuringiensis, NPV and CPV.

UNIT - V

Biostatistics - Basic principles; Measures of Central tendency - Mean, Median, Mode; Standard deviation and Standard Error; Simple hypothesis tests - Students 't' - test, 'F' - test and Chi - square test. Analysis of variance - one-way ANOVA and two-way ANOVA, Correlation and LinearRegression. Experimental designs Randomized Block Design (RBD) and Completely Randomized Design (CRD).

REFERENCE BOOKS:

1) Subbarao, N.S. 2000. Soil Microbiology 4th Edn.
2) Subbarao, N.S. 1995 Biofertilizers in Agriculture and Forestry.
3) Tilak, K.V.B.R. 1991. Bacterial Biofertilizers, ICAR Publications.
4) Atlas, R.M. and Bartha, R. 1998. Microbial Ecology: Fundamentals and Applications, Addison Wiesley Longman Publications.
5) Lynch and Poole, 1983 Microbial Ecology, ELBS Publications.
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(204MB24)

M.Sc. DEGREE EXAMINATION, MODEL QUESTION PAPER
MICROBIOLOGY - SECOND SEMESTER
AGRICULTURAL MICROBIOLOGY

Time: Three hours **Maximum: 70 marks**

Answer All Questions **5 × 14 = 70M**

UNIT-I

1) a) Give a brief account on microbial flora of rhizosphere soil and its effect.

OR

b) Write an account on plant growth promoting rhizobacteria and their significance.

UNIT-II

2) a) Write an account on importance of ecto and endo mycorrhiza as bio-fertilizers.

OR

b) Explain in detail about phosphate solubilizing microorganisms and their uses.

UNIT-III

3) a) Give an account on symptoms caused by plant pathogenic fungi.

OR

b) Explain about the causative agent, symptomology and control of late blight disease of potato

UNIT-IV

4) a) Write an account on plant quarantine and seed treatment methods to control plant diseases.

OR

b) Describe the biological control of plant diseases.

UNIT-V

5) a) Give an account on basic principles of biostatistics and measures of central tendency

OR

b) Describe in brief about Randomized Block Design and Completely Randomized Design for experimental setup.

CONTENTS

LESSON-1

MICROBIAL FLORA OF RHIZOSPHERE SOIL, RHIZOSPHERE EFFECT, ROOT EXUDATES, AND SOIL FUNGISTASIS

1.0. OBJECTIVE:

- This lesson gives a clear concept to the students about Microflora of Rhizosphere soil, Rhizosphere effect, root exudates and soil fungistasis.

STRUCTURE:

1.1. INTRODUCTION:

The rhizosphere is the narrow region of soil around the plant root that is influenced by several factors like the root exudates and the associated soil microorganisms. The rhizosphere is considered the most active region of soil as it receives the nutrients from the nutrients, in addition to the microorganisms that are present around the root. It is a dynamic environment fluctuating with the stages of root growth and senescence. Rhizosphere as a region was defined more than a century ago by Lorenz Hiltner as a soil compartment influenced by plant roots. The rhizosphere is an important part of soil microbiology which is responsible for various metabolic processes occurring in the soil like cycling of nutrients and uptake of carbon. The roots of crop plants create an interface between the plant and the soil environment, thus establishing an enormous reservoir of the microbial community. The area of rhizosphere usually extends a few millimeters from the root surface where the roots release various compounds like root exudates, mucilage, and sloughed-off root cells that support higher microbial populations and activities than in bulk soil.

1.2. MICROBIAL FLORA OF RHIZOSPHERE SOIL:

The microbial population in the rhizosphere consists of different groups of microorganisms like bacteria, fungi, parasites, viruses, and algae. The microbial population in the rhizosphere is known as the rhizosphere microbiome and the microbial population in such

an area much higher than the bulk soil (Fig. 1.1). In the rhizosphere, there is a microbial population distinct from the rest of the soil. Bacteria in the rhizosphere are larger and have higher proportions of Gram-negative and denitrifying bacteria than those in the bulk soil. Rhizosphere fungal populations, abundant in both pathogenic and mycorrhizal species, can be 10 to 20 times higher than those in the non-rhizosphere. Protozoa and other microfauna also thrive in the rhizosphere because that is where food is most plentiful. The type and population of microorganisms in the rhizosphere are highly influenced by the type of plant grown on the soil. Microbes in the bulk soil often experience long periods of nutrient deprivation; they have different survival strategies in dealing with starvation and stress. The rhizosphere bacterial community is recruited from the main reservoir of microorganisms present in the soil. Next to the recruitment of specific soil microbes into the rhizosphere microbiome, plant roots also influence specific functions of the microbiome. Some of the examples of microorganisms found in the rhizosphere region include *Bacillus, Arthrobacter, Pseudomonas, Agrobacterium, Alcaligenes, Clostridium, Flavobacterium, Corynebacterium, Micrococcus, Xanthomonas, Amanita, Tricholoma, Torrendia, Descomyces, Thelephora, Verticillium, Phytophthora, Rhizoctonia, Micromonospora, Thermoactinomycetes, Amycolaptosis, Actinomadura,* etc.

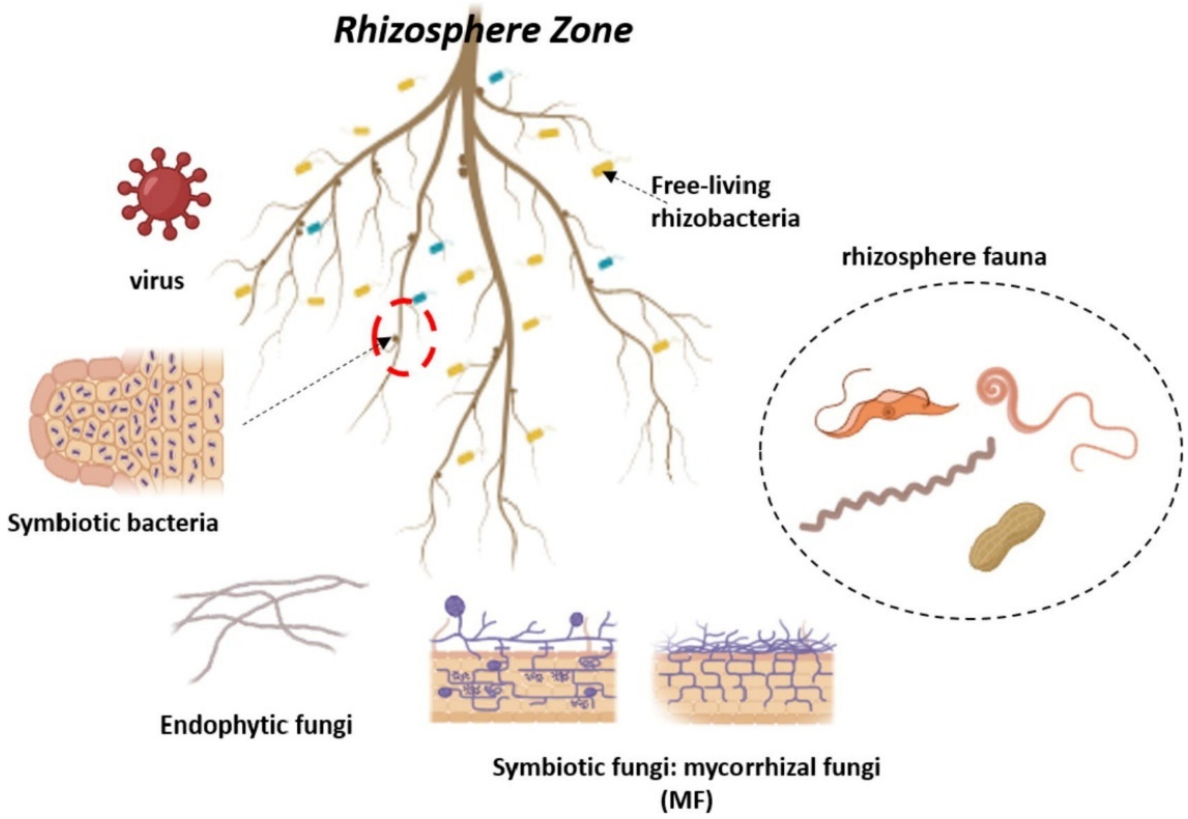

Fig. 1.1: Microbial Flora of Rhizosphere Soil

Microbiology of Rhizosphere:

The soil surrounding the plant root where root exudate migrates and microbiological activity is exceptionally high is called rhizosphere. The surface of root is called rhizoplane. Plant root produce and release various exudates containing sugar, amino acids, organic acids,

fatty acids, vitamins, nucleotides and other organic matters that promotes growth of microorganisms. Therefore, rhizospheric soil is characterized by greater number of microorganisms than soil away from plant roots. The intensity of rhizospheric effects depends on the distance to which root exudates can diffuse. The number of microorganisms decreases continuously as the distance from the plant root increases. The term rhizosphere to soil ratio (R:S) indicates number of microbes in rhizospheric soil divided by number of microbes in soil free of plant root. R:S ratio is greater for bacteria (20:1) and less for fungi and actinomycetes. Effects of rhizosphere are almost negligible for algae and protozoa. It is because algae are photosynthetic and do not depends upon organic matter present in root exudates. On the other hand, most, bacteria cannot utilize relatively resistant to organic matter of soil and depends on easily available decomposable matter of root exudates. Therefore, number of bacteria is exceptionally high in rhizosphere.

Examples of Rhizospheric Microorganisms:

Large number of bacteria, fungi and actinomycetes are found in rhizosphere.

i) **Bacteria:** Many nitrogen fixing bacteria and phosphate solubilizing and other bacteria are found in rhizosphere. For example: *Pseudomonas, Arthrobacter, Azotobacter, Agrobacterium, Flavobacterium, Cellulomonas, Rhizobium, Clostridium* etc.

ii) **Fungi:** Some fungi are found associated with root forming mycorrhiza and other occurs freely in soil. For example: *Marticella, Cephalosporium, Tricoderma, Penicillium, Gliodadium, Gliomastix, Fusarium* etc.

iii) **Actinomycetes:** *Frankia, Derxia* etc

Factor Affecting Rhizospheric Microorganisms:

Various factors affect the rhizospheric microbes and some of them are -

i) **Proximity of soil to root:** The number of rhizospheric organisms is greater near the root and their number continuously decreases with increase in distance from the root. It is because concentration of organic matter released by root in exudates decreases with increases in distance from the root.

ii) **Temperature and light intensity:** Low temperature and low light intensity decreases the rate of exudate secretion from the root so that number of rhizospheric organisms decreases. On the other hand, number of microbes in rhizosphere increases when temperature and light intensity increases as multiplication rate is high.

iii) **Type of soil:** Type of soil also influences rhizospheric effects. For example; R: S ratio is very high in sandy soil and low in clay soil. It is because sandy soil contains very little or no organic matter and the root region is the only place where organics matter is available and microorganisms can grow. Therefore, number of microbes is high around root in sandy soil. On the other hand, in fertile soil such as clay soil, organic matter is sufficiently available for growth, so microorganisms need not to depend on the root exudates for growth.

iv) **Age of Plant:** With age of plant, rate of exudates secretion is altered so that number of rhizospheric microbes' changes.

v) **Types of Plant and Location of Root:** Location of root affects number of rhizospheric microbes. Root cap and regions of root from where lateral root arises are primary sites of exudate secretion. Therefore, number of microbes is comparatively high around these locations. Amount and type of exudates secretion differs with species of plant that influences growth of rhizospheric microbes. For example; some plant root release antimicrobial chemicals such as glycosides, hydrocyanic acids and several antifungal agents that inhibits rhizospheric microbes.

vi) **Depth of Root:** In general number of rhizospheric microorganisms decrease with increase in depth of root, which is mainly due to anaerobic condition.

vii) **Root Respiration:** Plant root release carbon-dioxide during respiration that makes the soil acidic. Acidity of soil decrease number of rhizospheric bacteria.

viii) **pH of Soil:** pH of rhizosphere become acidic due to root respiration and by oxidation of sulphur caused by *Thiobacillsu* spp. Acidification of rhizospheric soil decrease number of microorganisms.

ix) **Pesticides and Antibiotics:** Spray of pesticides and antibiotics on agriculture crops decreases the number of rhizospheric organisms.

Role of Rhizospheric Microbes:

Rhizospheric microorganisms are important for plant growth. They promote plant growth by various ways as given below; some rhizospheric bacteria such as Rhizobium, *Azotobacter*, *Clostridium* etc. fix atmospheric nitrogen and make it available for plant gowth. Many phosphate solubilizing microbes such as *Bacillus polymyxa* found in rhizosphere release free phosphate from inorganic salt of phosphate. Free phosphate is important nutrient for plant growth. Several rhizospheric microbes (*Azotobacter*, *Arthrobacter*, *Pseudomonas*, *Agrobacterium*) produce growth hormone such as Gibberllin, Indole acetic acid (IAA) etc that promote plant growth. Many rhizospheric fungi are associated with plant root in the form of mycorrhiza. Mycorrhizal fungi promote plant growth by various ways. Rhizospheric microbes induce development of lateral root, root hairs development and mucilage secretion from plant root. Some rhizospheric microbes produce antibiotics and other antimicrobial chemicals that inhibit plant pathogens. Some time it may inhibit beneficial N_2 fixing and phosphate solubilizing bacteria. Microorganisms also increase rate of exudate secretion. Exudate secretion from plant root helps in formation of soil aggregate that improve soil fertility. Some rhizospheric microbes e.g. Pseudomonas produces Siderophore. Siderophore is a chelating agent that tightly binds iron and makes it unavailable for growth of pathogenic microorganisms.

Effect of Plant Root on Rhizospheric Microbes:

Plant root usually promote growth of rhizospheric microbes. Sometimes plant root gives minor unwanted effect to microorganism. Some of them are;

- Plant root produce exudate containing carbohydrate, amino acids, nucleotide, vitamins etc. that serves as food for growth of rhizospheric microbes.

- Some plant root produces antimicrobial chemicals such as glycosides, hydrocyanic acids and antifungal agents that inhibit growth of rhizospheric microorganisms.
- Plant root release CO2 during respiration that make habitat acidic and anaerobic.
- Some plant root produce chemicals that bring fungistasis. Fungistasis is referred to the inability of spore to germinate. For e.g. root of Allium produce alkylcystein sulfoxide that inhibit germination of sclerotia (spore) of *Sclerotium capivarum*.

1.3. RHIZOSPHERE EFFECT:

The rhizosphere effect is the influence of plant roots on the development of soil microorganisms as a result of the physical and chemical alteration of soil and the release of root secretions and exudates within the rhizosphere. The rhizospheric effect is observed on the basis of the microbial biomass of the rhizosphere when compared to the biomass of the bulk soil. The rhizosphere effect on soil microbial population can be measured by comparing the population density [colonies forming units (CFU)] between the rhizosphere soil (R) and the bulk soil (S), for which the "R/ S ratio" is employed. The rhizosphere effect is higher for bacteria > fungi > actinomycetes > protozoa. The microorganism diversity is higher near to the rhizoplane, which then decreases with an increase in distance from the rhizoplane. The interaction between plant nutrients in soil and plant exudates modifies the microclimate of the rhizosphere. The rhizosphere effect is a result of the interaction between the plant root and the microbial community of the region, where both factors influence each other. In the rhizosphere, microbial activity influences the plant root, and the plant root secretions influence the microbial biomass.

1.4. ROOT EXUDATES:

Root exudates (REs), a diverse array of bioactive metabolites secreted by plant roots in response to environmental stimuli, serve as key mediators of rhizosphere ecology and plant defense responses, offering a promising avenue for sustainable pest management and eco-friendly plant protection. While earlier reviews primarily focus on root exudates in the context of general rhizosphere dynamics and plant-microbial interactions, critical knowledge gaps persist in REs-plant-pest tripartite interactions, the mechanistic basis of REs-mediated plant defense, and their practical integration with integrated pest management (IPM) frameworks. This review provides a synthesis of the latest literature on the biochemical diversity and functions of REs, their environmentally-driven exudation dynamics, and their roles in induced systemic resistance (ISR) in plants and disrupting pest communication and development. Furthermore, we highlight their translational potential-including advances in RE-inspired green pesticide development, and emerging strategies that employ beneficial microorganisms to modulate REs profile for enhanced plant protection. By integrating these insights, this review underscores the potential of REs to redefine modern pest management strategies. We advocate for interdisciplinary research to further explore the ecological and evolutionary roles of REs, ultimately contributing to more resilient and sustainable agricultural systems (Fig. 1.2).

Root exudates, acting as substrates and signaling molecules for microbes, are another critical factor modulating the assembly of the rhizosphere microbiome. On the one hand, root exudates are known to have specialized roles in plant–plant communication. On the other hand, root exudates are the key regulators in plant-microbe cross-talk, and can modify both biological and physical interactions between roots and soil microorganisms by mediating

various positive and negative plant-microbe interactions. Root exudates, particularly those containing specific secondary metabolites, play crucial roles in shaping the rhizosphere microbiome by recruiting or repelling different community members. Sun et al. demonstrate that the root exudates of *Flaveria bidentis* could significantly increase the abundances of *Bacillus frigoritolerans* and *Bacillus megaterium* and promote their nitrogen-fixing and phosphate-solubilizing abilities, which further increases soil available phosphorus and nitrogen levels and promotes the invasiveness of *F. bidentis*. Yang et al. indicate that root exudates of *Rehmannia glutinosa* could stimulate the proliferation of *Fusarium oxysporum*, which alters the expression patterns of Leucine-rich repeat receptor-like protein kinases (RgLRRs), disorders the growth and development of *R. glutinosa*, and finally results in the formation of replant disease. However, intercropping with *Achyranthes bidentata* alleviates *Rehmannia glutinosa* replant disease by modulating root exudates and improving the rhizosphere microenvironment. Furthermore, An et al. indicate that alfalfa cultivars affect rhizosphere microbial biomass and community composition. Ultimately, the effects of plant domestication, plant genotype, plant development stage, and plant compartment on the assembly of rhizosphere microbiomes have also been reported to be associated with the changes in root exudation profile. Therefore, a deeper understanding of the spatiotemporal dynamics of root exudates is vital in disentangling the chemical communication between plants and microbes and modulating the rhizosphere microbiome for plant fitness and sustainable agriculture.

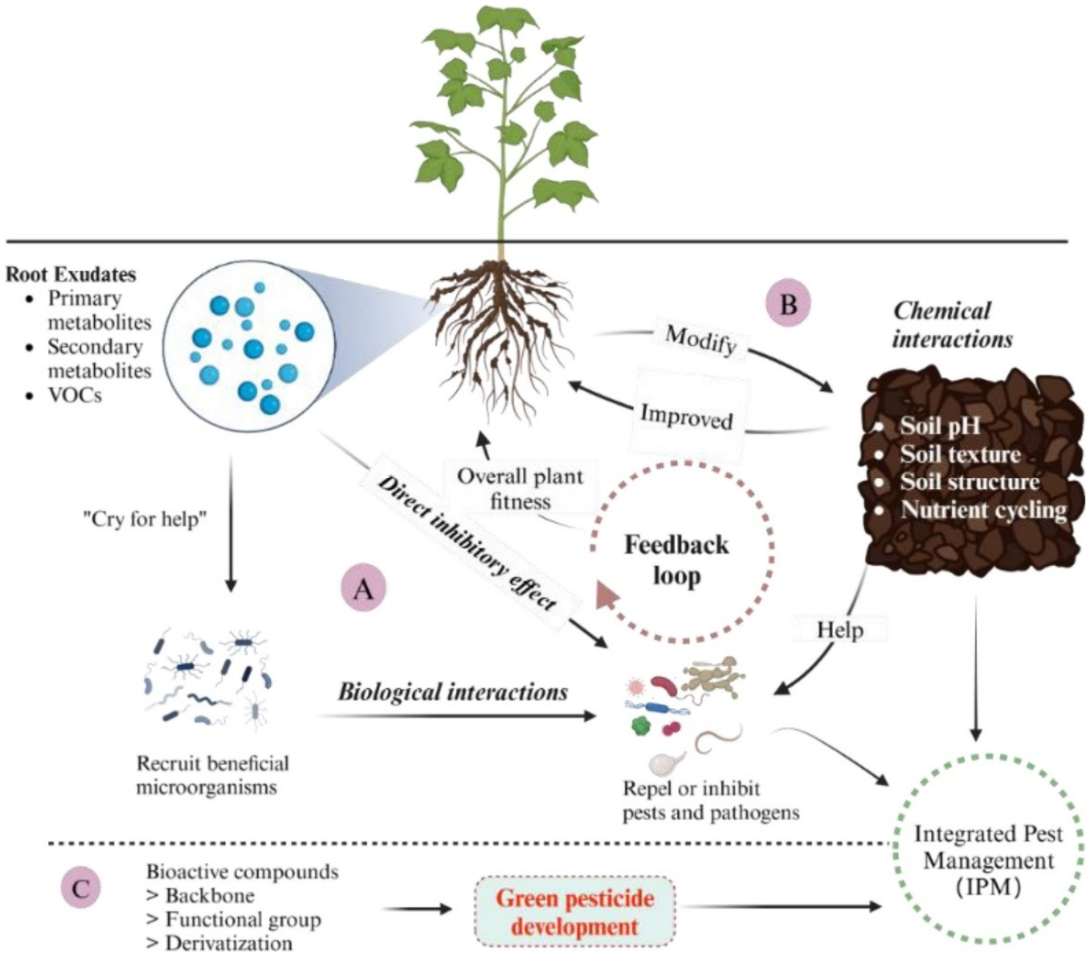

Fig. 1.2: Illustration of Root exudates (REs)-mediated interactions in the rhizosphere for integrated pest management IPM. (A) REs consist of a diverse array of primary and secondary metabolites (B) REs modify soil properties, enhance nutrient uptake, and create

conditions unfavorable to harmful microbes. (C) REs serves as a valuable source for green pesticide development due to their bioactive components and diverse chemical structures.

1.5. SOIL FUNGISTASIS:

Soil fungistasis is the widespread, natural inhibition of fungal spore germination and hyphal growth in soil, caused primarily by microbial activity and nutrient limitation. It acts as a defense mechanism limiting pathogen activity, often reversed by adding nutrients or root exudates. This phenomenon is stronger in neutral/alkaline soils and higher in topsoil.

Fungistasis in soil is a widespread phenomenon affecting most fungal propagules, though some are insensitive. In most instances, it is coexistent with the presence of living microorganisms, and is annulled by energy-yielding nutrients. Fungistasis with characteristics similar to that in soil may also occur on leaves of plants. Germination and growth of bacteria and actinomycetes is also restricted in soils. The characteristics of their inhibition appear to be the same as those for fungi. Therefore, the concept of a widespread microbial inhibition in soil can be applied to all three groups of microorganisms. Fungistasis can be detected by various direct methods, or indirectly by methods involving the use of porous or permeable carriers. It may be expressed as a restriction on the final amount of germination (the usual parameter), germination rate (with time), and rate of germ-tube or hyphal growth. Since the expression of fungistasis is often complete in soil, titration with nutrients may be required to distinguish between the sensitivities of different fungi. Fungistasis generally is expressed most strongly at soil moisture contents somewhat less than saturation. Its expression usually is maximal in neutral or slightly alkaline soils. In acidic conditions fungistasis may be lessened because of suppression of bacterial and actinomycete activity. Increased sensitivity of some fungi in soils of pH > 7.0 may be caused by a directly unfavorable effect of pH on the fungus. Fungal species with small spores tends to be highly sensitive to fungistasis. These spores tend to germinate slowly and to require exogenous nutrients for germination. By contrast, species with larger spores and sclerotia often do not require exogenous nutrients for germination. The larger spores tend to germinate rapidly and to exhibit low sensitivity, as compared with small spores. A few nutrient-independent spores are insensitive to fungistasis. At least a part of the difference in sensitivity is related to germination time; spores which germinate slowly compete poorly with the soil micro-flora for their nutrients. Fungistasis is often temporarily annulled by enriching the soil with energy-yielding nutrients. Usually, complex materials such as plant residues are most effective. A few weeks after such treatment, the level of fungistasis may, however, be increased. Annulment of fungistasis by compounds not utilized as energy sources has not yet been demonstrated. Several soils naturally suppressive to *Fusarium* wilt diseases were more fungistatic to *Fusarium* than soils conducive to wilt. Potential means by which fungistasis may be manipulated to control root-infecting fungi are (*a*) through stimulation of germination with nutrients, thus exposing the germ tube to lysis, and (*b*) by increasing the fungistatic level of soil through appropriate amendments.

Volatile substances identified in soils, some of which are potentially inhibitory to fungi include (*a*) ammonia, which apparently is evolved from ammonium salts in some arid soils of high pH, (*b*) ethylene, which has been identified in some soils of pH < 7.0 (though high levels of this gas seem to be tolerated by most fungi), (c) allyl alcohol, and (*d*) other unidentified substances. Non-volatile inhibitors include high molecular weight substances revealed by molecular sieve chromatography of soil extracts. Microbial metabolites such as those present in staled fungal cultures also have been proposed to account for fungistasis. In a few soils fungistasis persists after sterilization because of the presence of inhibitory concentrations of calcium carbonate, iron or aluminium. Inherent in the proposition that inhibitory substances provide the primary mechanism of fungistasis is the concept of a highly complex phenomenon, involving various highly specific inhibitory and counteracting stimulatory substances, with the outcome for the fungus depending on the kinds and relative amounts of each present. By the nutrient-deficiency hypothesis, the level of available nutrients in soil is insufficient to support germination of nutrient-dependent propagules, except in nutrient-rich microsites. Inhibition of nutrient-independent propagules is explained by loss of endogenous nutrients required for germination, through microbial nutrient competition. Evidence for this hypothesis is (*a*) the imposition of fungistasis on numerous nutrient-independent propagules during incubation on leaching model systems designed to simulate microbial nutrient competition in soil, (*b*) similar losses of endogenous nutrients occurring on soil and the leaching system, and (*c*) the fact that soils are chronically deficient in energy in relation to the microbial populations present, with the consequence that enforced inactivity is imposed upon most of the population at any given time for this reason alone, regardless of the presence or absence of fungistatic substances.

Aspects of Soil Fungistasis:

A temporary, non-lethal inhibition of fungal growth, also affecting bacteria and actinomycetes. It allows propagules to survive long-term. Microorganisms in the soil consume nutrients, creating a nutrient-deficient environment for fungal spores. Active microbial production of inhibitory substances, including volatiles like benzaldehyde, acetamide, and benzothiazole. Microbial activity generally disappears upon soil sterilization. Soil depth is stronger in topsoil layers. Most effective at moisture levels below saturation. Often observed to be higher during rainy seasons. Functions as a form of "general suppression" of soil-borne diseases. Fungistasis is annulled by adding nutrients (e.g., glucose) or root exudates from plants.

Positive Effect of Rhizospheric Microorganisms on Plants:

Rhizospheric microorganisms play an important role in the ecological fitness of the plant and the soil. Important microbial processes like plant protection, growth promotion, production of antibiotics, geochemical cycling, and plant colonization take place in the rhizosphere. Rhizospheric microorganisms increase the supply of mineral nutrients from the

soil to the plant. Another group of microorganisms in the rhizosphere stimulate plant growth indirectly by preventing the growth or activity of plant pathogens. These microorganisms are responsible for direct growth promotion by the production of phytohormones. Plant growth-promoting rhizobacteria act as biofertilizers by enhancing phytochrome production, phosphate solubilization, and siderophore production. The capacity of rhizospheric organisms to synthesize anti-fungal metabolites such as antibiotics, fungal cell wall-lysing enzymes, or hydrogen cyanide suppresses the growth of fungal pathogens.

1.6. SUMMARY:

The rhizosphere is considered the most active region of soil as it receives the nutrients from the nutrients, in addition to the microorganisms that are present around the root. It is a dynamic environment fluctuating with the stages of root growth and senescence. The soil surrounding the plant root where root exudate migrates and microbiological activity is exceptionally high is called rhizosphere. The surface of root is called rhizoplane. Plant root produce and release various exudates containing sugar, amino acids, organic acids, fatty acids, vitamins, nucleotides and other organic matters that promotes growth of microorganisms. Rhizospheric microorganisms are important for plant growth. They promote plant growth by various ways as given below; some rhizospheric bacteria such as *Rhizobium, Azotobacter, Clostridium* etc. fix atmospheric nitrogen and make it available for plant gowth. Many phosphate solubilizing microbes such as Bacillus polymyxa found in rhizosphere release free phosphate from inorganic salt of phosphate. Rhizospheric microorganisms play an important role in the ecological fitness of the plant and the soil. Important microbial processes like plant protection, growth promotion, production of antibiotics, geochemical cycling, and plant colonization take place in the rhizosphere. Rhizospheric microorganisms increase the supply of mineral nutrients from the soil to the plant.

1.7. TECHNICAL TERMS:

Rhizospheric microorganisms, *Bacillus polymyxa, Rhizobium, Azotobacter, Clostridium,* fix atmospheric nitrogen, Root exudates (REs).

1.8. SELF-ASSESSMENT QUESTIONS:

1) Define Rhizosphere? Explain Different types of Microflora of Rhizosphere soil?
2) Add a note on Rhizosphereic effect?
3) Write a short note on Root exudates?
4) Explain about Soil fungistasis?

1.9. SUGGESTED READINGS:

1) Subbarao, N.S. 2000. Soil Microbiology 4th Edn.
2) Subbarao, N.S. 1995 Biofertilizers in Agriculture and Forestry.
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Prof. A. Amruthavalli

LESSON-2

TECHNIQUES-SOIL PLATE, CONTACT SLIDE METHOD, AND FLUROSCENCE MICROSCOPY

2.0. OBJECTIVE:

- This lesson deals with concept of Soil plate method, contact slide method and Fluroscence microscopy.

STRUCTURE:

2.1. INTRODUCTION:

Soil is variable environment with diver's microbial community consists of bacteria, actinomycetes, molds, yeast, algae and protozoa. Necessary to use different types of culture media due to differences in dietary requirements for each type of microorganism to be isolate. The ability to view soil microbes *in situ* is important since it allows students to view the interrelationships between soil microbes and their interactions with soil particles. However, it is difficult to observe colloidal size microbes that exist within soil. A technique developed back in the 1930s is still a valuable learning tool today. This is the contact slide or buried-slide technique of Rossi et al. (1936), which is a simple technique for qualitatively assessing the spatial relationships between soil microorganisms.

2.2. SOIL PLATE METHOD:

Soil is variable environment with diver's microbial community consists of bacteria, actinomycetes, molds, yeast, algae and protozoa. Necessary to use different types of culture media due to differences in dietary requirements for each type of microorganism to be isolate. **Note:** Culture Media used the following, according to the type of microorganisms to be isolated: 1. Enumeration of bacteria used Nutrient agar. 2. Enumeration of Actinomycetes used Jensen's medium, characterized Actinomycetes are isolated in dishes as dry and dusty or chalky. Also characterized dishes distinctive odor similar to odor earth after rain. 3. Enumeration of fungi used sabouraud medium. There are two main methods of direct plate counting (Fig. 2.1): spread plate method and pour plate method: 1. The spread plate method consists of evenly spreading the diluted sample over an agar plate. Using this method yields

colonies that form on the surface of the agar. Procedure: 1. Make serial dilution of microorganism sample in series of tubes containing D.W. 2. Transfer 0.1ml from last dilution of microorganism culture by pipette. 3. Put it on the center of an agar plate. 4. Moist spreader with alcohol and sterilize by flaming. 5. Spread the sample on agar plat by spreader. 6. Sterilize it again. 7. Incubate the plate at 37°c for 24 hours, and then examine and count the present colonies distributed throughout the agar.

1) Count plates which show only about 30-300 colonies.
2) Used colony counter to enumerate the colonies. Determine No. of bacterial cells in soil sample from equation: No. of bacterial cells /1ml= No. of colonies × inverted dilution ×10
3) The pour plate method, a volume of 1 ml of the diluted sample is put into a sterile petri plate, and then melted agar is poured in and mixed with the sample. This method yields colonies that form colonies throughout the agar (growing both on the agar and in the agar, not just on the surface.
4) Procedure: 1. Put agar medium in water bath at 45°c. to liquefy. 2. Add 1gm of sample to first tube and make serial dilution from one to another tube. 3. Transfer 1ml from last dilution of microorganism culture by pipette, and then put in sterile petri dish. 4. Pour melted agar and mixed with the dilution sample. 5. Leave petri dish to solidify. 6. Incubate the plate at 37°c for 24 hour Determine No. of bacterial cells in soil sample from equation: No. of bacterial cells /1gm moist soil =No. of colonies × inverted dilution. No. of bacterial cells /1gm dry soil = No. of colonies ×inverted dilution Dry weight of 1gm soil sample the unit of measurement here (CFU) Colony forming unit where the colony may be the yields of the growth and multiplication of a single cell or more.

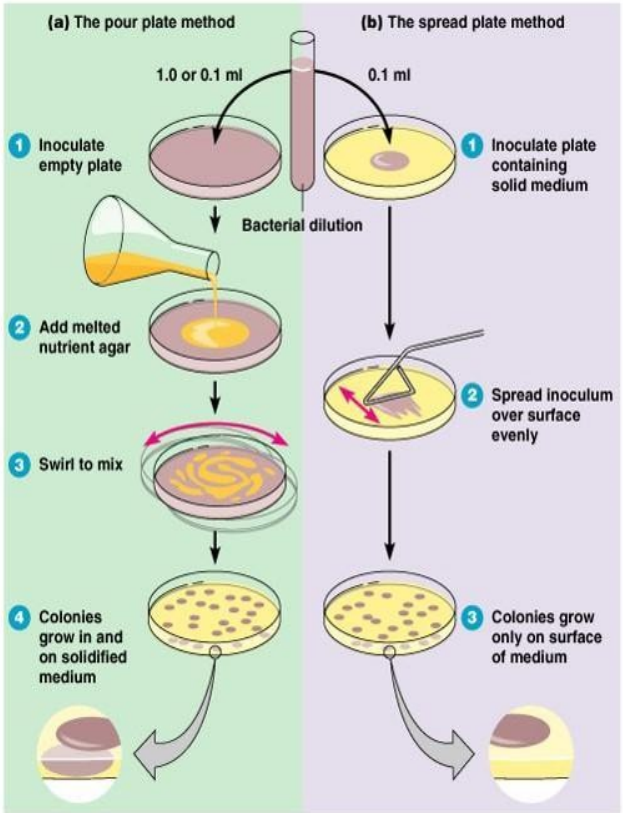

Fig. 2.1: Pour Plate and Spread Plate Methods

2.3. CONTACT SLIDE ASSAY:

The ability to view soil microbes *in situ* is important since it allows students to view the interrelationships between soil microbes and their interactions with soil particles. However, it is difficult to observe colloidal size microbes that exist within soil. A technique developed back in the 1930s is still a valuable learning tool today. This is the contact slide or buried-slide technique of Rossi et al. (1936), which is a simple technique for qualitatively assessing the spatial relationships between soil microorganisms. It is useful to illustrate the orientation of soil organisms to one another and to soil particles. It also allows students to see bacteria, actinomycetes, fungi and spores; the technique involves burying a glass slide in soil for a defined period of time (Figure 2.2 A-E). Nutrient amendments, such as the carbon source glucose and the nitrogen source ammonium nitrate, encourage the rapid proliferation of heterotrophic microorganisms.

After removing the slide from within the soil, the slide is fixed with acetic acid and stained to provide contrast, as the often-colorless organisms would otherwise not be visible under a microscope.

Viewed under a microscope, soil bacteria, actinomycetes, and fungi can be seen growing on soil particles, in pure colonies on the slide, and in juxtaposition to each other, often with bacteria lining the fungal hyphae.

1) Adjust soil moisture to a value close to “field capacity” (value provided by instructor).
2) Insert glass slides into a beaker of moist soil.
3) Incubate for one week.
4) Remove slides, stain with phenolic Rose Bengal.
5) View under microscope

Materials-I:

300 g of each soil, 1% glucose, NH_4NO_3, 2 polystyrene cups for each soil type, volume 250ml, Label tape and pens, Plastic wrap, four microscope slides for each soil type, Rubber bands, weighing paper, Deionized water in a wash bottle Analytical balance and benchtop balance (±0.01 g) Graduated cylinder.

Methodology-I:

1) Weigh out 150 g portions of each soil into two cups, recording the mass of the soil you added to each cup. Label one cup as “treatment” and the other as “control.” A 100 g sample of soil should be used for soils high in organic matter, as they are less dense than mineral soils.

2) Calculate the amount of moisture necessary to alter the moisture content of the soil samples to the moisture content specified by your instructor. This soil moisture content is often close to field capacity. Measure out this much-distilled water with a graduated cylinder and add it to each of two vials. Label one vial “treatment” and the other “control.”

3) Amend the water in the treatment vial with enough glucose for a final soil glucose concentration of 1% (w/w) on a dry weight basis in the treatment soil above. Also add 200mg of NH_4NO_3 to the treatment vial. Stir to dissolve the amendments. Do not amend the control vial.

4) Mix the contents of the treatment and control vials into their respective Cups by adding the liquid to the soil in small aliquots, and mixing with a spatula after each moisture addition. For heavy textured clay, soils avoid mixing, as this will "puddle" the soil.

5) For each cup, label two clean microscope slides, designating the soil and treatment for that slide. There will be two slides for each cup. Insert each slide vertically into its respective cup, leaving 2 cm of each slide projecting above the soil surface. Do not force the slides as they will break.

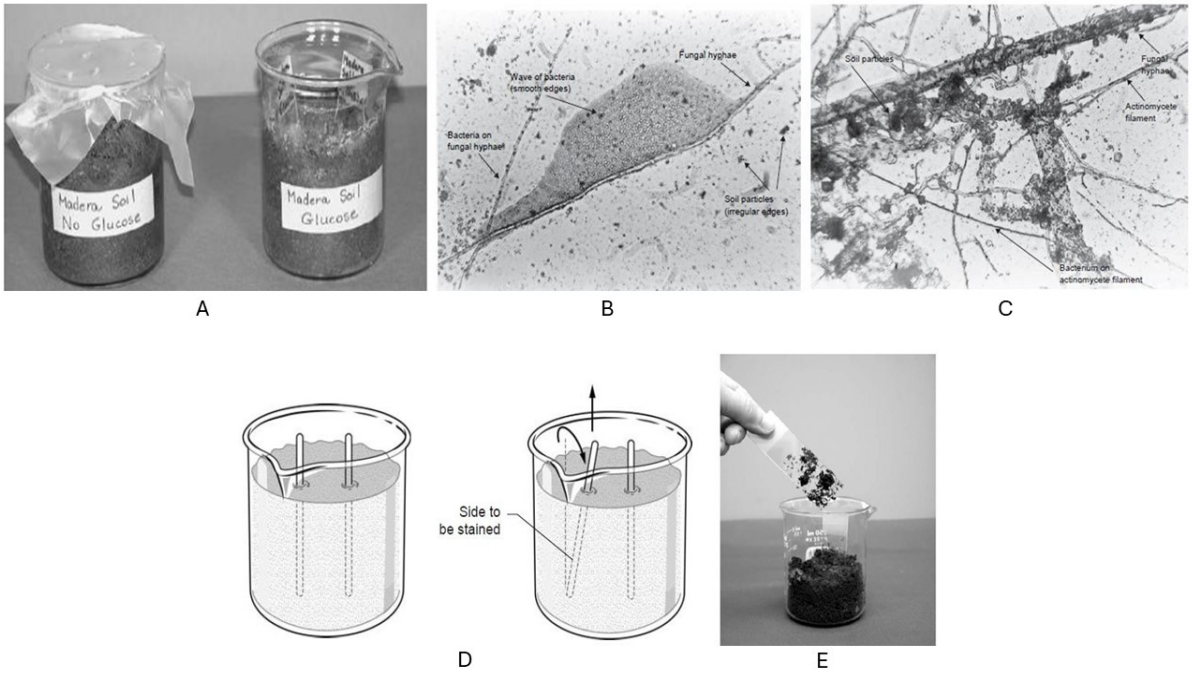

Fig. 2.2: Mechanism and Steps Involved in the Contact Slide Method

Cover the cups with plastic wrap, securing with a rubber band. Puncture the wrap or foil several times with a probe to allow air in and yet preclude excessive evaporation of moisture. Weigh each cup. Incubate the soil-filled cups at room temperature in a designated incubator for one week.

Materials-II:

Incubated cups from Period 1, 40% (v/v) acetic acid, Phenolic Rose Bengal stain, Staining racks with a pan to catch excess stain, Protective goggles, Microscopes, Immersion oil and paper towels.

II. Methodology:

1) Re-weigh the cup and calculate the soil moisture at the time of slide removal.

2) Remove the two slides from each cup after seven days by pressing each slide to an inclined position and withdrawing in a manner such that the upper face of the slide is not disturbed. Mark and identify the side to be stained

3) Gently tap the slide on the bench top to remove large soil particles from the slide surface. Clean the lower face with a damp paper towel and dry the slide at room temperature.

4) Wearing protective goggles immerse the slide in 40% (v/v) acetic acid for 1–3 min under a fume hood, holding the slide with forceps.

5) Wash off the excess acid under a gentle stream of water, and cover the surface with phenolic Rose Bengal from a dropper bottle, supporting the slide on a staining rack over a container to catch the excess stain. Be careful not to wash with such force as to remove microorganisms from the slide surface.

6) Stain for 5–10 minutes, but do not permit the slide to become dry. Add more stain as needed.

7) Gently wash the slide to remove excess stain. Dry and examine the slide microscopically using the oil immersion objective. Compare what you see with.

2.4. FLUORESCENCE MICROSCOPY:

A fluorescence microscope is similar to a regular light microscope, but it has several extra qualities that enhance its usefulness (Fig. 2.3). The typical microscope magnifies a sample using visible light (400-700 nanometers). On the other hand, Fluorescence microscopes use high-intensity light to stimulate fluorescent organisms in a sample. This fluorescent species emits a longer- wavelength, lower-energy light that magnifies the image. Therefore, a fluorescence microscope is an optical microscope that studies the properties of organic or inorganic substances by using fluorescence and phosphorescence instead of or in addition to reflection and absorption.

Fluorescence was first discovered in 1845 by Fredrick W. Herschel. However, the first working fluorescent microscope was developed by Oskar Heimstaedt in 1911.

Parts of Fluorescence Microscope:

Fluorescence microscope components includes

1) **Fluorescent Dyes (Fluorophore):** A fluorophore is a fluorescent chemical compound that can re-emit light upon light excitation. Fluorophores typically contain several combined aromatic groups, or plane or cyclic molecules with several π bonds. Many fluorescent stains have been designed for a range of biological molecules. Some are fluorescent tiny compounds that bind a biological molecule. Nucleic acid stains like DAPI and Hoechst, and phalloidin are examples.

2) **A Light Source:** Four main types of light sources are used, including xenon arc lamps or mercury-vapor lamps with an excitation filter, lasers, and high- power LEDs. Complex fluorescence microscopy techniques require lasers, while wide-field epifluorescence microscopes use xenon lamps, mercury lamps, and LEDs with a dichroic excitation filter.

3) **The excitation filter:** Typically, the exciter is a bandpass filter that transmits only the wavelengths absorbed by the fluorophore, reducing the excitation of other fluorescence sources and blocking excitation light in the fluorescence emission band.

4) **The dichroic mirror:** A dichroic filter or thin-film filter is a very accurate color filter used to selectively pass light of a small range of colors while reflecting other colors.

5) **The emission filter:** The emitter is typically a bandpass filter that passes only the wavelengths emitted by the fluorophore and blocks all undesired light outside this band – especially the excitation light. By blocking unwanted excitation energy (including UV and IR) or sample and system autofluorescence, optical filters ensure the darkest background.

Fig. 2.3: Structure of Fluorescence Microscope

Working Principle of Fluorescence Microscope:

Most cellular components are colorless and cannot be clearly distinguished under a microscope. The basic premise of fluorescence microscopy is to stain the components with fluorescent dyes, also known as fluorophores or fluorochromes, which are molecules that absorb excitation light at a given wavelength (generally UV), and after a short delay emit light at a longer wavelength. To observe the sample through a fluorescence microscope, it should first be labeled with fluorescent dyes/substances known as fluorophores. Higher energy shorter wavelength lights (UV rays or blue light) generated from xenon arc lamp or mercury vapor arc lamp passes through the excitation filter. The excitation filter allows only the short wavelength of light to pass through and removes all other non-specific wavelengths of light. The filtered light is reflected by the dichroic filter and falls on the fluorophore-labeled sample. The fluorochrome absorbs shorter wavelength rays and emits rays of longer wavelength (lower energy) that pass through the emission filter. The emission filter blocks

(suppresses) any residual excitation light and passes the desired longer emission wavelengths to the detector (Fig. 2.4). Thus, the microscope forms glowing images of the fluorochrome-labeled microorganisms against a dark background.

To the observer, the background is dark, as there is no visible light and only the labeled specimen (cells, microorganisms, etc.) appear bright (fluoresce).

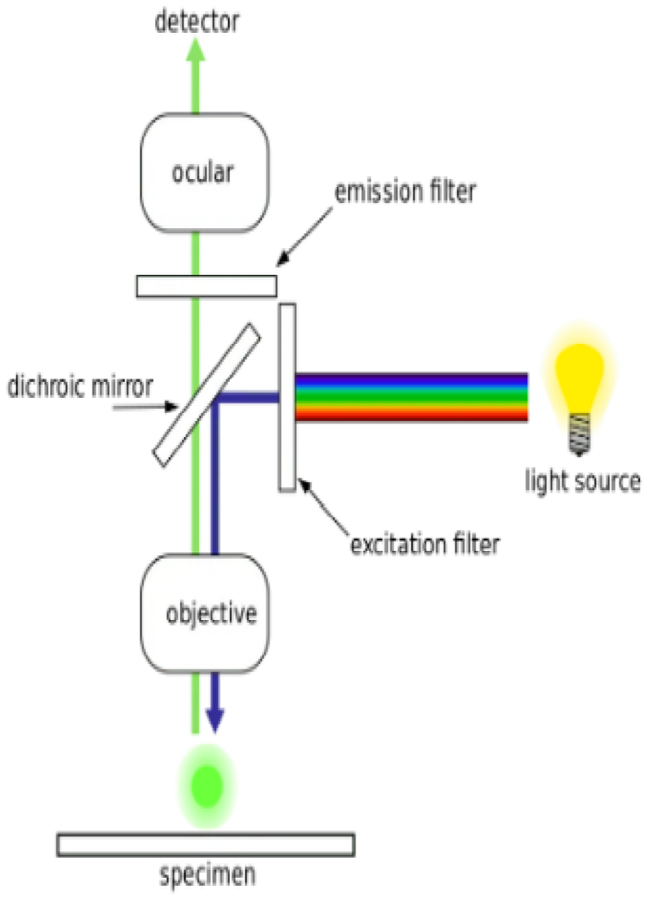

Fig. 2.4: Working Principle of Fluorescence Microscope

Types of Fluorescence Microscope:

Fluorescence microscopy is one of the most used imaging modalities in molecular biology and living specimens. To increase image contrast and spatial resolution, different type of fluorescence microscopy has been developed. However, there are 4 main types of fluorescence microscopy:

a) **Epifluorescence Microscopes:** It is the most common type of fluorescence microscope. In this microscope, excitation of the fluorophore and detection of the fluorescence are done through the same light path (i.e., through the objective).

b) **Confocal Microscope:** In this type of fluorescence microscope, high-resolution imaging of thick specimens (without physical sectioning) can be analyzed using fluorescent-labeled dye.

c) **Multiphoton Microscope:** In this type of microscope, multiphoton fluorescence excitation captures high-resolution three-dimensional images of specimens tagged with highly specific fluorophores.

d) **Total Internal Reflection Fluorescence (TIRF) Microscope:** Total internal reflection fluorescence microscopy (TIRFM) exploits the unique properties of an induced evanescent wave or field in a limited specimen region immediately adjacent to the interface between two media having different refractive indices.

Applications of Fluorescence Microscope:

1) Fluorescence microscopy is widely used in diagnostic microbiology and microbial ecology for enumerating bacteria in natural environments. Some organisms, such as Pseudomonas, fluoresce naturally when irradiated with ultraviolet light. Other organisms, such as *Mycobacterium tuberculosis* and *Treponema pallidum*, are treated with fluorochrome. Acid-fast bacilli (AFB) in sputum or CSF are detected when stained with auramine fluorescent dye. Detection of Trichomonas vaginalis, intracellular gonococci, and other parasites when stained by acridine orange. In immunodiagnosis of infectious diseases, using both direct and indirect antibody techniques. Immunofluorescence is especially useful in diagnosing syphilis and rabies.
2) It is used to identify structures in fixed and live biological samples.
3) Fluorescence microscopy is a common tool for today's life science research because it allows the use of multicolor staining, labeling of structures within cells, and the measurement of the physiological state of a cell.
4) It is used to imaging structural components of small specimens, such as cells
5) It is used to conducting viability studies on cell populations (are they alive or dead?)
6) It is used to imaging the genetic material within a cell (DNA and RNA)
7) It is used to viewing specific cells within a larger population with techniques such as FISH

Advantages of Fluorescence Microscope:

1) Fluorescence microscopy is the most popular method for studying the dynamic behavior exhibited in live-cell imaging.
2) This stems from its ability to isolate individual proteins with a high degree of specificity amidst non-fluorescing materials.
3) The sensitivity is high enough to detect as few as 50 molecules per cubic micrometer.
4) Different molecules can now be stained with different colors, allowing multiple types of the molecule to be tracked simultaneously.
5) These factors combine to give fluorescence microscopy a clear advantage over other optical imaging techniques, for both in vitro and in vivo imaging.

2.5. SUMMARY:

Fluorophores gradually lose their ability to fluoresce as they are illuminated in photobleaching. Photobleaching can severely limit the time a sample can be observed by fluorescence microscopy. However, several techniques exist to reduce photobleaching, such as using more robust fluorophores, minimizing illumination, or using photoreactive scavenger chemicals. Fluorescence microscopy has enabled the analysis of live cells, but fluorescent molecules generate reactive chemical species under illumination that enhances the phototoxic effect, to which live cells are susceptible. Fluorescence microscopy only allows observation of the specific structures labeled for fluorescence. For example, observing a tissue sample prepared with a fluorescent DNA stain by fluorescence microscopy only reveals the organization of the DNA within the cells and reveals nothing else about the cell morphologies.

2.6. TECHNICAL TERMS:

Enumeration of Bacteria, Immunodiagnosis, Nutrient Agar. Enumeration of Actinomycetes, Fluorescent DNA Stain, Fluorescence Microscopy.

2.7. SELF-ASSESSMENT QUESTIONS:

1) Explain the Soil Plate Method?
2) Explain in detail about Contact Slide Technique?
3) Discuss about Fluroscence Microscopy?

2.8. SUGGESTED READINGS:

1) Subbarao, N.S. 2000. Soil Microbiology 4th Edn.
2) Subbarao, N.S. 1995 Biofertilizers in Agriculture and Forestry.
3) Tilak, K.V.B.R. 1991. Bacterial Biofertilizers, ICAR Publications.
4) Atlas, R.M. and Bartha, R. 1998. Microbial Ecology; Fundamentals and Applications, Addison Wiesley Longman Publications.
5) Lynch and Poole, 1983 Microbial Ecology, ELBS Publications.

Prof. A. Amruthavalli

LESSON-3

PANT GROWTH PROMOTING RHIZOBACTERIA, PHYLLOSPHERE MICROFLORA AND THEIR SIGNIFICANCE

3.0. OBJECTIVE:

- This lesson deals with concept of Pant Growth Promoting Rhizobacteria, Phyllosphere Microflora and their Significance.

STRUCTURE:

3.1. INTRODUCTION:

Worldwide, considerable progress has been achieved in the area of PGPR biofertilizer technology. It has been also demonstrated and proved that PGPR can be very effective and are potential microbes for enriching the soil fertility and enhancing the agriculture yield. PGPR are excellent model systems which can provide the biotechnologist with novel genetic constituents and bioactive chemicals having diverse uses in agriculture and environmental sustainability. Current and future progress in our understanding of PGPR diversity, colonization ability, mechanisms of action, formulation, and application could facilitate their development as reliable components in the management of sustainable agricultural systems.

3.2. PANT GROWTH PROMOTING RHIZOBACTERIA (PGPR):

Plant Growth Promoting Rhizobacteria (PGPR) is a group of bacteria that enhances plant growth and yield via various plant growth promoting substances as well as biofertilizers. Given the negative environmental impact of artificial fertilizers and their increasing costs, the use of beneficial soil microorganisms such as PGPR for sustainable and safe agriculture has increased globally during the last couple of decades. PGPR as biofertilizers are well recognized as efficient soil microbes for sustainable agriculture and holds great promise in the improvement of agricultural yields.

Agriculture contributes to a major share of national income and export earnings in many developing countries, while ensuring food security and employment. Sustainable agriculture is vitally important in today's world because it offers the potential to meet our future agricultural needs, something that conventional agriculture will not be able to do. Recently there has been a great interest in eco- friendly and sustainable agriculture. PGPR are known to improve plant growth in many ways when compared to synthetic fertilizers, insecticides and pesticides. They enhance crop growth and can help in sustainability of safe environment and crop productivity. The rhizospheric soil contains diverse types of PGPR communities, which exhibit beneficial effects on crop productivity. Several research investigations are conducted on the understanding of the diversity, dynamics and importance of soil PGPR communities and their beneficial and cooperative roles in agricultural productivity. Some common examples of PGPR genera exhibiting plant growth promoting activityare *Pseudomonas, Azospirillum, Bacillus, Azotobacter, Burkholdaria, Enterobacte, Rhizobium, Erwinia, Mycobacterium, Mesorhizobium, Flavobacterium,* etc.

3.2.1. PGPR as Biofertilizers:

Free-living PGPR have shown promise as biofertilizers. Many studies and reviews have reported plant growth promotion, increased yield, solubilization of P have reported plant growth promotion, increased yield, solubilization of P living PGPR have shown promise as biofertilizers. Many studies and reviews have reported plant growth promotion, increased yield, solubilization of P (phosphorus) or K (potassium), uptake of N (nitrogen) and some other elements through inoculation with PGPR. In addition, studies have shown (phosphorus) or K (potassium), uptake of N (nitrogen) and some other elements through inoculation with PGPR. In addition, studies have shown with PGPR enhances root growth, leading to a root system with large surface area and increased number of root hairs. A huge amount of artificial fertilize replenish soil N and P, resulting in high costs and increased environ (phosphorus) or K (potassium), uptake of N (nitrogen) and some other elements through inoculation with PGPR. In addition, studies have shown that inoculation with PGPR enhances root growth, leading to a root system with large surface area and increased number of root hairs. A huge amount of artificial fertilizers is used to replenish soil N and P, resulting in high costs and increased environmental pollution.

Most of P in insoluble compounds is unavailable to plants. N_2-fixing and P-solubilizing bacteria may be important for plant nutrition by increasing N and P uptake by the crop plants, and playing a crucial role in biofertilization. N_2-fixation and P-solubilization, production of antibiotics, and other plant growth promoting substances are the principal contribution of the PGPR in the agro-ecosystems. More recent research findings indicate that the treatment of agricultural soils with PGPR inoculation significantly increases agronomic yields as compared to uninoculated soils. Several mechanisms of Plant Growth Promotion by PGPR have been suggested by which can promote plant growth; some important ones are as follows (Table 3.1):

Table 3.1: PGPR and Their Effect on Growth Parameters/Yields of Crop/Fruit Plants

PGPR	Crop Parameters
Rhizobiumleguminosarum	Direct growth promotion of canola and lettuce
Pseudomonas putida	Early developments of canola seedlings, growth stimulation of tomato plant
Azospirillum brasilense and *A. irakense*	Growth of wheat and maize plants
P. flurescens	Growth of pearl millet, increase in growth, leaf nutrient contents and yield of banana (*Musa*)
Azotobacter and *Azospirillum* spp.	Growth and productivity of canola
P. alcaligenes, *Bacillus polymyxa*, and *Mycobacterium phlei*	Enhances uptake of N, P and K by maize crop
Pseudomonas, Azotobacter and *Azospirillum* spp.	Stumulates growth and yield of chick pea (*Cicer arietinum*)
R. leguminismarum and *Pseudomonas* spp.	Improves the yield and phosphorus uptake in wheat
P. putida, P. flurescens, A. brasilense and *A. lipoferum*	Improves seed germination, seedling growth and yield of maize
P. putida, P. fluorescens, P. fluorescens,	Improves seed germination, growth parameters of
P. putida, A. lipoferum, A. brasilense	Maize seedling in greenhouse and also grain yield of field grown maize

3.2.2. Phytohormone Production:

The enhancement in various agronomic yields due to PGPR has been reported because of the production of growth stimulating phytohormones (Table 3.2) such as indole-3-acetic acid (IAA), gibberellic acid (GA3), zeatin, ethylene and abscisic acid (ABA).

Table 3.2: Examples of Different Phytohormone Producing PGPR

Phytohormones	PGPR
Indole-3-acetic acid (IAA)	*Acetobacter diazotrophicus* and *Herbaspirillum seropedicae*
Zeatin and Ethylene	*Azospirillum sp.*
Gibberellic acid (GA_3)	*Azospirillum lipoferum*
Abscisic acid (ABA)	*Azospirillum brasilense*

Recent studies confirm that the treatment of seeds or cuttings with non-pathogenic bacteria, such as *Agrobacterium, Bacillus, Streptomyces, Pseudomonas, Alcaligenes*, etc. induce root formation in some plants because of natural plant growth promoting substances produced by the bacteria. Although the mechanisms are not completely understood, root induction by PGPR is the accepted result of phytohormones such as auxins, growth inhibiting ethylene and mineralization. Environment friendly applications in agriculture have gained more importance particularly in horticulture and nursery production. The use of PGPR for nursery material multiplication may be important for obtaining organic nursery material because the use of all formulations of synthetic plant growth regulators, such as indole-3-butyric acid (IBA), is prohibited in organic agriculture throughout the world.

3.2.3. Phosphate Solubilization:

Rhizobium and phosphorus (P) solubilizing bacteria are important to plant nutrition. These microbes also play a significant role as PGPR in the biofertilization of crops. These bacteria secrete different types of organic acids (e.g., carboxylic acid) thus lowering the pH in the rhizosphere and consequently release the bound forms of phosphate like $Ca_3(PO4)_2$ in the calcareous soils. Utilization of these microorganisms as environment friendly biofertilizer helps to reduce the use of expensive phosphatic fertilizers. Phosphorus biofertilizers could help increase the availability of accumulated phosphate (by solubilization), increase the efficiency of biological nitrogen fixation and render availability of Fe, Zn, etc., through production of plant growth promoting substances.

3.2.4. Siderophore Production:

PGPR are reported to secrete some extracellular metabolites called siderophores. Siderophores are commonly referred to as microbial Fe-chelating low molecular weight compounds. The presence of siderophore producing PGPR in rhizosphere increases the rate of Fe^{3+} supply to plants and therefore enhances the plant growth and productivity of crop. Further, this compound after chelating Fe^{3+} makes the soil Fe^{3+} deficient for other soil microbes and consequently inhibits the activity of competitive microbes.

3.2.5. PGPR as Biocontrol:

Agents PGPR produce substances that also protect them against various diseases. PGPR may protect plants against pathogens by direct antagonistic interactions between the biocontrol agent and the pathogen, as well as by induction of host resistance. In recent years, the role of siderophore producing PGPR in biocontrol of soil borne plant pathogens has created great interest. Microbiologists have developed techniques for introduction of siderophore producing PGPR in soil system through seed, soil or root system. PGPR that indirectly enhance plant growth via suppression of phytopathogens do so by a variety of mechanisms. These include: The ability to produce siderophores (as discussed above) that chelate iron, making it unavailable to pathogens. The capacity to synthesize anti-fungal metabolites such as antibiotics, fungal cell wall-lysing enzymes, or hydrogen cyanide, which suppress the growth of fungal pathogens. The ability to successfully compete with pathogens for nutrients or specific niches on the root and the ability to induce systemic resistance. Among the various PGPRs identified, *Pseudomonas fluorescens* is one of the most extensively studied rhizobacteria because of its antagonistic action against several plant pathogens. Banana Bunchy Top Virus (BBTV) is one of the deadly viruses which severely affect the yield of banana (*Musa spp.*) crop in Western Ghats, Tamil Nadu, India. It has been demonstrated that application of *P. fluorescens* strain. significantly reduced the BBTV incidence in hill banana under greenhouse and field conditions. Different PGPR species as biocontrol agents against various plant diseases are given in Table 3.

Table 3.3: PGPR as Biocontrol Agents Against Various Plant Diseases

PGPR	Disease Resistance
Bacillus pumilus, Kluyvera cryocrescens, B. amyloliquefaciens and *B. subtilus*	Cucumber Mosaic Cucumovirus (CMV) of tomato (*Lycopersicon esculentum*)
B. amyloliquefaciens, B. subtilis and *B. pumilus*	Tomato Mottle Virus
B. pumilus	Bacterial wilt disease in cucumber (*Cucumis sativus*), Blue mold disease of tobacco (*Nicotiana*)
Pseudomonas fluorescens	Sheath blight disease and leaf folder insect in rice (*Oryza sativa*), Reduce the Banana Bunchy Top Virus (BBTV) incidence, Saline resistance in groundnut (*Arachis hypogea*)
B. subtilis and B. pumilus	Downy mildew in pearl millet (*Pennisetum glaucum*)
B. subtilis	CMV in cucumber

PGPR	Disease Resistance
B. cereus	Foliar diseases of tomato
Bacillus spp.	Blight of bell pepper (*Capsicum annuum*), Blight of squash
Burkholderia	Maize (*Zea mays*) rot
B. subtilis	Soil borne pathogen of cucumber and pepper (*Piper)*
Bacillus sp. and *Azospirillum*	Rice blast
Fluorescent *Pseudomonas* spp.	Rice sheath rot (*Sarocladium oryzae*)

Conclusion:

Worldwide, considerable progress has been achieved in the area of PGPR biofertilizer technology. It has been also demonstrated and proved that PGPR can be very effective and are potential microbes for enriching the soil fertility and enhancing the agriculture yield. PGPR are excellent model systems which can provide the biotechnologist with novel genetic constituents and bioactive chemicals having diverse uses in agriculture and environmental sustainability. Current and future progress in our understanding of PGPR diversity, colonization ability, mechanisms of action, formulation, and application could facilitate their development as reliable components in the management of sustainable agricultural systems.

3.3. PHYLLOSPHERE MICROFLORA AND THEIR SIGNIFICANCE:

The phyllosphere comprises the aerial parts of plants and is dominated by the leaves followed by stems, flowers, and fruits. The phyllosphere is a unique and dynamic habitat constituting irregular, and sometimes relatively large microbial community inhabitants in the ecosystem. Although the phyllosphere has been less intensively studied than the rhizosphere, it has received considerable attention in recent years, and interest in the microbiology of aerial surfaces is now acknowledged to extend beyond pathogens. The phyllosphere is the ambient region for microbes to colonize and establish its association with plants usually epiphytes. Phyllosphere is considered a heterogeneous and dynamic habitat as the parts of the plant are in direct contact with the environment and thus, are affected by even slight changes in the environment. While there has been some investigation of the microbial community of buds and flowers, most work on the phyllosphere has focused on leaves, a more dominant aerial plant structure. Leaves constitute a huge microbial habitat with larger surface areas and diverse microbial communities. The Phyllosphere area has comparatively less microbial number than the rhizosphere as the parts of the phyllosphere is hydrophobic and covered with wax that limits the exudates produced by the parts.

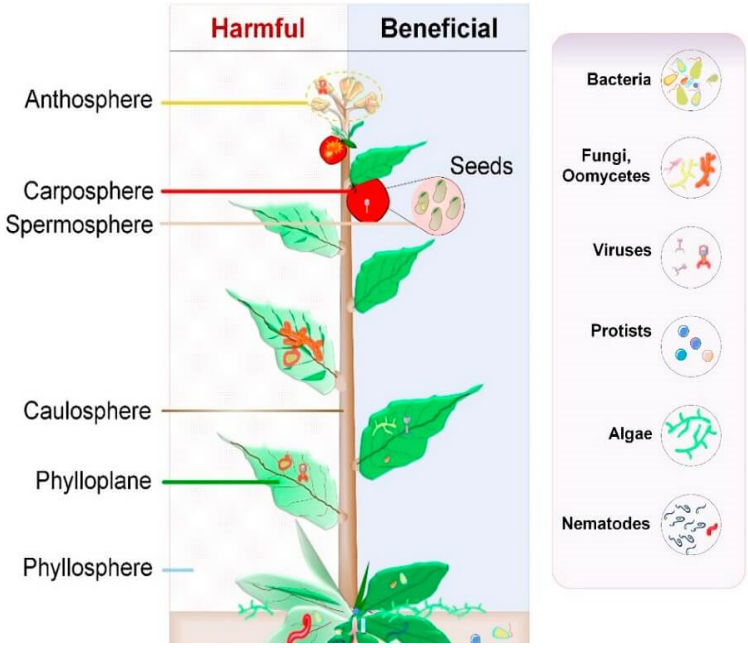

Fig. 3.1: Microorganisms found in the Phyllosphere (Phyllosphere microbiome). Image Source: **MDPI Microorganisms (https://doi.org/10.3390/microorganisms7080269)**

The phyllosphere is an important habitat for diverse groups of microbial communities including bacteria, filamentous fungi, yeasts, algae, and protozoans. A variety of bacteria, filamentous fungi, and yeasts naturally colonize the phyllosphere region, followed by less frequent protozoa and nematodes colonization. These microorganisms exhibit commensalism or mutualism (symbionts) or antagonism type of relationship on their host plants. Among the diverse community of microbes, bacteria are the predominant community on parts like leaves, with its range in between $10^2$and 10^{12}/g of the leaf. Generally, the phyllosphere contains four major species of bacteria such as Proteobacteria, Firmicutes, Bacteroides, and Actinobacteria. Predominant genera of bacteria found in the phyllosphere include *Methylibium, Hyphomicrobium, Methylocella, Proteobacteria, Actinobacteria, Bacteroidetes, Massilia, Flavobacterium, Pseudomonas,* and *Rathayibacter*. It has been found that the alpha-, beta- and gammaproteobacteria and firmicutes are the dominant bacterial inhabitants of the phyllosphere. Besides, acidobacteria, actinobacteria, and cyanobacteria are the less frequent colonizers of the phyllosphere environment. The cultivable yeast genera such as *Cryptococcus, Rhodotorula, Sporobolomyces,* and their species have mainly inhabited the plant leaf. Moreover, the abundance of filamentous fungi has been estimated to range from $10^2$to 10^8 CFU/g based on the culture-dependent methods. Genera such as *Alternaria, Penicillium, Cladosporium, Acremonium, Mucor* and *Aspergillus* are the frequent filamentous fungi colonizing as epiphytes and endophytes.

3.3.1. Phyllosphere Microbiome of Stem (Caulosphere):

The caulosphere (stems) is a wooden aerial plant part containing large groups of microorganisms. The stem is the not most ideal habitat for most microorganisms as the surface of the stem is hydrophobic as a result of the chitin and wax covering. However, some groups of microorganisms can still be found in the stem as it is exposed to the air and constantly changing environmental conditions. The microbes residing in the caulosphere need

adaptation techniques to withstand the change in temperature and moisture content. Fungi are the primary resident of the caulosphere, followed by bacteria and nematodes. Fungal species including *Saccharomyces, Candida, Hanseniaspora* and *Lachancea* are the common inhabitants. Bacteria in the stem are similar to those in the leaves which include bacteria like *Pseudomonas, Proteobacteria,* and *Flavobacterium.*

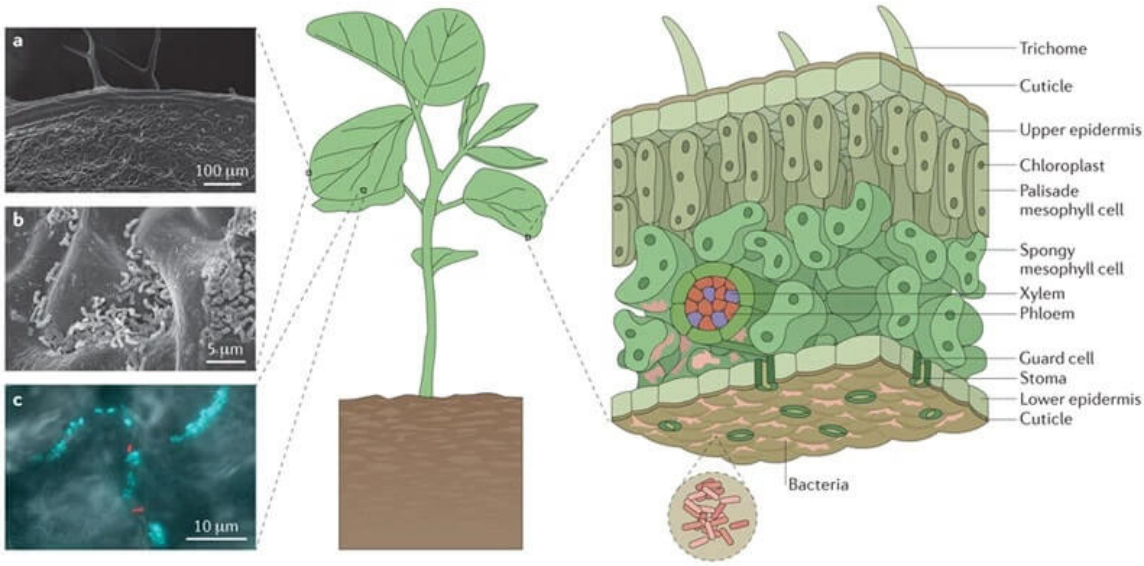

Fig. 3.2: The phyllosphere comprises the aerial parts of plants and is dominated by the leaves. Various microorganisms, and in particular bacteria (shown) and fungi (not shown), can colonize the surface of the leaf and the apoplast. Most of the leaf inhabitants are non-pathogenic bacteria, which are often referred to as commensals. **A.** Scanning electron micrograph showing the edge of an *Arabidopsis thaliana* leaf with trichomes visible on the upper side. **B.** Scanning electron micrograph of epiphytic *Sphingomonas* sp. Fr 1 on the surface of a leaf. The bacterial cells are located in the grooves formed by the junctions of the epidermal plant cells. **C.** Epifluorescence image of *Pantoea agglomerans* str. 299R constitutively expressing *cfp,* and *Pseudomonas syringae* pv. *tomato* str. DC3000 producing mCherry, on an *A. thaliana* leaf.

Image Source: Nature Reviews Microbiology.

The microbial communities of leaves are diverse and consist of many different genera of bacteria, yeasts, filamentous fungi, algae, and, less frequently, protozoa, and nematodes. Filamentous fungal communities are considered transient inhabitants of leaf surfaces, with spores as predominant forms, whereas rapidly sporulating species and yeasts colonize the habitat more actively. Bacteria are the most abundant inhabitants of the phylloplane, assuming that there are on average 10^6 –10^7 bacteria per square cm of the leaf surface. The arrangement of leaf epidermal cells determines the leaf physiology and the microenvironment, which allow the abundance and distribution of microorganisms on the leaf surface. Epiphytes make biofilm-like growth, preferably in the form of larger bacterial aggregates on the trichomes, veins, and epidermal cell groves, along with the leaf exudates, creating a nutrient-rich region. Different physical characteristics of the leaf surface make it a suitable habitat for many microorganisms. The presence of the outer cuticle on the leaves and its physiology helps the microbes to colonize the surface, and the leaching of nutrients along with water allows the epiphytes to utilize and develop colonies on the phyllosphere. Most

bacteria on leaf surfaces do not occur as solitary cells or small groups of cells, as fungi tend to, but form larger aggregates. These aggregates are particularly common at the depressions formed at the junctions of epidermal cells, along the veins, and at the bases of trichomes. Studies have shown that the bacterial aggregates on leaves consist of 1000 cells or more and that the size of the aggregates positively correlates with water availability. Fungal communities in the phyllosphere in temperate regions are hypervariable, and the communities exhibit even greater diversity in the phyllosphere of tropical trees. The types of microorganisms inhabiting the phylloplane region also depend on the type of plant species.

3.3.2. Phyllosphere Microbiome of Flowers (Anthosphere):

The anthosphere region, that is, the region around the flowers, is also an important dynamic habitat for microbial growth. In particular, the anthosphere region around flowers is colonized by a vast diversity of microorganisms, which are flower specific. However, some members of the genera *Pseudomonas and Acinetobacter* (Proteobacteria), *Metschnikowia* (Ascomycota), and *Cryptococcus* (Basidiomycota) are consistent members of the floral microbiome across many agricultural and ornamental plants. Another notable feature of this interface is the permanence; this habitat has a shorter life span when compared with other spheres. Studies on flower associated microbial communities have highlighted that the fungal population is the highest in the anthosphere, followed by bacteria. The floral components, namely, pollen, nectar, sepals, petals, stamens, style, ovary, and stigmas, act as short-span microsites for the colonization of microorganisms. It has been found that the floral surface organs are abundant with basidiomycetous yeasts, whereas nectar and pollen were filled with ascomycetous yeast species and nectar hosts most fungi compared with other floral parts. In pollen, the bacterial count is abundant, ranging between 10^6 and 10^9, and the diversity and composition vary from species to species because of the difference in nutrient composition, pollen viability, pollen structure, and moisture. The epiphytic bacteria in the atmosphere exist either single or in clusters with the particular formation of thin biofilms in certain habitats. Therapeutic metabolites producing endophytic fungi identified as *Pestalotiopsis disseminate*, *Phomopsis,* and *Coelomycete* sp. are also isolated from some medicinal flowers of the temperate region.

3.3.3. Phyllosphere Microbiome of Fruits (Carposphere):

Carposphere represents a unique and dynamic habitat, where the microbial communities are subjected to irregular, and sometimes comparatively large changes in temperature, UV radiation, relative humidity, and nutrient availability upon the plant surface. Fruits are essential phyllosphere habitat as it is rich in sugar and fruits act as a common source of food for many yeast species. Fruit skin inhabits diverse groups of microbes, encompassing both bacteria and fungi. The entry of microorganisms inside the fruit is comparatively less frequent due to the presence of a tough, waterproof covering. However, the type of microorganisms present on the fruit depends on the chemical composition of the fruit. It has been seen that fruits like lemons and orange harbor a large number of yeasts while grapes and apples have a higher number of bacterial cells. The microbial community of the carposphere is transient as fruits have a shorter lifespan than other phyllosphere parts. Microbial communities in the fruits might differ throughout their development as the microorganisms present in the buds of fruits might not be present in the developed fruit.

3.3.4. Factors Influencing their Growth and Activities:

Different environmental and plant factors influence the microbial communities in the phyllosphere region.

A. Light

The microorganisms in the phyllo sphere region, unlike that in the rhizosphere, are influenced by the light. Bacteria and fungi utilize sunlight to produce different chemical products that promote their growth and plant growth. Light also affects several aspects of plant physiology which influences plant secretions, affecting microbial growth. UV radiation affects the production of plant secondary metabolites, diversity of the microbial population, and the behavior towards biological control agents.

B. Temperature

Abiotic factors like temperature affect the growth of activities of phyllosphere microorganisms and the phyllosphere-plant interactions. Temperature fluctuations are observed with the changes in day and night regimes in the phyllosphere region. Temperature affects both the plants, microorganisms, and their interactions which affects the rates of physical, chemical, and biological processes in the region. Microbial colonizers of the phyllosphere region are subjected to diurnal and seasonal fluctuations of heat and moisture.

C. Plant species

The identity of the host plant has a significant influence on the identity of its microbiome. Different plant species growing adjacent to one another can harbor distinct microbiomes. Factors like the nutrient content of the fruits, flowers, and stems affect the growth and activities of the microbiome present on the plants. Plant age and developmental stage determine the interactions between plant and microorganisms, which might change with the changes in the plant development. The immune system of the plant plays a vital role in determining microbial assembly.

D. Microbe-Microbe Interactions

The extent to which microbe-microbe interactions can play roles in the microbiome composition is not well understood. But the outcome of microbe-microbe interactions could be explained as cooperation, parasitism, and competition. Each group of microorganisms plays a unique and vital role in microbiomes, and their absence could cause a significant alteration in microbiome composition and functioning. There can be direct microbe-microbe interactions, such as the hyper-parasitism (parasite of a parasite) of primary colonizers and opportunists that utilize the host's compromised plant defenses to colonize them and cause diseases.

3.3.5. Positive Effect of Phyllosphere Microorganisms on Plants:

Phyllosphere microorganisms often have a direct positive influence on plants by altering plant surface properties, enhancing nitrogen fixation, and promoting the growth of plants, the control of plant pathogens, and the degradation of organic pollutants. Phyllosphere microflora significantly influences the ecological relationship, adaptation, growth, resistance, and infection of the plant host. The phyllosphere microbiome affects leaf functions and longevity, seed mass, apical growth, flowering, and fruit development. Beneficial microbes

play an important role in increasing yields of the crop, removing contaminants, and producing novel substances. Phyllopsphere microbial communities produce plant growth factors like IAA and cytokines that promote nutrient uptake and crop yield.

3.4. SUMMARY:

The phyllosphere comprises the aerial parts of plants and is dominated by the leaves followed by stems, flowers, and fruits. The phyllosphere is a unique and dynamic habitat constituting irregular, and sometimes relatively large microbial community inhabitants in the ecosystem. The microbial communities of leaves are diverse and consist of many different genera of bacteria, yeasts, filamentous fungi, algae, and, less frequently, protozoa, and nematodes. Filamentous fungal communities are considered transient inhabitants of leaf surfaces, with spores as predominant forms, whereas rapidly sporulating species and yeasts colonize the habitat more actively. Carposphere represents a unique and dynamic habitat, where the microbial communities are subjected to irregular, and sometimes comparatively large changes in temperature, UV radiation, relative humidity, and nutrient availability upon the plant surface. Fruits are essential phyllosphere habitat as it is rich in sugar and fruits act as a common source of food for many yeast species. Fruit skin inhabits diverse groups of microbes, encompassing both bacteria and fungi.

3.5. TECHNICAL TERMS:

Rhizospheric microorganisms, *Bacillus polymyxa,* Rhizobium, Azotobacter, Clostridium, fix atmospheric nitrogen, Root exudates (REs).

3.6. SELF-ASSESSMENT QUESTIONS:

1) Define Rhizosphere? Explain Different types of Microflora of Rhizosphere Soil?
2) Add a note on Rhizosphereic Effect?
3) Write a short note on Root Exudates?
4) Explain about Soil Fungistasis?

3.7. SUGGESTED READINGS:

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LESSON-4

BIOFERTILIZERS – MYCORRHIZAE, *AZOTOBACTER* AND *AZOSPIRILLUM*

4.0. OBJECTIVE:

- This lesson deals with concept of Biofertilizers-Mycorrhiza-Ectomycorrhizas and arbuscular mycorrhiza, *Azotobacter* and *Azospirillum*.

STRUCTURE:

4.1. INTRODUCTION:

Biofertilizers provide "eco-friendly" organic agro-inputs. *Rhizobium*, *Azotobacter*, *Azospirillum* and blue-green algae (BGA) are perhaps the species with the longest history of use as biofertilizers. *Rhizobium* inoculant is used for leguminous crops. *Azotobacter* can be used with crops like wheat, maize, mustard, cotton, potato, and other vegetable crops. *Azospirillum* inoculations are recommended mainly for sorghum, millets, maize, sugarcane

and wheat. Blue-green algae belonging to the cyanobacteria genera *Nostoc, Anabaena, Tolypothrix* and *Aulosira* fix atmospheric nitrogen and are used as inoculants for paddy crops grown in both upland and lowland conditions. *Anabaena*, in association with the water fern *Azolla*, can contribute nitrogen up to 60 kg/ha/season and can also enrich soils with organic matter. Seaweeds are rich in various types of mineral elements (potassium, phosphorus, trace elements, etc.), hence they are extensively used as a form of manure replacement by people of coastal districts. Mycorrhiza is an association formed between the plant root and a fungal species as a result of non-disease-producing infection. The term 'mycorrhiza' indicates a symbiotic relationship between the roots of green plants and fungi. The mycorrhizal association is considered a mild form of parasitism, more accurately called mutualism, where both the plants and the fungi are benefitted from each other. Mycorrhizal fungi are crucial for mineral uptake by plants while fungi also receive nutrients from plants in return. About 90% of all land plants depend on mycorrhizal fungal for minerals like phosphorus. Mycorrhizal associations are born out of necessity, in the case of plants growing in soil that is deficient in nutrients. In some cases, mycorrhiza might even be harmful to the plant species that might range from mild to severe.

4.2. MYCORRHIZAE:

Mycorrhizal fungi are species of fungi that intimately associate with plant roots forming a symbiotic relationship, with the plant providing sugars for the fungi and the fungi providing nutrients such as phosphorus, to the plants. Mycorrhizal fungi can absorb, accumulate and transport large quantities of phosphate within their hyphae and release to plant cells in root tissue. A mycorrhiza ("fungus – root") is a type of endophitic, biotrophic, mutualistic symbiosis prevalent in many cultivated and natural ecosystems. There are three major groups of mycorrhizae: Ectomycorrhiza, Ectendomycorrhiza and Endomycorrhiza. Ectomycorrhiza and endomycorrhiza are important in agriculture and forestry. In Thailand, endomycorrhiza biofertilizer has been investigated for ten years. Initially the mycorrhizal biofertilizer production is for economic crops such as fruit trees (durian, longan, sweet tamarind, mangosteen, papaya). Now the biofertilizer can be used for vegetables and rubber. Endomycorrhiza (vesiclular arbuscular mycorrhiza; VA mycorrhiza; now known as arbuscular mycorrhiza) play a very important role on enhancing the plant growth and yield due to an increase supply of phosphorus to the host plant. Mycorrhizal plants can absorb and accumulate several times more phosphate from the soil or solution than non–mycorrhizal plants. Plants inoculated with endomycorrhiza have been shown to be more resistant to some root diseases.

Mycorrhizae, the term mycorrhizae were coined for symbiotic associations formed by fungi with roots (Gr. myces = fungus, rhizo = roots). Mycorrhizae are wide spread under natural conditions and occur nearly in all soils from mine spoils to agricultural soils as well as soil under horticultural or fruit crops. More than 95% of plant taxa form mycorrhizal associations. The association is generally mutualistic in that the fungi obtain a carbon source from host, whilst the latter benefits from enhanced nutrient uptake through transfer from soil via the fungi. They are formed by most vascular plants except for a few monocotyledons like cyperacea or juncacae and dicotyledons like chenopodiacea or brassicaceae. Mycorrhizae are usually divided into three morphologically distinct groups depending on whether or not there is fungal penetration of root cells: endomycorrhiza, ectomycorrhiza and ectoendomycorrhiza. Of the three groups, endomycorrhizae are important as biofertilizer.

Endomycorrhizae are formed by nearly 90% of the land plants. In this association the fungi form external hyphal networks in the soil and grow extensively within the cells of the root cortex. This network of fungal hyphae within the root cortex is known as hartig net. Fungi belonging to basidiomycetes, ascomycetes or zygomycetes are involved depending on the type of endomycorrhizal association. Specific types of endomycorrhizae are formed by members of the Ericaceae (Ericoid mycorrhizae) and orchidaceae (orchidaceous mycorrhizae), but the type of mycorrhizae which is widespread is the arbuscular mycorrhizae (earlier referred as vesicular-arbuscular mycorrhizae). It is formed by 120 species of zygomycetes, all belonging to the order Glomales (*Glomus*, *Acaulospora*, *Gigaspora*, *Sclerocystis*, *Entrophospora* and *Scutellospora*). None of these fungi has yet been successfully cultured axenically.

The effect of mycorrhizae in increasing plant growth has been well documented by different workers for many plants. The beneficial effect of mycorrhizae on plant growth has mostly been attributed to an increase in the uptake of nutrients, especially phosphorus. Mycorrhizal fungi improve the soil phosphorus availability by solubilizing inorganic forms of phosphorus or by mineralization of organic phosphorus. External hyphae of mycorrhiza also has the capacity to take up and deliver various other nutrients to plants like NH4+, NO3-, K, Ca, SO42-, Cu, Zn and Fe. In experimental chambers, the external hyphae of AM can deliver up to 80% of plant P, 25 % of plant N, 10% of plant K, 25% of plant Zn and 60% of plant Cu. Mycorrhiza also produce ectoenzymes which provide host plant with the potential to access organic N and P forms that are normally unavailable to AM fungi or to nonmycorrhizal roots. The environment, or the volume of soil that is influenced biologically and biochemically by living root, is known as rhizosphere. Root exudates and secretions create a rhizosphere effect that manifests itself in the intense microbial activity that is associated within the immediate vicinity of the root. Root associated bacteria, also called rhizobacteria, can be beneficial, neutral or deleterious to the growth of the plant.

Mycorrhiza is an association formed between the plant root and a fungal species as a result of non-disease-producing infection. The term ‘mycorrhiza’ indicates a symbiotic relationship between the roots of green plants and fungi. The mycorrhizal association is considered a mild form of parasitism, more accurately called mutualism, where both the plants and the fungi are benefitted from each other. Mycorrhizal fungi are crucial for mineral uptake by plants while fungi also receive nutrients from plants in return. About 90% of all land plants depend on mycorrhizal fungal for minerals like phosphorus. Mycorrhizal associations are born out of necessity, in the case of plants growing in soil that is deficient in nutrients. In some cases, mycorrhiza might even be harmful to the plant species that might range from mild to severe. The exchange of nutrients between the plants and the fungi is an essential part of nutrient cycles, ecology, evolution, and the overall physiology of plants. The primary function of the fungi in such association is to provide water and nutrients from the soil to the plant while the plants share the nutrients formed via photosynthesis to the plants.

4.2.1. Types (Ectomycorrhiza and Endomycorrhiza) (Fig. 4.1):

Mycorrhizal associations are of two types depending on the presence of fungi on the plant body.

a) Ectomycorrhiza:

Ectomycorrhiza is fungi that form an external association with plant roots and do not reach the cells of the root. Ectomycorrhiza is formed by various basidio-, asco- and zygomycetes as well as by fungi imperfecti. Their morphology, physiological abilities, adaptability, and their benefit to the plants vary. The hyphae of the ectomycorrhizal mycobiont penetrate between cells of the cortex of the root to form a branched structure called the "Hartig net", as well as forming a compact "mantle" (sheath) surrounding the rootlet. The extensive mycelium facilitates more efficient growth for the phycobiont due to improved nutrient and water uptake. Furthermore, considerable amounts of nutrient salts can be stored in the fungal mantle. Ectomycorrhiza usually forms symbiotic associations with hard woody plants, and it accounts for about 5 to 10% of the mycorrhizal associations formed in terrestrial plants. Rootlets of plants colonized by ectomycorrhizal fungi often exhibit more ramification and initiate characteristic, often club-shaped, swollen short roots. These morphological changes are caused by auxins and growth regulators synthesized by the mycobionts.

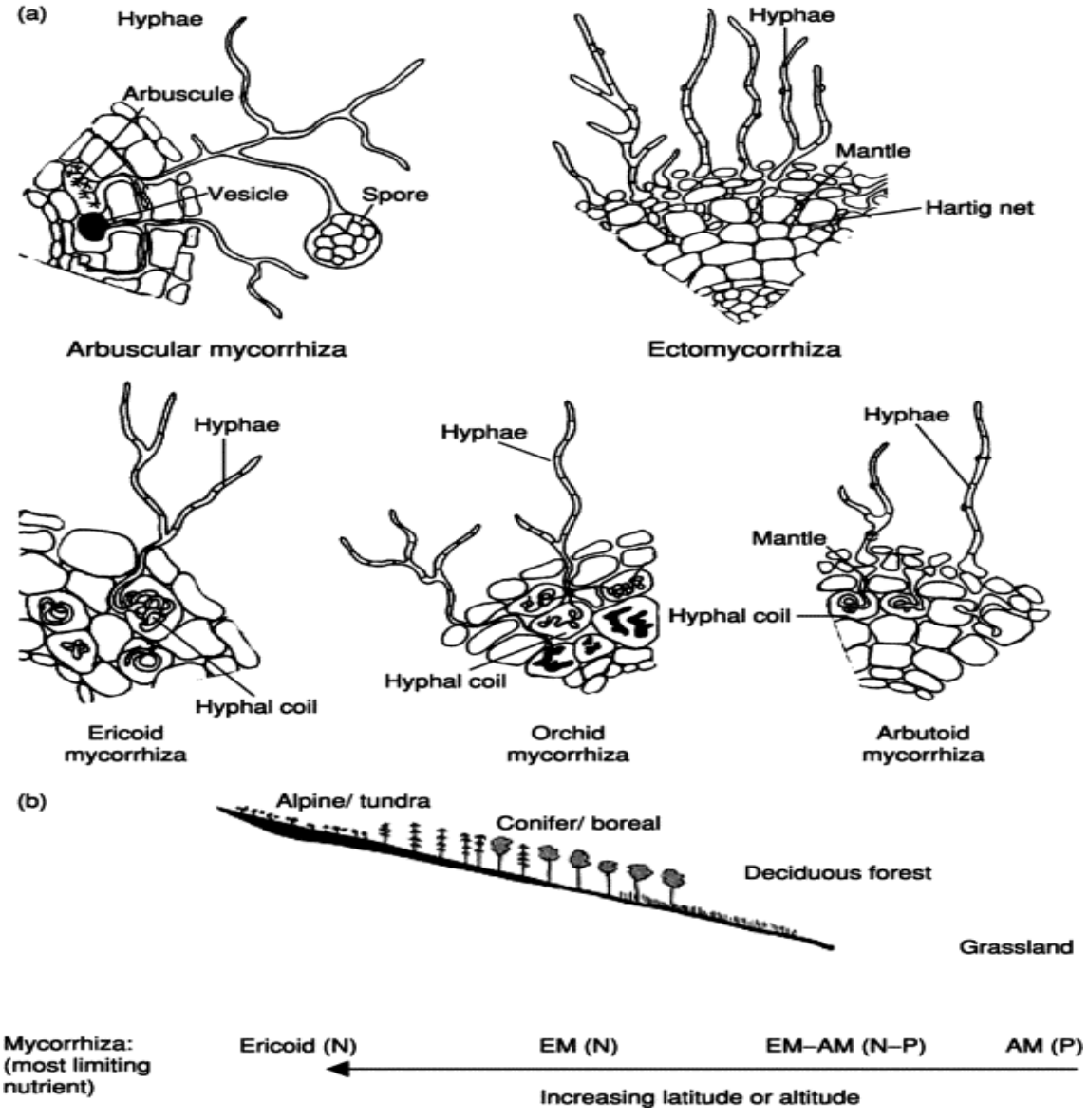

Fig. 4.1: Types of Mycorrhiza

b) Endomycorrhiza:

Endomycorrhiza is fungi that form an association with plant roots by penetrating the cortical cells of the root tissue. Endomycorrhiza accounts for about 80% of all the fungal associations with terrestrial plants. It occurs in many plant species ranging from vegetables, flowers, fruits, and trees. The hyphae of endomycorrhiza extend out of the root representing a more invasive relationship between the plant root and the fungi. Two distinct root structures are seen in the case of endomycorrhiza in the form of vesicles (for storage) and arbuscules (nutrient exchange). Endomycorrhiza is further classified as arbuscular, ericoid, or orchid, based on the host plants and structural nature of the symbiosis found in each group. Endomycorrhiza, like ectomycorrhiza, is involved in absorbing nutrients (especially phosphorus) from soil to provide them to the plants while receiving nutrients from the plant.

4.2.2. Arbuscular Mycorrhiza:

They are most widely distributed (7090%) type. VAM is produced by aseptate mycelial fungi belong to Endogonaceae under Mucorales of Zygomycotina and those members produced zygospores, e.g. *Acaulospora*, *Gigaspora*, *Glomus*, they are found in bryophytes, pteridophytes, gymnosperm (except Pinaceae) and most of the angiosperms, these are aseptate and forms intracellular hyphae in cortex. Two characteristic structures are present. The arbuscules are repeated dichotomously branched haustoria which grow intracellularly and live for four days and then get lysed releasing the stored food as oil droplets, mostly polyphosphate. Arbuscules transfer mineral nutrients from fungi and transfer sugars from host to fungi. The vesicles are thin or thick-walled vesicular structures produced intra-cellularly. Vesicles act as storage organ of P as phospholipids. There is no fungus mantle, but only a loose and very sparse network of septate hyphae spread into the soil. These hyphae bear different types of spores, chlamydospores, or aggregation of spores in sporocarp or zygospores. The superficial hyphae bear branches that penetrate the epidermis and then grow intercellularly only in cortex. Intercellular hyphae form arbuscules inside the parenchyma of cortex. The cell membrane of the penetrated cell is invaginated and covers the arbuscules (Fig. 4.2).

Arbuscular Mycorrhizal (AM) fungi (or Vesicular-Arbuscular Mycorrhizal, VAM fungi), belonging to the Phylum Glomeromycota are symbionts with terrestrial plant roots. It is now generally recognized that they improve not only the phosphorus nutrition of the host plant but also its growth, which may result in an increase in resistance to drought stress and some diseases. Therefore, AM fungi offer a great potential for sustainable agriculture, and the application of AM fungi to agriculture has been developed. In fact, in some countries the AM fungal inoculum has been commercialized. Since it is laborious and cost-consuming for production of AM fungal inoculum because of their obligate biotrophic nature, the ways to increase the function of the indigenous AM fungi in soil have also been developed. In this manual, the introductory techniques with AM fungi are presented.

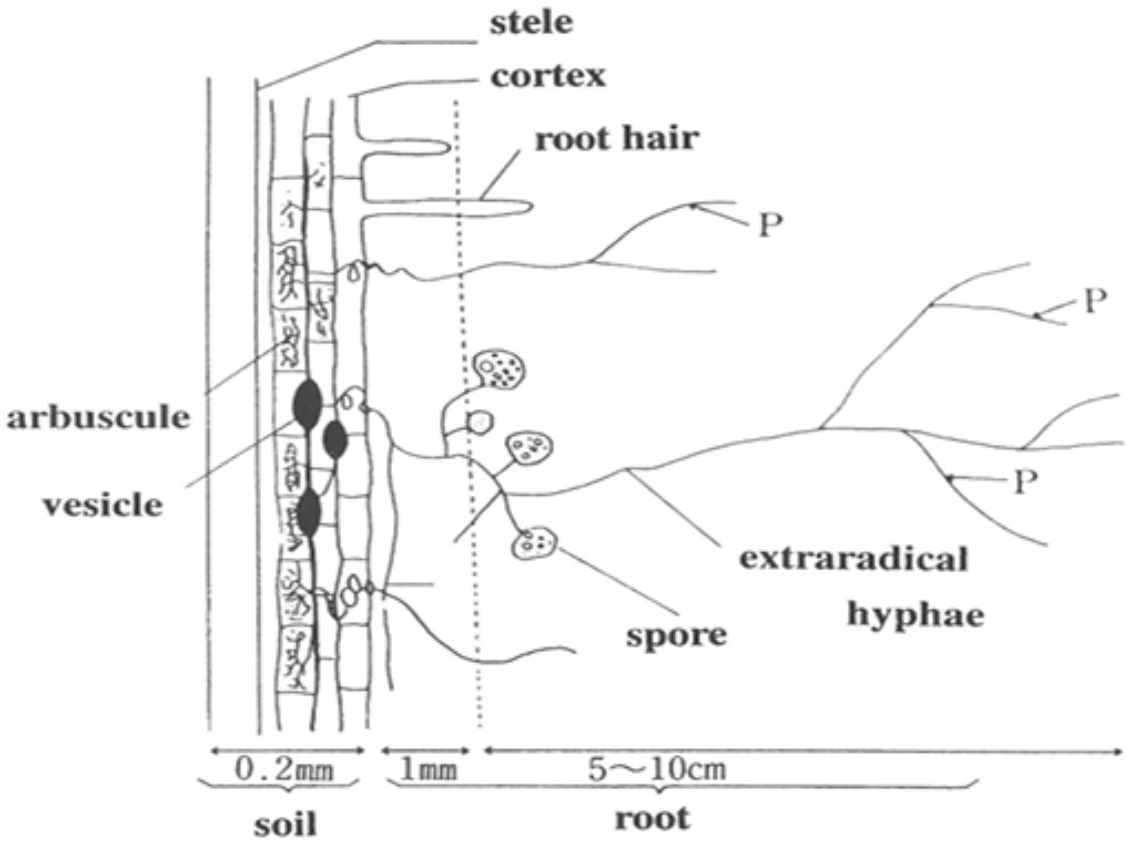

Fig. 4.2: Arbuscular Mycorrhizal Fungi Colonizing Roots and their Hyphal Extension into Soil

Because of morphological characteristics such as no hyphal septum, AM fungi had long been recognized as a member of Zygomycota. Recent molecular phylogenetic studies showed that Zygomycota is poly-phyletic and that AM fungi should be separated from other Zygomycota. A new Phylum Glomeromycota has been proposed for AM fungi. Current classification system is summarized in Fig. 4.2. This classification is mainly based upon the sequence data of rRNA gene. However, some new genera have been raised with relatively small numbers of isolates, so further study may revise the present classification system. In this manual, the morphological characteristics of representative genera are shown in the following sections. Although there is a recent trend that the sequence data of AM fungi is over-emphasized for the identification, the conventional morphological observation is still important and should not be neglected for identification.

Table 4.1: Morphological Character of Spores of AM Fungi

Shape:	(i.e. globular, spherical, irregular etc)
Size:	Globular: diameter (minimum – average – maximum) Irregular shape: length x width (minimum – average – maximum)
Colour:	(compare with Standard Colour Chart)
Hyphal attachment:	(i.e. sporiferrous saccule, bulbous suspensor etc) sporiferrous saccule = *Acaulospora, Entrophospora, Archaeospora* bulbous suspensor = *Gigaspora, Scutellospora*
Auxiliary cell:	(presence = *Gigaspora, Scutellospora*, none)
Sporocarp:	(presence, none)
Germination shield:	(presence =*Scutellospora*, absence)
Surface ornamentation:	(i.e. smooth, rough, reticulate etc)
Vesicle:	(presence or absence in mycorrhizal roots)

* These characters should be recorded with careful observation of many spores.

Benefits of Mycorrhizal Biofertilizer:

Mycorrhiza plays a very important role on enhancing the plant growth and yield due to an increase supply of phosphorus to the host plant. Mycorrhizal plants can absorb and accumulate several times more phosphate from the soil or solution than non–mycorrhizal plants. Plants inoculated with endomycorrhiza have been shown to be more resistant to some root diseases. Mycorrhiza increase root surface area for water and nutrients uptake. The use of mycorrhizal biofertilizer helps to improve higher branching of plant roots, and the mycorrhizal hyphae grow from the root to soil enabling the plant roots to contact with wider area of soil surface, hence, increasing the absorbing area for water and nutrients absorption of the plant root system. Therefore, plants with mycorrhizal association will have higher efficiency for nutrients absorption, such as nitrogen, phosphorus, potassium, calcium, magnesium, zinc, and copper; and also increase plant resistance to drought. Benefits of mycorrhizal biofertilizer can be seemed as follows: Allow plants to take up nutrients in unavailable forms or nutrients that are fixed to the soil. Some plant nutrients, especially phosphorus, are elements that dissolve were in water in neutral soil. In the extreme acidic or basic soil, phosphorus is usually bound to iron, aluminum, calcium, or magnesium, leading to water insolubility, which is not useful for plants. Mycorrhiza plays an important role in phosphorus absorption for plant via cell wall of mycorrhiza to the cell wall of plant root. In addition, mycorrhiza help to absorb other organic substances that are not fully soluble for plants to use, and also help to absorb and dissolve other nutrients for plants by storage in the root it is associated with. Enhance plant growth, improve crop yield, and increase income for the farmers. Arising from improved water and essential nutrients absorption for plant growth by mycorrhiza, it leads to improvement in plant photosynthesis, nutrients translocation, and plant metabolism processes. Therefore, the plant has better growth and yield, reduce the use of chemical fertilizer, sometimes up to half of the suggested amount, which in turn increases income for the farmers. As in the trial involving mycorrhizal biofertilizer on asparagus it was observed that, when the farmers used suggested amount of chemical fertilizer together with mycorrhizal biofertilizer, it was found that the crop yield improved by more than 50%, and

the farmers' income increased 61% higher than when chemical fertilizer alone was used. Improve plant resistance to root rot and collar rot diseases. Mycorrhizal association in plant roots will help plant to resist root rot and collar rot diseases caused by other fungi. It can be used together with other agricultural chemicals. Mycorrhiza are endurable to several chemical substances; for example; pesticide such as endrin, chlordane, methyl parathion, methomyl carbofuran; herbicide such as glyphosate, fuazifopbutyl; chemical agents for plant disease elimination such as captan, benomyl, maneb triforine, mancozed and zineb.

Functions of Mycorrhiza:

The most important function of mycorrhiza is to exchange nutrients between the surrounding and the host plants. Considerable amounts of nutrient salts can be stored in the fungal mycelium which then facilitates rapid activation of metabolism at the beginning of the growing season for plants at suboptimal locations like at high altitude. The fungal mycelium can further act as a significant barrier against infections caused by other microorganisms. Endomycorrhiza is essential for the formation of lateral roots and the absorption of essential minerals like sulfur and phosphorus. Mycorrhiza also alters nitrogen acquisition which improves plant growth. Ectomycorrhiza is known to be involved in the decomposition process and the mobilization of nitrogen from organic matter. These associations also enhance the tolerance of plants to difficult environmental conditions and salinity. Mycorrhizae are involved in phytoremediation processes by decreasing aluminum toxicity, improving plant oxidant systems.

4.3. *AZOTOBACTER*:

The genus *Azotobacter* was discovered in 1901 by Dutch microbiologist and botanist Martinus Beijerinck, who was one of the founders of environmental microbiology. He selected and described the species *Azotobacter chroococcum* – the first aerobic, free-living nitrogen fixer. *Azotobacter* is a genus of usually motile, oval or spherical bacteria that form thick-walled cysts (and also has hard crust) and may produce large quantities of capsular slime. They are aerobic, free-living soil microbes that play an important role in the nitrogen cycle in nature, binding atmospheric nitrogen, which is inaccessible to plants, and releasing it in the form of ammonium ions into the soil (nitrogen fixation). In addition to being a model organism for studying diazotrophs, it is used by humans for the production of biofertilizers, food additives, and some biopolymers. The first representative of the genus, *Azotobacter chroococcum*, was discovered and described in 1901 by Dutch microbiologist and botanist Martinus Beijerinck. *Azotobacter* species are Gram-negative bacteria found in neutral and alkaline soils,[1][2] in water, and in association with some plants.

4.3.1. Morphology:

Cells of the genus *Azotobacter* are relatively large and measure 2–4 μm in diameter. They are usually oval but may take various forms from rods to spheres. In microscopic preparations, the cells can be dispersed or form irregular clusters or, occasionally, chains of varying lengths (Fig. 4.3). In fresh cultures, cells are mobile due to the numerous flagella. Later, the cells lose their mobility, become almost spherical, and produce

a thick layer of mucus, forming the cell capsule. The shape of the cell is affected by the amino acid glycine, which is present in the nutrient medium peptone. Under magnification, the cells show inclusions, some of which are colored. In the early 1900s, the colored inclusions were regarded as "reproductive grains", or gonidia – a kind of embryo cells. However, the granules were later determined to not participate in the cell division. The colored grains are composed of volutin, whereas the colorless inclusions are drops of fat, which act as energy reserves.

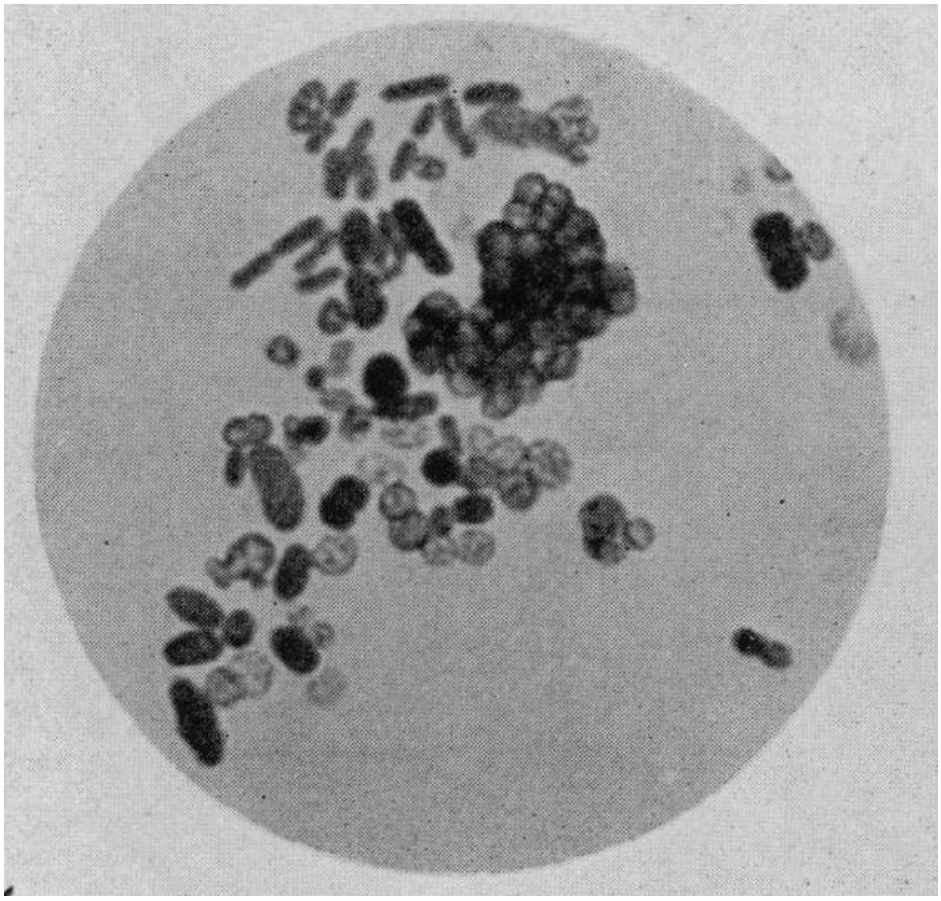

Fig. 4.3: Structure of *Azotobacter*

4.3.2. Physiological Properties:

Azotobacter respires aerobically, receives energy from redox reactions, using organic compounds as electron donors, and can use a variety of carbohydrates, alcohols, and salts of organic acids as sources of carbon. *Azotobacter* can fix at least 10 μg of nitrogen per gram of glucose consumed. Nitrogen fixation requires molybdenum ions, but they can be partially or completely replaced by vanadium ions. If atmospheric nitrogen is not fixed, the source of nitrogen can alternatively be nitrates, ammonium ions, or amino acids. The optimal pH for the growth and nitrogen fixation is 7.0–7.5, but growth is sustained in the pH range from 4.8 to 8.5. *Azotobacter* can also grow mixotrophically, in a molecular nitrogen-free medium containing mannose; this growth mode is hydrogen-dependent. Hydrogen is available in the soil; thus, this growth mode may occur in nature.

While growing, *Azotobacter* produces flat, slimy, paste-like colonies with a diameter of 5–10 mm, which may form films in liquid nutrient media. The colonies can be dark-brown,

green, or other colors, or may be colorless, depending on the species. The growth is favored at a temperature of 20–30°C. Bacteria of the genus *Azotobacter* are also known to form intracellular inclusions of polyhydroxyalkanoates under certain environmental conditions (e.g. lack of elements such as phosphorus, nitrogen, or oxygen combined with an excessive supply of carbon sources). *Azotobacter* species are free-living, nitrogen-fixing bacteria; in contrast to *Rhizobium* species, they normally fix molecular nitrogen from the atmosphere without symbiotic relations with plants, although some *Azotobacter* species are associated with plants. Nitrogen fixation is inhibited in the presence of available nitrogen sources, such as ammonium ions and nitrates. *Azotobacter* species have a full range of enzymes needed to perform nitrogen fixation: ferredoxin, hydrogenase, and an important enzyme nitrogenase. The process of nitrogen fixation requires an influx of energy in the form of adenosine triphosphate. Nitrogen fixation is highly sensitive to the presence of oxygen, so *Azotobacter* developed a special defensive mechanism against oxygen, namely a significant intensification of metabolism that reduces the concentration of oxygen in the cells. Also, a special nitrogenase-protective protein protects nitrogenase and is involved in protecting the cells from oxygen. Mutants not producing this protein are killed by oxygen during nitrogen fixation in the absence of a nitrogen source in the medium. Homocitrate ions play a certain role in the processes of nitrogen fixation by *Azotobacter*.

4.3.3. Nitrogenase:

Nitrogenase is the most important enzyme involved in nitrogen fixation. *Azotobacter* species have several types of nitrogenase. The basic one is molybdenum-iron nitrogenase. An alternative type contains vanadium; it is independent of molybdenum ions and is more active than the Mo-Fe nitrogenase at low temperatures. So it can fix nitrogen at temperatures as low as 5 °C and its low-temperature activity is 10 times higher than that of Mo-Fe nitrogenase.[47] An important role in maturation of Mo-Fe nitrogenase plays the so-called P-cluster.[48] Synthesis of nitrogenase is controlled by the *nif* genes. Nitrogen fixation is regulated by the enhancer protein NifA and the "sensor" flavoprotein NifL which modulates the activation of gene transcription of nitrogen fixation by redox-dependent switching. This regulatory mechanism, relying on two proteins forming complexes with each other, is uncommon for other systems.

4.3.4. Importance and Applications:

Nitrogen fixation plays an important role in the nitrogen cycle. *Azotobacter* also synthesizes some biologically active substances, including some phytohormones such as auxins, thereby stimulating plant growth. They also facilitate the mobility of heavy metals in the soil, thus enhancing bioremediation of soil from heavy metals, such as cadmium, mercury and lead. Some kinds of *Azotobacter* can also biodegrade chlorine-containing aromatic compounds, such as 2,4,6-trichlorophenol, which was previously used as an insecticide, fungicide, and herbicide, but later was found to have mutagenic and carcinogenic effects. Owing to their ability to fix molecular nitrogen and therefore increase the soil fertility and stimulate plant growth, *Azotobacter* species are widely used in agriculture, particularly in nitrogen biofertilizers such as azotobacterin. They are also used in production of alginic acid, which is applied in medicine as an antacid, in the food industry as an additive to ice cream, puddings, and creams.

4.4. AZOSPIRILLUM:

Azospirillum is a Gram-negative, microaerophilic, non-fermentative and nitrogen-fixing bacterial genus from the family of Rhodospirillaceae. *Azospirillum* bacteria can promote plant growth.

4.4.1. Characteristics:

The genus *Azospirillum* belongs in the Alphaproteobacteria class of bacteria. *Azospirillum* are gram-negative, do not form spores, and have a slightly twisted oblong-rod shape. *Azospirillum* have at least one flagellum and sometimes multiple flagella, which they use to move rapidly. *Azospirillum* are aerobic, but many can also function as microaerobic diazotrophs, meaning, under low oxygen conditions, they can change inert nitrogen from the air into biologically usable forms. At least three species, *A. melinis*, *A. thiophilum*, and *A. humicireducens* are facultative anaerobes, and can live, if necessary, without oxygen. Growth of *Azospirillum* is possible between 5 °C and 42 °C and in substrates with a pH of 5 to 9, with optimal growth occurring around 30 °C and 7 pH. Microbiologists use nitrogen-free semi-solid media to isolate *Azospirillum* from samples. The most commonly used media is called "NFb".

4.4.2. Ecological and Agricultural Significance:

Azospirillum are found in freshwater and soil habitats, especially in close relationships with plant roots. Associations with plants are thought to be largely beneficial. Over 113 species of plants in 35 different plant families have been documented to have benefited from association with a species of *Azospirillum*. In addition to vascular plants, the growth of the algae *Chlorella vulgaris* was positively affected by the presence of *Azospirillum*. Since the 1970s, *Azospirillum* strains have been researched for their effects in improving agricultural yields and improving growth of wild plants. In 2009, the first commercial inoculants containing *Azospirillum* came on the market, and by 2018, over 3 million doses were applied annually to crops by farmers, mainly in South America.

4.4.3. Plant Growth Promotion:

Fig. 4.4: *Azospirillum* promoting growth of fine root hairs

Azospirillum promote plant growth through a variety of mechanisms. Many *Azospirillum* species excrete plant hormones that alter how the roots of plants grow. Affected roots frequently grow more branches and fine root hairs, which may help the plants to acquire water and nutrients more efficiently. In addition to these changes, *Azospirillum* can also alter the forms of plant nutrients such as nitrogen and phosphorus to make them more available to plants. However, how much nitrogen *Azospirillum* contribute to crop plants via biological fixation is debated. *Azospirillum* also make antioxidants that protect the plant roots from stresses due to drought and flooding.

Plant growth can also be promoted indirectly by *Azospirillum* reducing plant disease. *Azospirillum* competes with pathogens on the roots for space and for trace nutrients such as iron. The plants' immune systems can also be primed by *Azospirillum* to resist attack by pathogens, a process known as induced systemic resistance.

4.5. SUMMARY:

A biofertilizer is a substance containing living micro-organisms which, when applied to seeds, plant surfaces, or soil, colonize the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant. Biofertilizers add nutrients through the natural processes of nitrogen fixation, solubilizing phosphorus, and stimulating plant growth through the synthesis of growth-promoting substances. The micro-organisms in biofertilizers restore the soil's natural nutrient cycle and build soil organic matter. Through the use of biofertilizers, healthy plants can be grown, while enhancing the sustainability and the health of the soil. Biofertilizers can be expected to reduce the use of synthetic fertilizers and pesticides, but they are not yet able to replace their use. As of 2024, more than 340 biofertilizer products have been approved for commercial use in the US.

4.6. TECHNICAL TERMS:

Biofertilizer, Arbuscular Mycorrhizal (AM), *Azospirillum*, Nitrogen fixation, nitrogen cycle. *Azotobacter*.

4.7. SELF-ASSESSMENT QUESTIONS:

1) Define Biofertilizer? Explain Ectomycorrhiza and Arbuscular Mycorrhiza (AM)?
2) Explain in detail about *Azotobacter*?
3) Explain Arbuscular Mycorrhiza (AM)?
4) Discuss about *Azospirillum*?

4.7. SUGGESTED READINGS:

1) Subbarao, N.S. 2000. Soil Microbiology 4th Edn.
2) Subbarao, N.S. 1995 Biofertilizers in Agriculture and Forestry.
3) Tilak, K.V.B.R. 1991. Bacterial Biofertilizers, ICAR Publications.
4) Atlas, R.M. and Bartha, R. 1998. Microbial Ecology; Fundamentals and Applications, Addison Wiesley Longman Publications.
5) Lynch and Poole, 1983 Microbial Ecology, ELBS Publications.

Dr. J. Madhavi

LESSON – 5

N_2 - FIXING CYANOBACTERIA, LEGUME - *RHIZOBIUM* ASSOCIATION, LEGUME ROOT NODULES, AND PHOSPHATE SOLUBILIZING MICROORGANISMS

5.0. OBJECTIVE:

- This lesson deals with concept of N_2-Fixing Cyanobacteria, Legume Rhizobium association and Development, Structure and Functions of Legume Root Nodules and Phosphate solubilizing microorganisms.

STRUCTURE:

5.1. INTRODUCTION:

Nitrogen is an essential nutrient for plant growth, yet most plants cannot directly utilize atmospheric nitrogen. Biological nitrogen fixation plays a crucial role in converting atmospheric nitrogen into forms usable by plants, thereby sustaining soil fertility and agricultural productivity. Among the most important nitrogen-fixing systems are free-living cyanobacteria and the symbiotic association between legumes and *Rhizobium*. This association involves the formation of specialized structures called root nodules, within which the nitrogenase enzyme complex of rhizobia reduces atmospheric nitrogen to ammonia. The specificity of legume–*Rhizobium* interactions is reflected in cross-inoculation groups, which determine successful symbiosis. Understanding the development, structure, and functions of legume root nodules provides insight into the efficiency of biological nitrogen fixation and its

significance in sustainable agriculture and ecosystem functioning. Phosphorus is a major nutrient required for the growth of plant. There are large reserves of phosphorus in soils but very little amount is available to the plant. There are several microorganisms that can solubilize the insoluble form of phosphorous and make it available to plants.

5.2. N_2-FIXING CYANOBACTERIA:

BGA are oxygen evolving, nitrogen fixing prokaryotes using sunlight as the energy source for N2 fixation. Occurrence varies with climatic factors and soil conditions. Abundant in rice fields as there are high levels of copper sulphate and combine nitrogen in the irrigation water, low occurrence of BGA is reported in Australian paddy fields. Predominant genera are *Anabaena, Nostoc, Calothrix, Aulosira, Aphanothece* and *Gloeotrichia*. The cyanobacterial nitrogen fixation has a switch on mechanism which gets activated when the combined nitrogen level falls below 40 ppm which enables algal biomass to produce more of biologically fixed nitrogen as soon as the nitrogen fertilizer level is reduced in the ecosystem due to loss and utilization. It has been observed that the removal of algae from paddy field water greatly reduced the in-situ nitrogen fixation. Application of BGA by farmers can save approximately 40-60 Kg urea as an average consumption of BGA has been found to be 20-30 kg N/ha/season. Biological nitrogen fixation is the conversion of atmospheric nitrogen (N_2) into ammonia (NH_3) carried out by certain prokaryotes using the enzyme nitrogenase. Fixed nitrogen is used to synthesize Amino acids, Proteins, Nucleic acids and Chlorophyll. Cyanobacteria are free-living nitrogen-fixing organisms. They play a vital role in Nitrogen Cycle, Soil Fertility, and aquatic productivity. Nitrogen fixation may occur in specialized cells (Heterocysts) under anaerobic conditions.

Examples: *Anabaena, Nostoc, Aulosira, Calothrix, Tolypothrix, Oscillatoria* (under special conditions).

Heterocysts are large, thick-walled, pale-colored cells. Occur at regular intervals in filamentous cyanobacteria, specialized for nitrogen fixation. Contains thick multilayered wall to prevent oxygen diffusion. Photosystem II is absent and presence of nitrogenase enzyme. Polar nodules are present for transport of nutrient and cyanophycin granules for nitrogen storage. Provide anaerobic environment. Fix atmospheric nitrogen and supply the fixed nitrogen to vegetative cells, and receive carbohydrates from vegetative cells. Nitrogenase is a complex enzyme system which is **h**ighly sensitive to oxygen. It consists of Fe-protein (iron protein) and Mo-Fe protein (molybdenum-iron protein). Oxygen-evolving photosynthesis is absent and high respiratory activity consumes oxygen. Micro-anaerobic conditions are maintained.

5.2.1. Types of Nitrogen-Fixing Cyanobacteria:

Heterocystous Cyanobacteria:

Possess specialized cells called heterocysts. Fix nitrogen efficiently in the presence of light. Examples: *Anabaena, Nostoc* (Fig. 5.1).

Non-Heterocystous Cyanobacteria:

Lack heterocysts. Fix nitrogen only under anaerobic conditions and dark conditions. Examples: *Oscillatoria, Lyngbya.*

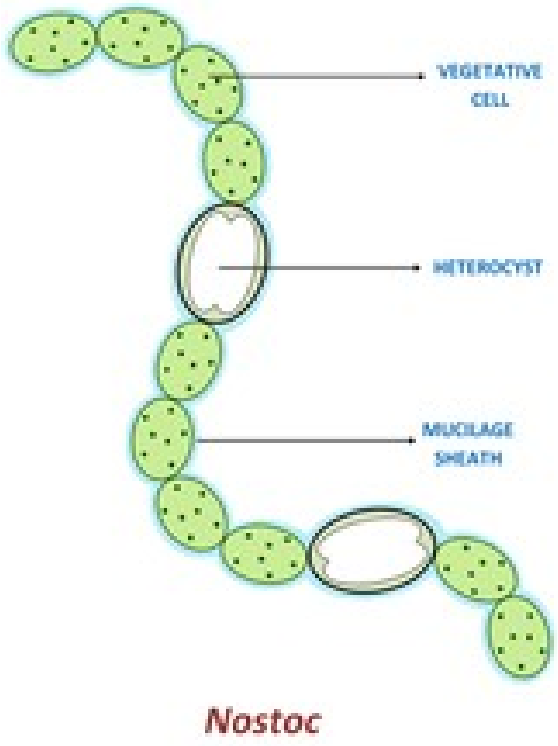

Fig. 5.1: Symbiotic Nitrogen-Fixing Cyanobacteria (Nostoc)

5.2.2. Molecular Mechanisms of Nitrogen Fixation in Cyanobacteria:

The nitrogenase enzyme complex is central to all forms of biological nitrogen fixation, including in cyanobacteria, where it facilitates the conversion of atmospheric dinitrogen (N_2) to ammonia (NH_3). This ammonia serves as a vital source of nitrogen for plant assimilation and supports ecosystems that depend on nitrogen availability. This enzymatic machinery consists of two main components, the Fe protein (dinitrogenase reductase) and the MoFe protein (dinitrogenase), and represents a finely tuned process that is essential for a sustainable nitrogen cycle. The Fe protein, functioning as an electron carrier, plays a pivotal role in the nitrogenase complex. It receives electrons from donor molecules such as ferredoxin or flavodoxin, acting as a conduit for the transfer of these electrons to the MoFe protein. The MoFe protein, housing the iron-molybdenum cofactor (FeMo-co) within its catalytic site, serves as a hub for the reduction of N_2 to NH_3 and catalyzes the multi-step process that transforms inert atmospheric nitrogen into a biologically accessible and valuable nutrient. In Fig. 5.2, inorganic nitrogen mainly includes dissolved nitrogen (N_2), ammonium nitrogen (NH_4^+), nitrite nitrogen (NO_2^-), and nitrate nitrogen (NO_3^-). Molecular nitrogen dissolved in water is only absorbed by nitrogen-fixing bacteria and nitrogen-fixing cyanobacteria in the water. Nitrogen can be converted into a form that plants can use through nitrogen fixation. Generally, phytoplankton utilizes ammonium nitrogen first, followed by nitrate nitrogen, and finally nitrite nitrogen. Nitrogen in the triple-state refers to the triple bond in dinitrogen gas (N_2). In contrast, the three inorganic forms of nitrogen, ammonium (NH_4^+), nitrate (NO_3^-), and nitrite (NO_2^-), are commonly referred to as available nitrogen.

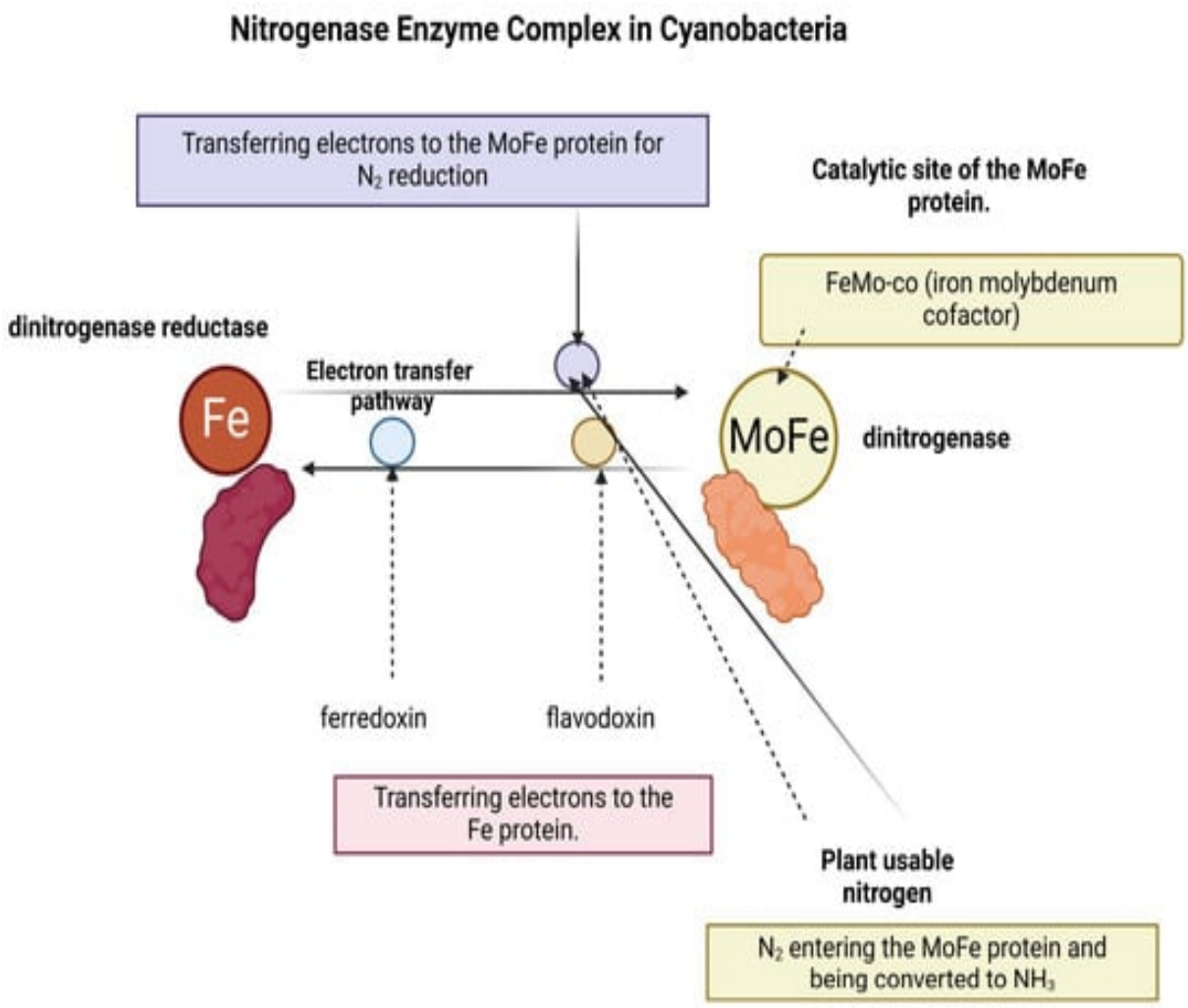

Fig. 5.2: The nitrogenase complex, comprising the Fe protein and MoFe protein with the FeMo-cofactor, facilitates electron transfer and catalyzes the reduction of atmospheric N_2 to NH_3, providing a plant-usable nitrogen form essential for the nitrogen cycle.

5.3. LEGUME-*RHIZOBIUM* ASSOCIATION:

Legume–*Rhizobium* association is a mutualistic symbiotic relationship that occurs between leguminous plants (family Fabaceae) and soil bacteria belonging to genus *Rhizobium* which leads to the formation of root nodules. The major biological system of symbiotic nitrogen fixation. Leguminous plants are belonging to family Fabaceae (Leguminosae), Examples: Pea, Bean, Gram, Groundnut, Soybean, Clover and Alfalfa. A characteristic feature is ability to form root nodules.

5.3.1 *Rhizobium*-General Features:

Gram-negative, rod-shaped, aerobic, motile bacteria in soil. Free-living in soil but symbiotic inside the nodules. Do not fix nitrogen freely in soil. Fix nitrogen only in symbiotic association with the host plant. Symbiosis is mutually beneficial. Plant benefits: Receives fixed nitrogen in usable form. Bacteria benefit: Receive carbohydrates, a protected environment supply of minerals.

Sequence of Events:

Root hairs release flavonoids. Rhizobium is attracted chemotactically. Bacteria produce Nod factors. Root hair curls around bacteria. Formation of an infection thread. *Rhizobium* multiplies inside the infection thread. Bacteria enter cortical cells. Formation of nodule primordium and development of mature root nodules.

5.3.2. Nitrogenase Rhizobia Complex, Cross-Inoculation Groups:

The reduction of nitrogen to ammonia is catalyzed by the enzyme complex nitrogenase, the synthesis of which requires nif gene. Nitrogenase complex consists of two major protein components; a Mo-Fe protein (Dinitrogenase, $\alpha_2\beta_2$ heterotetramer, MW 220,000) joined with one or two Fe proteins (Dinitrogenase reductase, homodimer, MW 64,000). Fe-protein is first reduced by ferredoxin, it then binds ATP. ATP binding changes the conformation of the Fe protein and lowers its reduction potential, enabling it to reduce the Mo-Fe protein. ATP is hydrolyzed when this electron transfer occurs. Finally, reduced Mo-Fe protein donates electrons to atomic nitrogen. Reductive processes are extremely sensitive to O_2 and must occur under anaerobic conditions even in aerobic microorganisms. Protection of the nitrogen fixing enzyme is achieved by means of a variety of mechanisms, including physical barriers, such as heterocysts in some cyanobacteria, O_2 scavenging molecules, such as leg-haemoglobin in nodules, and high rates of metabolic activity. The nitrogenase enzyme complex that reduces N_2 to NH_3 is oxygen labile. Carries out the reaction: $N_2 + 8H+ + 8e- +$ 16 ATP $2\ NH_3 + H_2 +$ 16 ADP + 16 Pi At least 8 electrons and 16 ATP molecules, 4 ATPs per pair of electrons, are required. The electrons come from ferredoxin that has been reduced in a variety of ways: by photosynthesis in cyanobacteria, respiratory processes in aerobic nitrogen fixers, or fermentations in anaerobic bacteria. Nitrogenase also reduces protons to H_2. The H_2 reacts with diimine (HNNH) to form N_2 and H_2. This futile cycle produces some N_2 even under favorable conditions and makes nitrogen fixation even more expensive. Once molecular nitrogen has been reduced to ammonia, the ammonia can be incorporated into organic compounds. In the symbiotic nitrogen fixer Rhizobium, it appears that ammonia diffuses out of the bacterial cell and is assimilated in the surrounding legume. The primary route of ammonia assimilation seems to be the synthesis of glutamine by the glutamine synthetase-glutamate synthase system.

Infection:

Whether native to the site or introduced through inoculation, rhizobia must be able to survive in the soil until they infect the roots of a plant. Generally, these microorganisms survive well in soil, but their numbers can be reduced by acidity, drought, high temperatures, or other stress conditions. If the rhizobia are compatible with a given legume species, they will multiply in the root zone and attach to the root hairs of the plants. The root hairs are fine structures on the roots that absorb water and nutrients. After the rhizobia attach, they use the root hair as an entry point into the plant (Figure 5.3). In some cases, rhizobia may also enter through "cracks" or breaks in the root surface where lateral roots emerge. The rhizobia enter the plant by forming an infection tunnel, or infection thread, through several cell layers to the site where a nodule will develop. Once inside the plant, the rhizobia are protected to some extent from stresses in the outside environment.

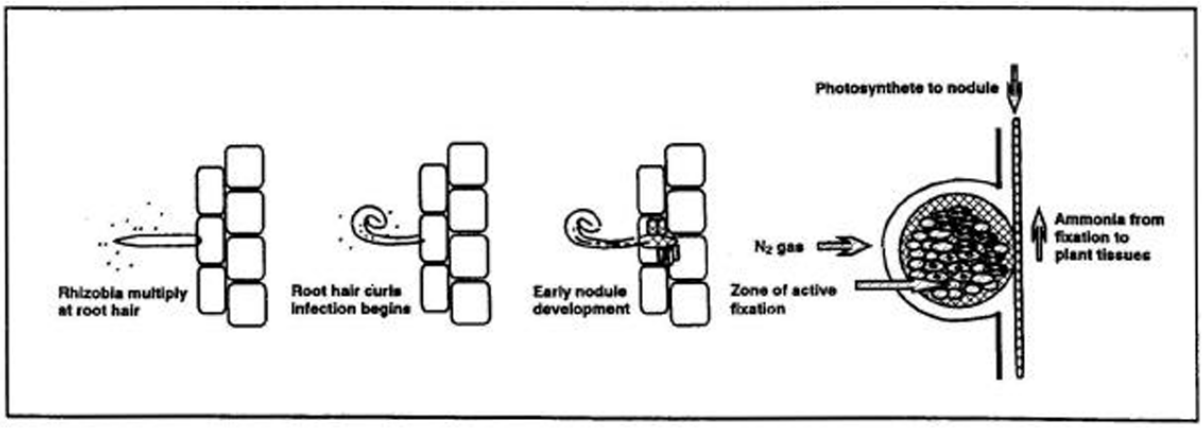

Fig 5.3: Stages of Infection, Nodule Development and Nodule Formation

Nodule Development:

The rhizobia end their journey at the site of the future nodule. There, special plant tissues develop around them. These include connective (vascular) tissues through which the plant feed sugars to the rhizobia and the rhizobia feed nitrogen back to the plant. As these and other tissues develop, the root begins to swell and the nodule becomes visible. In the field, nodules are visible within 21 to 28 days from emergence of the plant. The time from planting to the appearance of nodules varies depending on plant growth and availability of mineral nitrogen in the soil. Nodules differ in shape, size, color, texture, and location. Their shape and location depend largely on the host legume. Figure 5.4 shows some of the common nodule shapes, including spherical, finger-like, and fan-shaped. A few species belonging to the genera *Sesbania*, *Aeschynomene*, and *Neptunia* also form nodules on the plant skins.

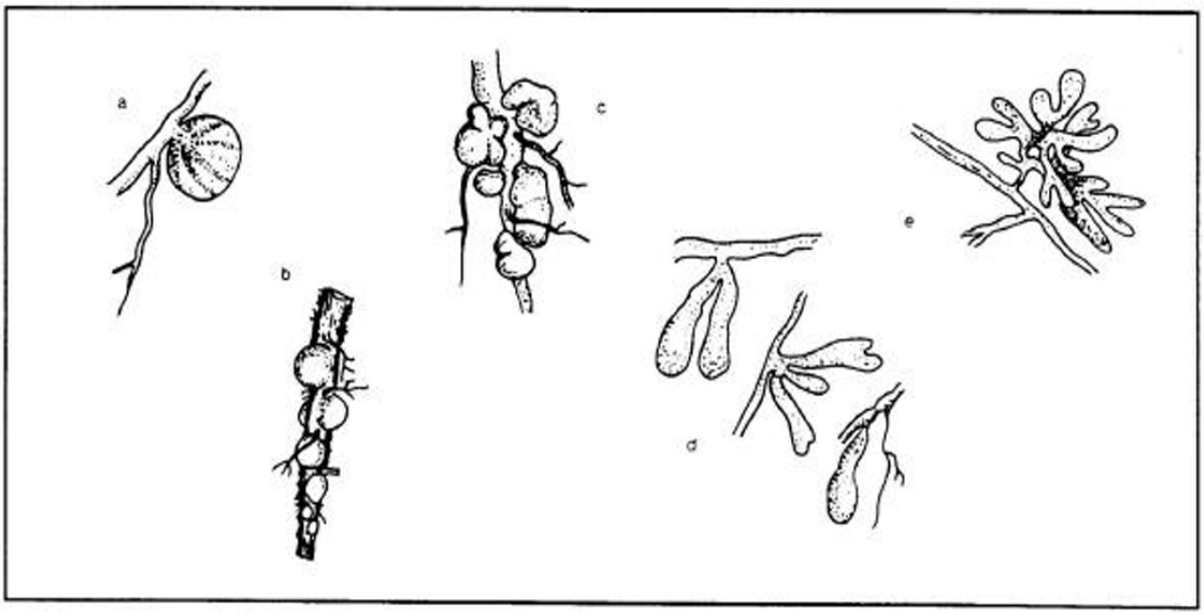

Fig. 5.4: Some representative shapes of leguminous nodules. Spherical: a. globose and streaked, e.g., *Glycine max*, *Calopogonium* and *Vigna radiata* and *Psophocarpus*. Finger-like forms: d. elongate and lobed, e.g., *Leucaena* and *Mimosa*. Fanshaped: e. coralloid, e.g., *Crotalaria* and *Calliandra*.

Nodule Function:

Within the developing nodule, the rhizobia become swollen. At this stage they are called bacteroids. In a cycle depicted in Figure 4-3, Nitrogen gas (N_2) from the soil atmosphere reaches the bacteroids through pores in the nodule. The bacteroids produce the enzyme nitrogenase, which they use to convert N_2 to NH_3 (ammonia). The ammonia attaches to a compound provided by the plant, forming amino acids. These amino acids move out of the nodule to other parts of the plant where they undergo further changes. They are mainly used to produce proteins. The bacteroids need large amounts of energy to support their nitrogen-fixing activity. The plant provides energy as sugars, produced through photosynthesis. It is estimated that the legume-rhizobia symbiosis requires about 10 kg of carbohydrates (sugars) for each kg of N_2 fixed. Clearly, the plant must be healthy to supply enough energy to support BNF. In addition to sunlight, it must have enough water and other nutrients. As discussed in Module 3, legume plants will generally produce nodules in response to several different strains of rhizobia, but not all these strains will be fully effective in fixing nitrogen. Some will be poor nitrogen fixers, many mediocre, and a few will be very good. Some strains may even induce nodulation but will not fix nitrogen at all. Inoculant obtained from a reputable source should contain only rhizobial strains that are highly effective nitrogen fixers. Nodules produced by effective rhizobia are usually large. They tend to be located in the upper portion of the root system on the primary and lateral roots. In annual legumes, the number and size of nodules reach a peak about the time of flowering. Nitrogen fixation is also at its peak at this time. By contrast, nodules produced by ineffective rhizobia tend to be small. They are often quite numerous, scattered throughout the root system. Young, healthy nodules that are providing nitrogen to the plant are often pink or red inside. As they age, they may contain white, green, and red areas, all within a single nodule. Ineffective nodules tend to be white or light green inside throughout the growing season, and they are often smooth textured (Fig. 5.5).

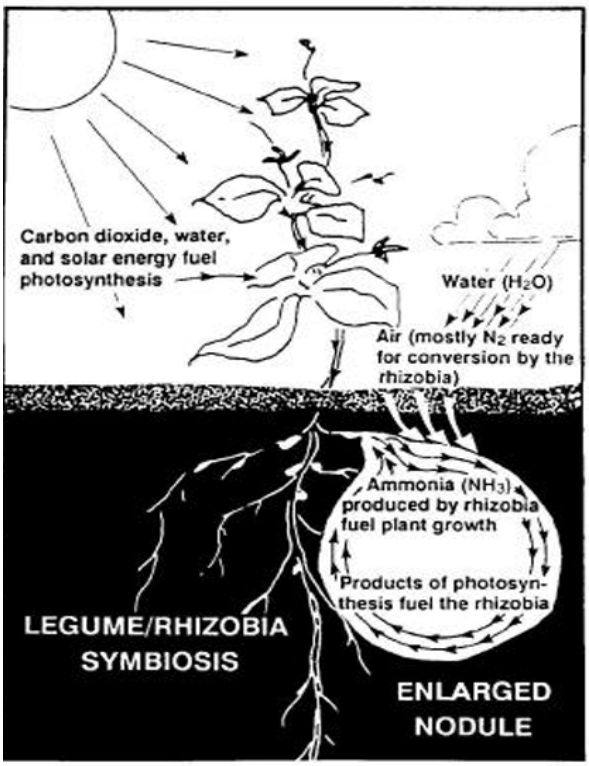

Fig. 5.5: The Legume-Rhizobia Symbiosis

Nodule Senescence:

Nodules eventually age and decay. Their life span is largely determined by four factors: the physiological condition of the legume, the moisture content of the soil, the presence of any parasites, and the strain of rhizobia forming the nodule. As an annual legume approaches maturity, it fills developing seeds with nutrients and storage compounds. As the plant puts more energy into seed production, the nitrogen-fixing activity of the bacteroids decreases. Eventually the nodules stop functioning and disintegrate, releasing bacteroids into the soil. Given favorable conditions, these rhizobia may survive and infect new plants during the next cropping season. However, in intensive agricultural systems it is usually necessary to add rhizobial inoculant with every crop. Plants may shed their nodules early if affected by severe drought. Forage legumes also shed nodules after heavy grazing, but these species can often produce new nodules. Finally, some crops may be susceptible to parasites, such as weevil larvae, that feed on root nodules.

5.4. DEVELOPMENT, STRUCTURE AND FUNCTIONS OF LEGUME ROOT NODULES:

The majority of legumes is Symbiotic biological nitrogen fixers and contains symbiotic nitrogen fixing bacteria *Rhizobium*, within small, knob like protuberances called root nodules on their roots, producing nitrogen compounds that help the plant to grow and compete with other plants. Only a specific *Rhizobium* species infects a particular leguminous plant. When the legume plant dies, the fixed nitrogen is released, making it available to other plants; this helps to fertilize the soil. The root nodules vary in their shape and size. They may be spherical, elongated, flat and grooved or may have finger like projections. Their size varies from pin head to one centimeter in diameter and are brown, red or pink in colour. There may be hundreds of nodules on the roots of a single plant. Root nodules acts as a site of N_2 fixation in legumes.

Depending upon growth, the genus Rhizobium has been divided into two major groups: (1) *Rhizobium*: It is a generic name which includes all fast-growing species. (2) *Bradyrhizobium*: It is also a generic name which includes all slow growing species. The Symbiotic nitrogen fixing leguminous bacteria (both the above genera) are gram negative, non-spore forming micro-aerobic and host specific. The root nodules in legume plants are produced due to infection of bacteria *Rhizobium*. This free-living soil bacterium usually grows near the roots of the legumes and is unable to fix nitrogen in free condition. It fixes nitrogen only when it enters into the root and is present inside root nodules. The roots of leguminous plants secrete some special types of lectins. The plant lectin of a given species can interact with polysaccharides of only a particular plant. The actual site, where the interaction takes place leading to the formation of nodules lies between the root tips and young root hair. The bacteria enter into the host through young root hairs. Prior to entry, it secretes some hormone like substances which causes deformation and curling of young root hairs. First a tubular infection thread is formed in the root hair cell and the bacteria enter into this thread, a new cell wall is formed which separates the bacteria from the contents of the host cells. The tubular infection thread contains mucopolysaccharides in which the bacteria get embedded and start multiplying. The infection threads containing bacteria and from root hair cell reaches to the cells of the inner layers of cortex where the bacteria are released. These bacterial cells induce the cortical cells to multiply which results in the formation of nodules on the surface of the roots. The bacterial cells also multiply and colonize inside the multiplying host cells. As the available space in the host cells is completely filled, the

bacterial cells become dormant and are called bacteroids. The bacteroids usually occur inside the cytoplasm in groups. Each group of bacteroids is surrounded by a membrane called peribacteroid membrane. The space surrounded by peribacteroid membrane is called peribacteroid space. A red pigment leg-haemoglobin is filled outside the peribacteroid space in the cytosol of the nodule cells. It means that the dormant bacteroids float in this red pigment. Leg-haemoglobin is a proteinaceous pigment, and the characteristic red, pink or brown colour of the nodules is due to this pigment. It has the ability to combine very rapidly with o2 thus acts as a very efficient scavenger. It protects the key enzyme nitrogenase, against oxidative inactivation and at the same time allows the bacteroids to carry on oxidative ATP generation which is essential for nitrogen fixation. The pigment is a symbiotic compound is made up of apoprotein part and heme molecule. The apoprotein part is synthesized under the genetic direction of the plant and heme pert under rhizobial gene. Recent studies have shown that leg-haemoglobin is not essential requirement but plays a helpful role in enhancing the efficiency of nitrogen fixation in leguminous plants. Nodules establish a direct vascular connection with the host for exchange of nutrients. A special vascular system develops in the host, supplying product of photosynthesis to the nodule tissue and carry away fixed nitrogenous compounds to other parts of the plant (Fig. 5.6 and 5.7).

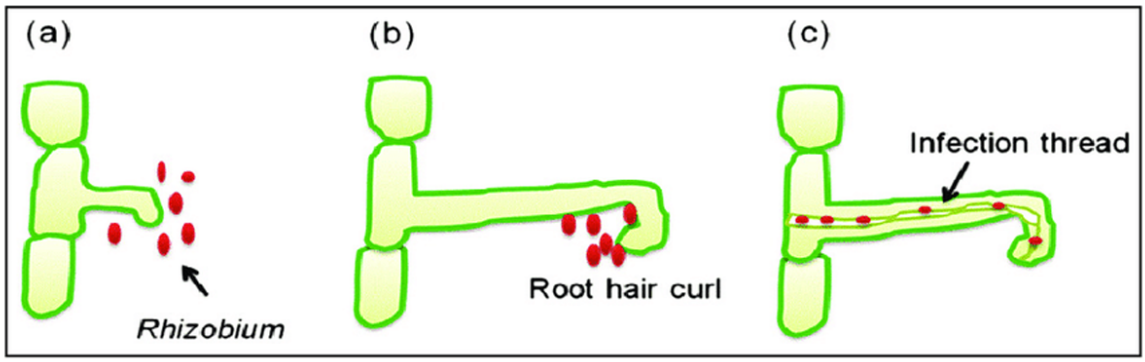

Fig. 5.6: Formation of Infection Thread

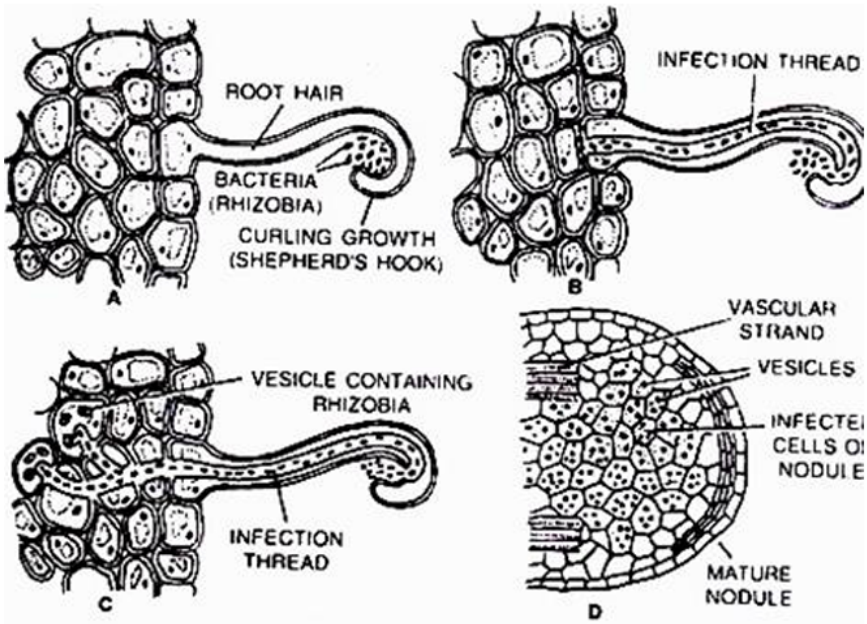

Fig. 5.7: Formation of Root Nodules

5.4.1. Factors Affecting Nitrogen Fixation:

Legumes are diverse in growth habit, size, and length of growing season. They also differ in the amounts of nitrogen they can fix, even under ideal conditions. The NifTAL/FAO manual gives a more extensive list of legume species and the amounts of nitrogen they fix. Legumes can obtain nitrogen from three sources-soil nitrogen, native rhizobia, and rhizobia introduced as inoculants. In most cases, legumes will obtain some of their N from the soil, even if they fix high amounts of N_2. As long as other plant health factors (water, pests, nutrients, etc.) are not limiting, the amount of nitrogen fixed by legume plant depends on the abundance and longevity of the root nodules, the effectiveness of BNF within the nodules, and the level of available soil nitrogen and as a general principle, nitrogen fixation goes up as soil nitrogen goes down, and vice-versa. Given high levels of nitrogen in the soil, plants may not form nodules at all, or they may reduce or cease nitrogen-fixing activity in the nodules already formed.

5.5. PHOSPHATE SOLUBILIZING MICROORGANISMS AND THEIR USES:

Phosphorus is second only to nitrogen in mineral nutrients most commonly limiting the growth of crops. Phosphorus is an essential element for plant development and growth making up about 0.2 % of plant dry weight. Plants acquire P from soil solution as phosphate anions. However, phosphate anions are extremely reactive and may be immobilized through precipitation with cations such as Ca^{2+}, Mg^{2+}, Fe^{3+} and Al^{3+}, depending on the particular properties of a soil. In these forms, P is highly insoluble and unavailable to plants. As the results, the amount available to plants is usually a small proportion of this total. Several scientists have reported the ability of different bacterial species to solubilize insoluble inorganic phosphate compounds, such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite, and rock phosphate. There are microorganisms in soil that can solubilize the unavailable phosphorus and make it available to plant. They are called Phosphate solubilizing microorgamisms (PSM). A group of fungi associates with the roots of higher plants and mobilize the phosphorus from soil to the plant system. Phosphate solubilizing microorganisms majority of agricultural soils contain large reserves of phosphorus of which a considerable part has accumulated as consequence of regular applications of P-fertilizer. Mostly they are associated with the plant rhizosphere, so they are called as rhizobacteria. This group of bacteria has been termed plant growth promoting rhizobacteria, and among them are strains from genera such as *Alcaligenes*, *Acinetobacter*, *Arthrobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Paenibacillus*, *Pseudomonas*, *Rhizobium*, and *Serratia*. The phenomenon of fixation and precipitation of P in soil, which is highly dependent on pH, causes a low efficiency of soluble P fertilizers. In acidic soils P is precipitated as Al and Fe phosphates whereas in calcareous soils high concentration of Ca results in P precipitation. The soil is a habitat for diverse group of organisms that employ variety of solubilization reactions to release soluble phosphorus from insoluble phosphates. The potential of these phosphate solubilizing microorganisms has been realized and are utilized as bioinoculants for crop grown in soils poor in available P and amended with rock phosphate or tricalcium phosphate. Phosphorus solubilizing microorganisms include various bacterial, fungal and actinomycetes forms which help to convert insoluble inorganic phosphate into simple and soluble forms. Members of *Pseudomonas, Micrococcus, Bacillus, Flavobacterium, Penicillum, Fusarium, Sclerotium* and *Aspergillus* are some of the phosphate-solubilizing micro-organisms. They normally grow in a medium containing

insoluble tri-calcium phosphate [$Ca_3(PO_4)_2$], apatite, rock phosphate, $FePO_4$ and $AlPO_4$ as sole source of phosphate.

The initial isolation of phosphate solubilizers is made by using Pikovaskaya medium suspended with insoluble-phosphates such as tri-calcium phosphate. The production of clearing zones around the colonies of the organism is an indication of the presence of phosphate-solubilizing organisms (Fig. 5.8). Such cultures are isolated, identified and the extent of solubilization determined quantitatively. Several rock phosphate dissolving bacteria, fungi, yeast and actinomycetes were isolated from soil samples collected from rock phosphate deposits and rhizosphere soils of different leguminous crops. The most efficient bacterial isolates were identified as *Pseudomonas striata*, *Pseudomonas rathonis* and *Bacillus polymyxa* and fungal isolates as *Aspergillus awamori*, *Penicillium digitatum*, *Aspergillus niger* and a yeast-*Schwanniomyces occidentalis*. These efficient micro-organisms have shown consistently their capability to solubilize chemically-fixed soil phosphorus and rock phosphate from different sources - Mussorie, Udaipur, Matoon, Singhbhum, Morocco, Gafsa and Jordan. In addition, these microorganisms were found to mineralize organic phosphorus to soluble form due to enzymatic activity. The efficient cultures have shown capacity to solubilize insoluble inorganic phosphate such as rock phosphate, tri-calcium phosphate, iron and aluminium phosphates by production of organic acids. They can also mineralize organic phosphatic compounds present in organic manure and soils. Inoculation of PSM to seeds or seedlings increases the grain yield of crops. They are known to add 30-35 kg P_2O_5 ha^{-1}. The inorganic phosphate solubilization by microbes can be attributed to acidification, chelation, and exchange reaction in growth medium as well as to the proton transfer during ammonium assimilation.

Applications of Phosphate Solubilizing Microorganisms

- **Biofertilizers:** Inoculating seeds, roots, or soil to boost phosphorus availability, often increasing crop yields by 10% to 40%.
- **Agricultural Sustainability:** Used to complement or replace chemical fertilizers, reducing environmental pollution.
- **Stress Tolerance:** Enhancing plant resilience against abiotic stresses, such as drought or heavy metal toxicity.
- **Soil Health Enhancement:** Improving soil nutrient profiles and increasing the population of beneficial microbes.
- **Synergy with Other Microbes:** Working in tandem with Arbuscular Mycorrhizal Fungi (AMF) to optimize phosphorus uptake (Fig. 5.9).

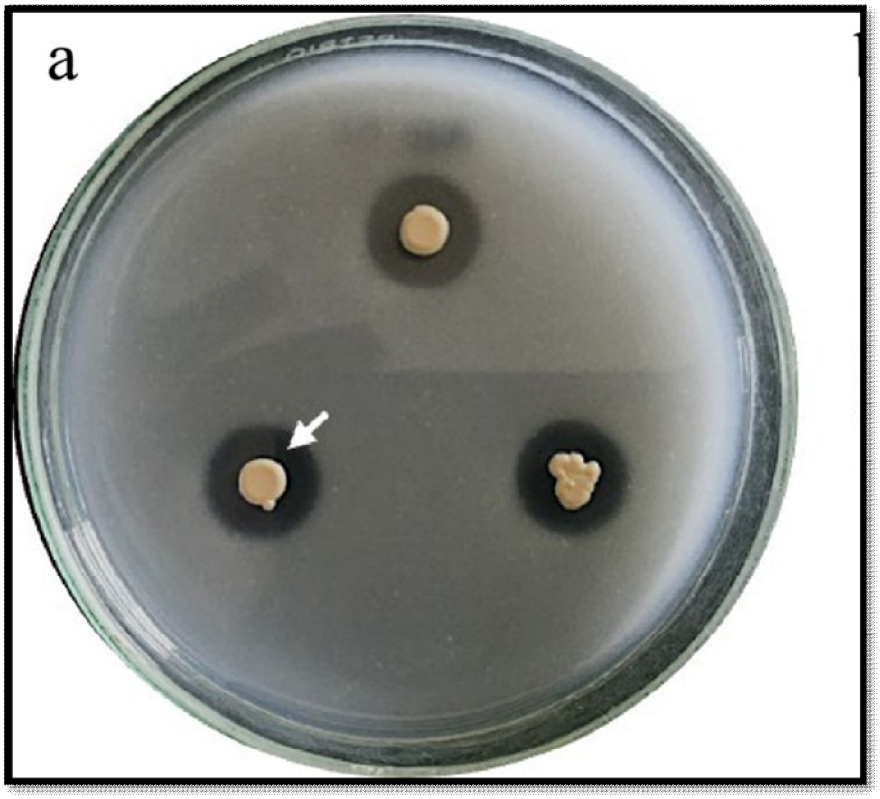

Fig-5.8: Phosphate Solubilization by Bacteria (Formation of a Clear Zone Around the Culture)

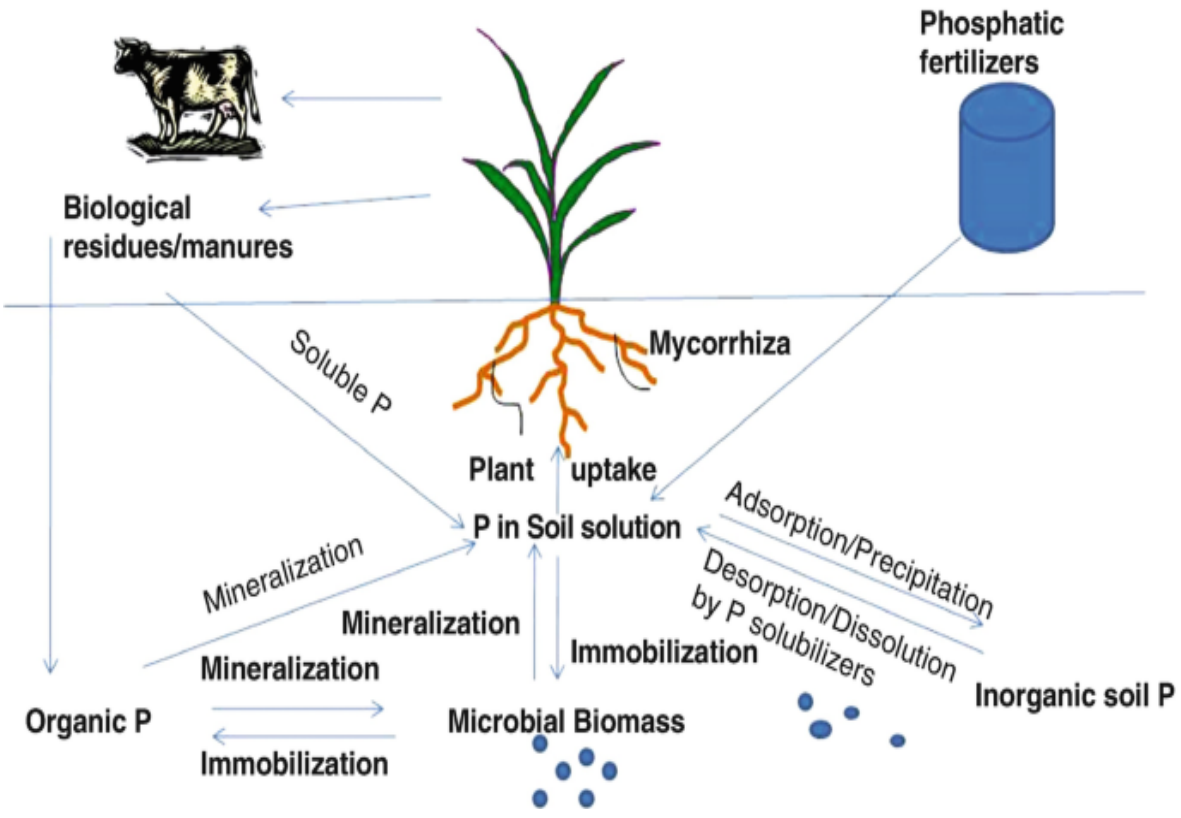

Fig-5.9: Applications of Phosphate solubilizing microorganisms

5.6. SUMMARY:

Atmospheric nitrogen is reduced to ammonia by a reaction catalyzed by nitrogenase enzyme which requires ATP, reducing power, and micro-aerobic conditions. Ammonia is converted into amino acids, amides, translocated to other parts of the plant. Fix atmospheric nitrogen and supply nitrogen to host plant. Improve the plant growth and yield, enhance protein synthesis. Reduce the dependence on nitrogen fertilizers and improve soil fertility. BGA are oxygen evolving, nitrogen fixing prokaryotes using sunlight as the energy source for N_2 fixation. Occurrence varies with climatic factors and soil conditions. Abundant in rice fields as there are high levels of copper sulphate and combine nitrogen in the irrigation water, low occurrence of BGA is reported in Australian paddy fields. Predominant genera are *Anabaena*, *Nostoc*, *Calothrix*, *Aulosira*, *Aphanothece* and *Gloeotrichia*. The cyanobacterial nitrogen fixation has a switch on mechanism which gets activated when the combined nitrogen level falls below 40 ppm which enables algal biomass to produce more of biologically fixed nitrogen as soon as the nitrogen fertilizer level is reduced in the ecosystem due to loss and utilization. It has been observed that the removal of algae from paddy field water greatly reduced the in-situ nitrogen fixation. Application of BGA by farmers can save approximately 40-60 Kg urea as an average consumption of BGA has been found to be 20-30 kg N/ha/season. The phosphate solubilizing microorganism are very important in agricultural soils which can enhance the availability of phosphorus to plants and there by the plan growth. Majority of agricultural soils contain large reserves of phosphorus of which a considerable part has accumulated as consequence of regular applications of P-fertilizer. The phenomenon of fixation and precipitation of P in soil, which is highly dependent on pH, causes a low efficiency of soluble P fertilizers. In acidic soils P is precipitated as Al and Fe phosphates whereas in calcareous soils, high concentration of Ca results in P precipitation.

5.7. TECHNICAL TERMS:

Nitrogen fixation, BGA, *Anabaena*, *Nostoc*, *Calothrix*, *Aulosira*, *Bradyrhizobium*, nitrogen fertilizers, Phosphate solubilizing microorganisms.

5.8. SELF-ASSESSMENT QUESTIONS:

1) Explain Development, Structure and Functions of Legume Root Nodules?
2) Explain in detail about N – Fixing Cyanobacteria?
3) Explain Molecular Mechanisms of Nitrogen Fixation in Cyanobacteria?
4) Discuss about Legume-Rhizobium Association?
5) Discuss Nitrogenase Rhizobia Complex?
6) Write about Phosphate solubilizing microorganisms?

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Dr. J. Madhavi

LESSON-6

CONCEPT AND SYMPTOMS OF PLANT DISEASES

6.0. OBJECTIVE:

- To acquaint the students with the knowledge of plant disease symptoms which make them to identify the diseases practically in fields.

STRUCTURE:

6.1. INTRODUCTION:

If any part of the plant is invaded by the microorganism like fungi, bacteria and viruses, the characteristic visible external morphological and non-visible internal physiological changes occurs in the plant. Any of these altered situations in the plant is regarded as 'disease'. The causal agent, bacteria or fungi or viruses, is referred as pathogen. The external and internal alterations in the infected plants are collectively called as symptoms - external symptoms and internal symptoms. These symptoms are usually characteristic of the pathogen infected. Often the symptoms appearing as a result of a disease, the cause of the disease and the injuries caused to the plant have been considered synonymous. However, they signify only the condition of the plant due to disease of the cause of the disease.

Julius Kuhn (1858) had defined the plant disease as abnormal changes in the physiological processes which disturb the norm activity of the plant organs. A similar definition was given by H.M. Ward in 1896 who defined the disease as a condition in which the functions of the organism are improperly discharged or, in other words, it is a state which is physiologically abnormal and threatens the life of the being or the organ. In 1918, E.J. Butler had defined disease as variation from normal physiological activity which is sufficiently permanent or extensive to check the performance of natural functions by the plant or completion of its development. Wheeler (1975) broadly considered plant disease as all the malfunctions which result in unsatisfactory performance of the plant or which reduce the

ability of the plant to survive and maintain its ecological niche. Stakman and Harrar (1957) defined plant disease as "a physiological disorder or structural abnormality that is harmful to the plant or to any of its part or products that reduces the economic value". Singh *et al* (1989) put up a definition based on the views expressed by several scientists. They defined the disease as "a sum total of the altered and induced biochemical reactions in a system of the plant or plant part brought about by any biotic or abiotic factors or by a virus leading to malfunctioning of its physiological processes and ultimately manifesting gradually at cellular and/or morphological level. All these alterations should be of such magnitude that they become a threat to the normal growth and reproduction of the plant".

6.2. SYMPTOMS OF PLANT DISEASES:

For practical purposes, the diseases are classified according to their symptoms, plant organs they affect or the mode of survival and dispersal of their propagules. A disease may be localized affecting only the special organs or parts of the plant, or it may be systemic affecting the entire plant, i.e., the pathogen moves through the entire plant. Based on the symptoms, the diseases may be called rusts, smuts, wilt, blight, canker, mildew, root rot and fruit rots. When the disease symptoms appear most conspicuously on special organs, they may be called leaf spot, stem lesions, root rot, fruit rot etc. The diseases may be grouped as cereal diseases, forage crop diseases, flax diseases, root crop diseases, plantation crop diseases, etc., based on the host plants. When a disease-causing agent survives and spread through soil it is known as soil-borne pathogen. Diseases or pathogens perpetuated through seed are known as seed-borne diseases. When dispersal is through the agency of air, the pathogen is known as air-borne. The same air-borne pathogen may be soil-borne, seed-borne and air-borne.

During invasion, the pathogens induce reactions in the body of the host. As a result of these reactions certain abnormalities appear on the plant. In addition, the pathogen itself may become visible on the host surface giving it an abnormal appearance. The abnormalities, signs or evidence of the disorder are known as symptoms of the disease. Since the origin of these symptoms is mainly from internal disorders and many kinds of pathogens can produce same type of disorder the symptoms resulting from host-pathogen interactions are not very reliable basis for identification of plant diseases although they do help to some extent. On the other hand, when the pathogen itself becomes visible on the host surface, it gives more reliable information about its own identity and identification of the disease becomes easy.

6.2.1. Symptoms Caused by Fungi:

Some of the important external symptoms of plant diseases caused by plant pathogenic fungi are leaf spots, mildew, rusts, smuts, white rust, blotch, sclerotia etc.

Leaf Spots:

Leaf spots are the necrotic symptoms of plant diseases. Necrosis is most commonly defined as cell death that results from exposure to highly toxic compounds, severe cold or heat stress, or traumatic injury that lead to the immediate damage to membranes or cellular organelles. The characteristic appearance of the dead area differs with different hosts and host organs and with different parasites. The leaf spots vary in color, size, shape, margin and

pattern. They may be isolated or coalesce based on severity of the disease. Spots may occur on leaves, stems, leaf sheaths, petioles, floral parts and fruits. In leaf spot diseases, the cells are killed in definitely limited areas and dead tissues usually become brown. Sometimes, they may appear as concentric rings as target spots e.g. *Alternaria* leaf spots. Yellowing may precede the death of the cells. Yellow halo is often present around the necrotic spots - E.g. early leaf spot of groundnut. In some leaf spot symptoms, the dead central portion of spot falls off giving an appearance of shot-hole.

Mildews:

In these diseases, the pathogen is seen as white, grey, brownish or purplish growth on the host surface. In 'downy mildew', the superficial growth of the fungus is seen on the lower surface of the leaf while in powdery mildew enormous numbers of spores are formed on the upper surface of the leaf, giving the hos surface a dusty or powdery appearance. Black, minute fruiting bodies may also develop in the powdery mass e.g. downy mildew of peas, crucifers etc., and late blight of potato, blight of *Colocasia* etc.

Rusts:

These diseases produce rusty symptoms. The rusts appear as relatively small pustules of spores, usually breaking through the host epidermis. The pustules may be dusty or compact and red, brown, yellow or black in color. The color is due to mass of spores of the fungus. Mostly basidiomycetous fungi produce this type of symptoms – e.g. rust of beans, pea, coffee, rusts of wheat etc.

Smut:

In plant diseases known as smut, the affected parts of the plant show a black or purplish-black dusty mass of spores. In some smuts, this mass of spores may be compact and held under the epidermis appearing as black streaks or blotches on the host surface. The symptoms of smut usually appear on floral organs, particularly the ovules and the pustules are usually considerably large than those of rusts. Smut symptoms may also appear on stems, leaves as well as roots. Mostly basidiomycetous fungi produce this type of symptoms. E.g. loose smut and flag smut of wheat, leaf smut of rice, loose smut of sorghum etc.

White Rust:

On leaves of crucifer and many other plants, numerous white, blister-like pustules are found. They break open the epidermis and expose white powdery masses of spores. Often, such symptoms have been called white rust. Since there is nothing common between them and the true rusts, they are more appropriately called as white blisters. *Albugo* and *Cystopus* species cause this type of symptoms.

Blotch:

It is a spot or mark which is larger and irregular. They appear as tiny purplish-reddish spots on leaf blades and leaf sheaths. These symptoms consist of a superficial growth giving the fruit a blotched appearance as in the sooty blotch of apple fruits. Initially, the symptoms on leaves appear as dark green circular patches and later leaf tissue necrosis takes place resulting in dark brown spots.

Sclerotia:

A sclerotium is a compact, often hard, mass of dormant fungus mycelium. In some diseases, as in ergot of cereals, the sclerotium assumes a characteristic horn-like shape but in others, the shape may be more variable. The sclerotia are most often dark colored (dark brown to black). Presence of these sclerotia on the host surface or within the tissues helps in identification of the pathogen and the disease.

Wilt:

In many diseases, the characteristic symptom is gradual or sudden drying or wilting of the entire plant. The leaves lose their turgidity, become flaccid and droop. Later the young growing tip or the entire plant wilts. Wilting may be the result of injury to the root system, to partial plugging of the wager conducting vessels or to toxins produced by the pathogen and carried to delicate tissues – e.g. *Fusarium* wilt of cotton.

Streaks or Stripes:

These are elongated but relatively narrow lesions containing dead cells. They are also initially yellowish in color before cell death occurs. Usually occurs on leaf blade, leaf sheath, stem and other parts. These symptoms are limited in number and size.

Blight:

Leaf spots and leaf blights resemble each other considerably in appearance but the area covered is different. In the leaf blight, a large area of leaf or the whole leaf is involved and sometimes evens the entire plant. The blighted ones may soon fall off. E.g. early blight of potato.

Damping off:

It is a condition in which the tender stem of seedlings is attacked near the soil surface. The affected portion becomes constricted due to necrosis of the cortical tissues and the stem is unable to bear the load of upper portion. The seedlings will collapse.

Rot:

The affected tissues die, decompose to a great extent and turn brown. The condition is brought about by fungi and bacteria which dissolve the middle lamella between cell walls by means of enzymes – e.g. *Rhizoctonia* root rot of cotton, fruit rot of cucurbits, stem rot of papaya etc.

Anthracnose:

A particular group fungus causes ulcer-like lesions on fruit, pods, leaf veins, petioles and stem – e.g. anthracnose of grapes and mango caused by *Colletotrichum* species.

6.2.2. Symptoms Caused by Bacteria:

In contrast to fungal pathogens, bacteria are incapable of mechanically penetrating the cutinized plant tissues. They can make entry through non-cutinized areas such as root hairs, stigmas, natural openings like stomata and wounds. In most bacterial diseases (bacterial blight of rice, bacterial leaf streak of rice, fire blight of pear and apple, bacterial cankers of stone fruits) masses of bacterial cells come out as ooze on the affected host surface where they may be seen as drop or smear.

Local Lesions:

Bacterial lesions or localized spots occur on the leaf blade and sometimes in the petiole, stem etc. Initially, the leaf sports appear as water-soaked lesions, later spread rapidly to form circular, irregular or angular spots restricted by veins and vein lets. This necrosis may spread rapidly in certain cases such as in fire blight of apples and pears.

Soft Rots:

The soft rot symptoms are found mainly on fleshy parts. The major effect is the softening of tissues due to disintegration of cells and dissolution of middle lamella as a result of the action of enzymes and very often dirty liquid oozes out of the effected parts – e.g. black rot of crucifers, brown rot of potato etc.

Vascular Disease:

In some of the bacterial leaf spots, the organism moves into the vascular tissues and becomes systemic. In others, the invasion is concentrated in the vascular tissues, causing typical wilt of the effected plants. Due to vascular infection there may be hypoplasia, leading to the stunting of the entire plant. Common examples are cucurbit bacterial wilt, brown rot of potato, ring rot of potato, bacterial wilt of tomato, etc. The pectinolytic and cellulolytic enzymes produced by these bacteria are responsible for the wilt symptoms.

Tumors and Galls:

In many diseases, the effect of invasion by the pathogen is the hyperplasia and hypertrophy of invaded tissues. As a result, tumors develop on the affected organs. The formation of root nodules in leguminous roots in response to bacterial action has also a similar effect. Crown gall caused by *Agrobacterium tumefaciens* is the best example.

Scabs and Cankers:

These are the corky outgrowths which are formed on leaves, twigs and all other plant parts above the ground. These outgrowths are the result of the reactions of the host tissues to the pathogen. Such reactions are mostly localized and often confined to the parenchymatous tissues of the plant.

Scab is formed by epidermal infection and is not deep seated. The powdery scab of potatoes is a familiar example. Cankers, on the other hand, are deep seated and involve the cambium layer. A canker is a dead area in the bark or cortex of the stem of woody and sometimes on leaves also. Usually, they are large with a definite margin. Some cankers are only superficial or they may involve all the tissues except the fibers. The surface of the cankers may be smooth but generally in later stages of development, they become rough with a sunken center. On woody parts, cankers cause splitting of the bark which may peel away. In some diseases, the fruiting bodies of the fungus are seen after the bark is destroyed. E.g. Citrus canker.

6.2.3. Symptoms Caused by Viruses

The symptoms of plant virus diseases are of major importance in the identification of the virus. Because, major viruses have been named on the basis of chief symptoms they produce on the host on which they were first reported. The symptoms produced by viral infections may be externally visible on all the aerial parts or internally inside the cell of the infected plant. The external visible symptoms caused by virus infection may be considered under two headings – those resulting from the primary infection in the inoculated cells of the host plant, and those caused by secondary or systemic infection as the virus which moves from the site of primary inoculation into the other parts of the plant.

The symptoms which develop near the site of entry are known as local symptoms and are usually in the form of local lesions. Local lesions are mostly seen in mechanically transmitted viruses. *Chenopodium amaranticolor* serve as local lesion host of several viruses. Characteristically, plant viruses are systemic in nature moving from one cell to another through plasmodesmata and long distance spread through the vascular system. The most common symptom of the virus infection is reducing plant size. Degree of stunting is usually correlated with the severity of the symptoms. Almost all parts of the plant show reduction in size. Some of the common symptoms of viral infections are – mosaic, chlorosis, ring spot, necrosis, abnormalities in leaf, stems, roots and flowers.

Mosaic:

The most common symptom of virus infection is the development of a pattern of dark green areas and light green areas giving a mosaic effect in infected leaves. These symptoms are due to disturbance and decreased chlorophyll – e.g. mosaic disease of tomato, cabbage etc. On contrary, when uniform reduction of chlorophyll symptom is referred to be as yellows which is believed to be caused by Mycoplasmas.

Chlorosis:

Sometimes due to virus infection, the whole leaf may become yellow, due to loss of chlorophyll and breakdown of chloroplasts. Interspersion of various degrees of chlorosis with normal green color of leaf results in mosaic with yellow and green colors. Sometimes, the area adjacent to the vein of the leaf remains green in contrast to the reminder of the leaf and is referred as vein-banding. If the chlorosis is confined to the area of vein, it is called vein-clearing. E.g. Yellow vein mosaic of bhendi.

Ring Spots:

These are the brown colored spots on the leaf lamina due to the death of the tissue. These are localized spots comprises various types of chlorosis and necrosis. Chlorotic ring spots contain circular chlorotic areas and necrotic ring spots shows necrosis in rings alternating with normal green areas. The centers of either type of spots finally become necrotic. Viruses producing this type of ring spot symptoms are mostly transmitted by soil microorganisms. The plants that show this type of symptoms, example virus infected tobacco ring spot on *Nicotiana rustica*, show the tendency to recover from the disease after an initial shock period.

Necrosis:

Necrosis may also occur chlorophyll-less leaves, hytpcotyls, seeds, fruits, tubers and whole plant. If the necrosis resulted in the collapse of the superficial layers of cells, it is called as etching. Sometimes, death of epidermal cells may cause bronzing symptoms. These types of spots are common with Poty virus X and Poty virus Y.

Enations:

Some virus infections are characterized by tumor like outgrowths on leaves or roots. The outgrowths on the leaves are commonly referred to as enations and these appear like 'warts' on the upper and lower surfaces of the leaves. They are common in pea plants infected with pea enation mosaic virus. Such growth is caused by abnormal cell proliferation which is probably due to virus induced changes in hormone concentration. Foliar outgrowths and vein swellings are generally occur on underside of leaves – e.g. Tobacco leaf curl disease, Fiji disease of sugar cane. Tumors of swellings on stems and roots are seen in sweet clover caused by wound tumor virus and cocoa caused by swollen shoot virus.

Hypoplasia:

In contrast to hypertrophy, the decrease in number of cells is referred to as hypoplasia. An example of hypoplasia is stem pitting virus infection in apple and citrus, caused by apple stem pitting virus. This is caused by the failure of some cambial cells to differentiate normally, which results in a wedge of phloem being embedded in the developing xylem. The pitting is visible as elongated pits or furrows on the surface of the stem when the bark is removed.

Flower Breaking:

Virus-induced color break symptoms in tulip petals caused by tulip mosaic virus. Such color break symptoms are also common in flowers of other infected plants, including wall flowers (*Cheiranthus cheiri*) and stocks (*Mannihot indica*) infected with turnip mosaic virus, and gladiolus infected with bean yellow mosaic virus.

Symptoms on Fruits:

Fruits from the infected plant may be dwarf, smaller, misshapen or of changed texture. For example, fewer fruits are produced in plums infected with prune dwarf virus, and sugar beet curly top virus infection results in smaller cantaloupe fruits.

Wilt:

In certain virus-infected plants, the outer whorls of leaves droop and become yellow and inner whorls also become pale yellow in color. The leaflets soon turn to brown and start drying up from their tips.

Stunting:

In this**,** all parts may affect and involves reduction in size of leaves, flowers, fruits and shortening of petioles and internodes – e.g. bean yellow mosaic virus on bean. The extent of stunting varies according the occurrence of infection in development stage of plant. Sometimes, some parts of plants may be more stunted than those of some parts in other plants.

Fruit Abnormalities:

Variety of symptoms may develop on fruits in certain viral infections.E.g. – Mottling on cucumber and papaya due to mosaic type of virus.

Star crack in apples and plums due to plum pox virus.

6.3. SYMPTOMS RESULTING FROM INTERNAL DISORDERS:

Color Changes:

Changes of color from the normal or discoloration is one of the most common symptoms of plant diseases. The green pigment may disappear entirely and its place taken by yellow pigment. When this yellowing is due to lack of light, it is called as Etiolation. This etiolation takes place in partial or complete absence of light resulting in long, weak stems and smaller leaves due to larger internodes. A similar condition may also results from the influence of low temperature, lack of iron, excess of lime or alkali in the soil and infection of viruses, fungi and bacteria. These conditions interfere with synthesis of chloroplasts. Such a yellowing is known as chlorosis. Change of color to red, purple or orange is chromosis. Sometimes, the leaves are devoid of any pigment and look bleached or white. This condition is known as albinism.

Overgrowth and Hypertrophy:

Many pathogens, through their biochemical activity induce hormonal imbalance. This results in excessive growth of host tissues and causes abnormal increase in size of affected organs. It is brought about by one or both of the two processes known as hyperplasia and hypertrophy. Hyperplasia is the abnormal increase in the size of plant organs due to increase in the number of cells at a given location is much higher than normal. In hypertrophy, the increased size of the organ is due to increase in size of cells of a particular tissue. The pathogen may dissolve the intervening walls between the cells and its products may cause the cells to increase in size. Often the cells increase in size to accommodate the fungal structure. The overgrowth and its effect are seen in galls, curl, pockets or bladder, hairy root, witches' broom, intumescence, etc. E.g. club root of cabbage, wart disease of potato, stem gall of coriander etc.

Atrophy or Hypotrophy or Dwarfing:

In many diseases one of the results is the inhibition of growth resulting in stunting or dwarfing. The internodes fail to elongate resulting in 'Rosetting' condition where the leaves appear close together. The whole plant may be dwarfed or only certain organs may be so affected. Sometimes, hypertrophy and atrophy occur together in the same organ such as flowers.

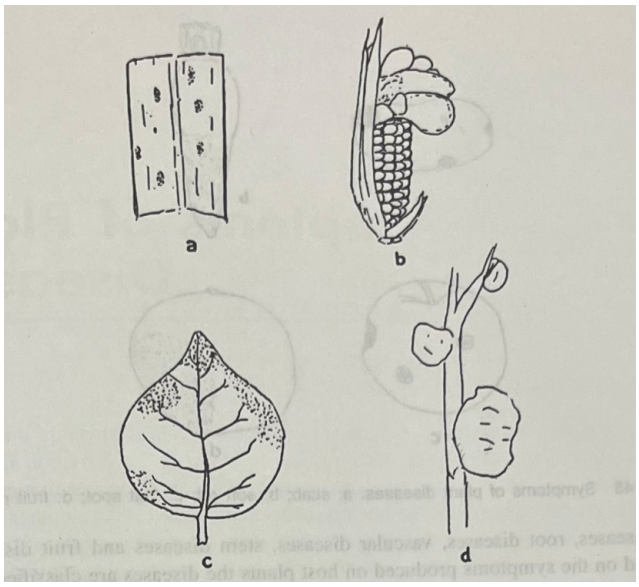

Fig. 6.1: Symptoms of Plant Diseases:

a) Rust; b) Smut; c) Powdery mildew; d) Stem galls

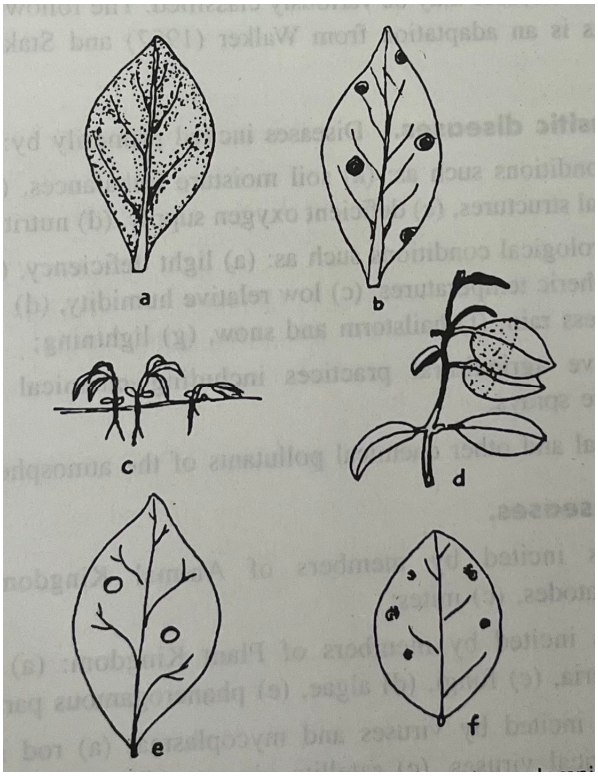

Fig. 6.2: Symptoms of Plant Diseases:

a) Mosaic; b) Leaf spots; c) Damping off; d) Twig blight; e) Shot-hole; f) Canker

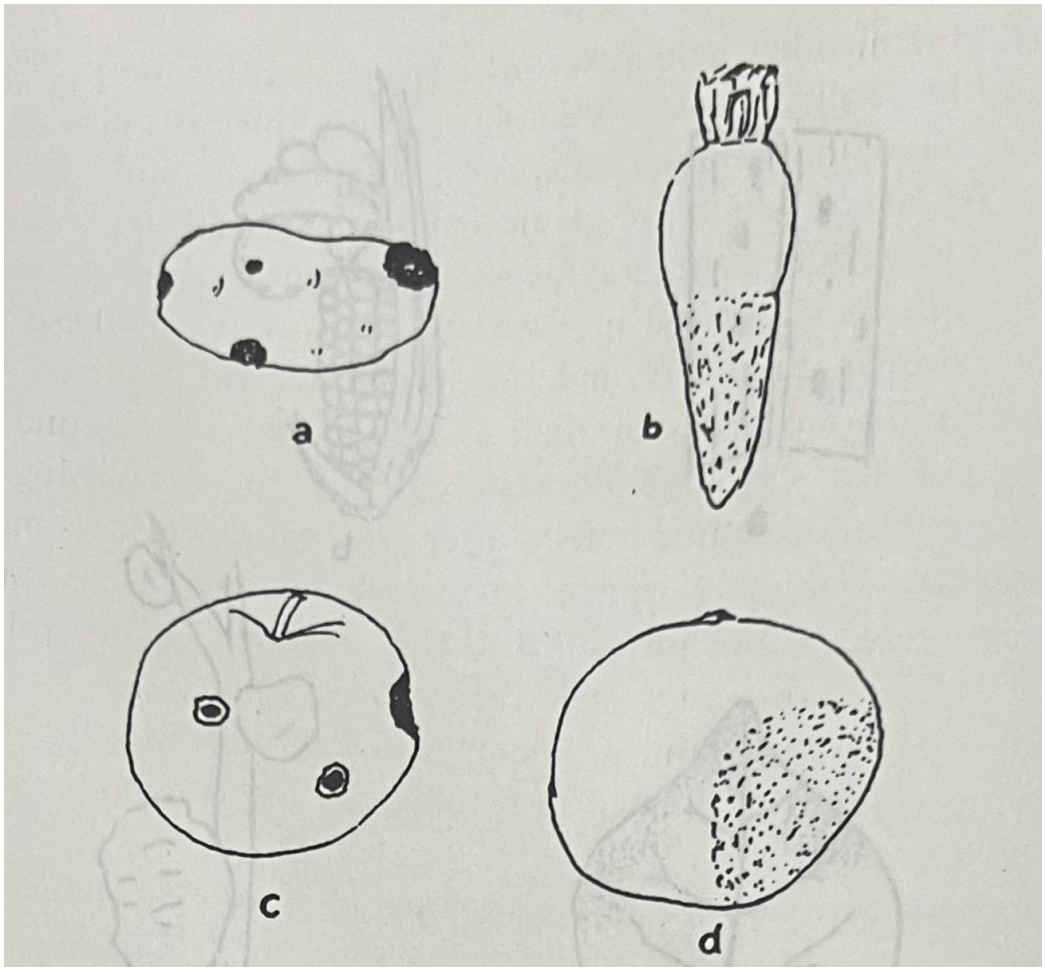

Fig. 6.3: Symptoms of plant diseases:

a) Scab; b) Soft rot; c) Fruit spot; d) Fruit rot

6.4. SUMMARY:

The visible and non-visible alterations in structure and functions in plant due to invasion of pathogen are called diseases. The external manifestation of infection is called symptom. Plant diseases are recognized by the symptoms (external or internal) produced by them or by sick appearance of the plant. The term plant disease signifies the condition of the plant due to disease or cause of the disease. Symptomology is the one which deals with (a) the presence of readily apparent morphological abnormalities of the host in response to a causal agent (symptoms) and (b) an indication of disease other than a reaction of the host, such as the mycelium or fruiting body of a causal fungus (sign). The fungal pathogens usually produce symptoms like rusts, smuts, wilt, blight, mildew, root rot and fruit rot. Soft rots, wilts, tumors, galls, scabs and cankers are the common symptoms caused by bacterial pathogens. However, the viral pathogens produce mosaics, chlorosis and malformations in the infected plants.

6.5. TECHNICAL TERMS:

Symptoms, Rusts, Smuts, Blight, Rots, Spots, Mildews, Tumors, Galls, Wilts, Cankers, Mosaics, Chlorosis, Necrosis, Hypertrophy, Hypotrophy.

6.6. SELF ASSESSMENT QUESTIONS:

1) Write an essay on symptoms caused by plant pathogenic fungi.
2) Give an account on symptoms caused by plant pathogenic bacteria.
3) Describe the symptoms caused by plant pathogenic viruses.

6.6. SUGGESTED READINGS:

1) Plant Pathology, Mehrotra, R.S. (1980), Tata McGraw-Hill Pub. Comp. Ltd.
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Dr. K. Nagaraju

LESSON-7

PLANT DISEASES-LATE BLIGHT OF POTATO, POWDERY MILDEW OF CUCURBITS, SMUT OF SORGHUM

7.0. OBJECTIVE:

- Students will acquaint with the symptoms of the plant diseases and thereby able to identify the crop diseases in the fields.

STRUCTURE:

7.1. INTRODUCTION:

Late blight of potato is a devastating disease caused by the water mold Phytophthora infestans, rapidly destroying foliage and tubers in cool, wet conditions, famously triggering the Irish Potato Famine and remaining a major threat to food security. Powdery mildew is a serious disease of cucurbits in India. It occurs throughout India almost every year, spreading rapidly in cool dry weather and cause heavy defoliation and yield losses. Important hosts are pumpkin (*Cucurbita pepo*), ash gourd (*Benincasa hispida*), bottle, gourd (*Lagenaria vulgaris*), ribbed gourd (*Luffa acutangula*), bitter gourd (*Momordica charantia*), water melon (*Citrullus vulgaris*) cucumber (*Cucumis sativus*), snake gourd (*Trichosanthes anguina*) etc. Sorghum smut is a fungal disease that replaces grains with black, powdery spore masses (smuts), primarily affecting yield. The most common destructive disease that affects the sorghum in India is the grain smut which is also known as covered smut, kernel smut or short smut.

7.2. LATE BLIGHT OF POTATO:

Potato (*Solanum tuberosum* L.) is an important vegetable crop in India and is a staple food in Europe, Canada, Australia, USA, USSR and other countries. In India, potato is cultivated extensively in hilly tracts during December - March or March - June. In plain regions, crop is grown during September - January. Late blight of potato disease is one of the serious diseases of potato and caused a wide spread famine during 1845 in Europe that

resulted in subsequent migration of people from Europe, especially from Ireland. In India, the disease is mostly confined to Northern hills, Gangetic plains of Uttar Pradesh, parts of West Bengal, some parts of South India including Nilgiris and Southern Karnataka.

Symptoms:

Generally, symptoms appear on above ground parts of potato plant and later on underground parts like tubers. Firstly, symptoms appear as small, water-soaked, light brown lesions or patches on leaves. Under favourable climatic conditions with humid and cloudy weather, the lesions spread fast over entire leaflet and petiole. The lesions which are dirty brown in the beginning soon turn to black and whitish fungal growth can be seen on lower surface of leaves on close examination. The pathogen also infects the tubers in the soil causing dry rot and brown discolouration of tissues. The skin of tubers becomes slightly sunken and turns to dark colour with rusty brown colour appearance just beneath the skin of tuber. Dry rot symptoms may or may not appear at the time of digging of tubers but develops during 1st or 2nd month of storage. In wet soils, tubers may rot quickly and thereby wet rot occurs. Severely diseased plants wilt within few days after the 1st symptom appear on leaf. In field conditions, the disease spreads like wild fire causing severe damage to crop yield.

Fig. 7.1: Late Blight of Potato

Pathogen:

The causal organism of late blight of potato is *Phytophthora infestans* (Mont.) de Bary. The pathogen belongs to class Oomycetes, order Peronosporales and family Pythiaceae. In India, at least 18 races of fungus viz., *P. infestans, P. parasitica, P. himalayensis* etc., have been identified as causative agents. The fungus present both as intercellular and intracellular in the host. The mycelium of the fungus is freely branched, hyaline, aseptate and 4-8µm in diameter. Mycelium sends globular or branched haustoria into the host cells for the absorption of food material. Generally, aerial sporangiophores emerge in clusters of 4 or 5 from the stomata. Sometimes, they may also come out piercing the epidermal cells of the host. Sporangia develop at the tips of these sporangiophores. Sporangia are hyaline, ovoid or lemon shaped, thin-walled and papillate, measuring 22-32 µm x 16-24 µm in size. These sporangia disseminate through wind currents, germinate through germ tubes when they fall on host plant and cause further infection. Sexual reproduction is oogamous type involving oogonia and antheridia. Antheridium develops first and later oogonium penetrates the antheridium from one side to other causing the process of fertilization which is referred to as Amphigynous. Oogonia are ovoid or spherical, smooth, reddish brown and 31-46 µm in diameter. Oogonia consists thick walled oospores that germinate by germ tubes.

Disease Cycle:

The predisposing factors that influence the sporangia germination are the excessive humidity of above 90% RH, temperature of 12-13^{o} C and optimum soil moisture of 15-20% saturation. The disease incidence and spread of disease always depend on weather conditions. Primary infection occurs through infected tubers and secondary infection takes place by the conidia that spread by wind and irrigation water, falling on the host foliage. If the infected tubers, after storage during off-season, are used as seed material for the next season cause further infection as the fungus return to soil. On the other hand, the fungus can also infect collateral hosts (*Solanum* spp.) and play a role in the spread of the fungus.

Control Measures:

Selection of healthy tubers for seed purpose – as a prophylactic measure, seed tubers that are free from late blight lesions should be obtained from disease free areas. Before planting, careful examination of seed tubers and pre-treatment of dipping with 1% Bordeaux mixture or other suitable fungicide is recommended.

Fungicidal spray - Zineb or Phenyl tin compound sprays at 15-day interval from one-month crop to the maturation of crop is advisable. Ridomil spray at 7kg/ha in combination with Dithane M-45 gives positive results in controlling the disease. Copper fungicides like Bordeaux mixture, Perenox, Blitox-50, and Fytolan as sprays are also advocated. Dithiocarbamates like Dithane D-14, Dithane Z-78, Dithane M-22 are proved to be more effective than copper fungicides.

During storage of tubers as a source of seed, treatment of tubers with mercurial compounds before storage at 40^{o} F temperature is recommended in Ireland.

Finally, the ultimate and effective disease control measure is the use the seed of disease resistant varieties.

7.3. POWDERY MILDEW OF CUCURBITS:

Cucurbit group includes Pumpkin (*Cucurbita pepo*), Cucumber (*Cucumis sativus*), Ash gourd (*Benincasa hispida*), Snake gourd (*Trichosanthes anguina*), Bitter gourd (*Momordica charantia*), Ribbed gourd (*Luffa acutangula*), Giant pumpkin (*Cucurbita maxima*), Bottle gourd (*Lagenaria siceraria*), Water melon (*Citrillus vulgaris*) etc. These crops are grown for their fruits mostly during certain seasons of the year. These vegetable crops are subjected to one or more diseases causing considerable damage to plants. Powdery mildew is one of the most common diseases of cucurbits, vegetables and also pulses. Disease is worldwide in distribution and, it is the commonest disease of cucurbits in India.

Symptoms:

Initially, the symptoms appear as tiny, white, superficial spots on leaves and stems. Later, they enlarge to white powdery coating indicating the fungal growth over the leaves mostly confined to upper surface. However, powdery appearance also occurs on lower surface of leaves and stem of the affected plants. Affected leaves become distorted, loose their green appearance and develop pale yellow colour at diseased areas. In severe infection condition, leaves and stem dry off and wither resulting in further plant growth arrest. Usually, the fruits are not directly attacked by the pathogen but they become malformed or sunburned due to the early loss of foliage. In infected plants, the fruits become small and result in considerable reduction in fruit yield.

Fig. 7.2: Powdery Mildew of Cucurbit

Pathogen:

The primary causal organisms for powdery mildew of cucurbits are two species of fungi – *Erysiphe cichoracearum* and *Podosphaera xanthii* (also known as *Sphaerotheca fuliginea* or *Prodoshaera fusca*). These fungi thrive in warm, dry conditions and spread through airborne spores, leading to the characteristic white powdery patches on cucurbit leaves and other plant parts. The fungal mycelium of *Erysiphe cichoracearum* spread superficially on leaf surface and produces haustoria that penetrate into the host cells for deriving food material. Conidiophores are produced almost right angles to the host surface. Conidiophores are short, hyaline, thin walled and bear chains of conidia. Each conidium is

oblong and measures 24-30 X 15-20 µm in size. At later stages, as the disease advances, cleistothecia (fruiting bodies) are formed and remain scattered on surface. They are usually spherical or globose in shape, dark coloured with thick walled appendages and measure 80-140 µm in diameter. Each cleistothecium contains many asci ranging from 10-25 in numbers. Each ascus is sub-cylindric or ovate or broadly ovate, more or less stalked with a size range of 60-90 X 25-50 µm. Each ascus contains 2-4 ascospores that are hyaline, elliptic, thin and measures 20-28 X 12-20 µm size.

Disease Development and Cycle:

The disease is soil-borne disease as the cleistothecia survive the off season along with plant debris in soil. On availability of suitable host plant, the ascospores present in cleistothecia germinate and cause fresh infections. Conidia disseminate by wind and cause secondary spread and infection at a rapid rate. Conidia can germinate even under dry and low humidity conditions. The fungus can perpetuate in its conidial stage by multiplying on the collateral hosts and become virulent during optimum climatic conditions and infects the normal host plants. The conditions like dense plant growth, low light intensity, warm temperatures and high RH favour the disease development. Unlike other fungal diseases, powdery mildew disease spread will not be hindered by dry weather conditions.

Control Measures:

Best method of controlling the disease is dusting of finely powdered sulphur on foliage once or twice during the season. Weekly spray of copper sulphate at 1gm/litre or copper oxychloride at 0.5% concentration is also effective. Use of 1% barium polysulphide in green house with 24-25^{o} C temperature and 80% humidity as a protective measure is effective against *E. cichoracearum*. Sprays with colloidal sulphur and thiram are also effective against *S. fulliginea*. Systemic fungicides like Benomyl, Bavistin, Trizoles etc. are also equally effective on pathogen.

Best Management Practices:

1) Resistant varieties – the best and simplest method of disease control is using resistant varieties for planting. However, as there are several fungal races that cause powdery mildew, some resistant cultivars may become susceptible to a specific fungal race.

2) Proper cultural practices – planting cucurbits in sunny location with good air circulation is always advisable. Planting new crops next to those that are already infected by powdery mildew should be avoided. Old and heavily diseased leaves should be removed to improve air circulation and to reduce inoculum.

3) Application of traditional fungicides – most of the fungicides used to control powdery mildew are preventive in nature. So, they must be applied at very early stages of an epidemic. To avoid the development of resistance in pathogen towards fungicides, rotation of fungicides in application program is advisable. At the same time, two fungicides having same mode of action should not be used in the same rotation program. Always better to use a protectant fungicide with multi-site activity and low resistance risk.

4) Bio rational products – these are the plant derived materials, also called as botanicals, used as alternatives to traditional pesticides and antimicrobials. E.g. Neem oil, vegetable oils, capsaicin etc.

5) Biological agents – use of microorganisms or their by-products. E.g. *Bacillus subtilis* strain QST 713 and *Bacillus thuringiensis*.

7.4. SMUT OF SORGHUM:

The most common destructive disease that affects the sorghum in India is the grain smut which is also known as covered smut, kernel smut or short smut. Severe losses are caused by this disease in the regions where the crop is extensively cultivated. In certain areas, up to 25% of the plants have been found affected. Huge losses are reported in USA, Myanmar, South Africa, Italy, Venezuela and other countries.

Symptoms:

Depending on the susceptibility of the host variety, individual grains or majority of the grains or all the grains of the ear are affected. Each grain is transformed into a spore sac (sorus) which varies in shape and size according to the variety of the host crop. Generally, the grain is replaced by an oval or cylindrical, dirty grey sac, sometimes conical at the tip and measures 4-12 mm in length. The sac is surrounded by the unaltered glumes at the base. Sometimes, the stamens develop normally, but more commonly they are absent or involved in the sorus, being represented by 3 conical protrusions from sides of the sorus. The stigma is often not transformed and spores are not formed in other parts of the host.

In some varieties of sorghum, the shape and size of smutted grains are not affected and elongated sacs are not formed but the grain is full of smut powder. Such sori easily escape from notice and are dangerous source of contamination of healthy grains during threshing. In such cases, the covering of the sorus is generally reddish.

The wall of the sorus varies according to the nature of attack. In the long protruding sacs, the wall is almost entirely composed of fungal tissues consisting of small-celled pseudoparenchyma. Only at the base, the wall is composed partly of host tissues. These sacs rupture and shed their spores much more easily than in the unelongated sacs. In the hidden forms, the wall is rough and rigid and usually remains unbroken until after harvest.

The interior of the sorus is completely filled with the spore powder, except a slender, sometimes curved, central column of hard tissues, the columella which is hallowed into depressions at the surface. These depressions are filled with black spore mass. The columella is composed of the host tissues and consists of parenchyma traversed by fibro vascular bundles. Sometimes, the columella is branched at the tip.

Fig. 7.3: Grain Smut

Causal Organism:

Grain smut of sorghum is caused by *Sphaecelotheca sorghi* (Link.) Clinton. The spores of the species are round to shortly oval, dark brown in mass but olive brown singly, smooth walled, and 5-9 microns in diameter. They are often united in loose balls which breakup into individual spores when placed in water.

Germination of spores may take place immediately or after they have been kept dry for up to six and a half years. In herbarium specimens, the spores have been found viable for as long as 13 years. In India, their germination has been seen after 2 years of storage. Two types of germination process occur in water. In one case, a promycelium of 3 cells is formed and sporidia are budded off laterally and at the apex. The apical sporidium appears as the fourth cell of the promycelium. These sporidia are spindle shaped, 10-12.5 X 2-3 microns in size and do not bud off secondary sporidia. In the other case, the promycelium directly develops into a branched or unbranched infection hypha. The different races of pathogen are distinguished by their pathogenic ability on certain different hosts.

Disease Cycle:

The pathogen is extremely seed-borne and seedling infection occurs at the time of germination and emergence of seedlings. During threshing, the sori are broken and the spores get lodged on the surface of healthy seeds. They remain dormant until the next season when they germinate with the germination of the seed. The infection can take place only during the period between germination and emergence of seedlings above the soil surface. This period varies according to host variety, soil temperature, soil moisture and depth of planting.

Sorghum germinates best at 36° - 40° C temperature and the rate decreases progressively down to 10° C temperature. The primary shoot grows more rapidly in high soil moisture than in dry conditions. Infection occurs best on slow germinating seeds checked by cold since the optimum temperature for spore germination is only 20° - 30° C. The germ tube enters mainly through the mesocotyl. Further development of the parasite within the host is like any other smut fungus. High temperatures following the sowing results in less smut incidence than in the moderate temperatures, regardless of soil moisture conditions existing before or after emergence.

Management of the Disease:

Seed treatment with suitable fungicides is quite effective as the seed is externally seed borne. The use of clean seed from cobs free from smut sori is an additional precaution. Immersion of seeds in 0.5 per cent formalin for 2 hours followed by quick drying, in 0.5-3.0 per cent copper sulphate solution for 10-15 minutes followed by drying and sowing had been the old and very effective recommendations. Dry seed dressing with Agrosan GN (1:500) is the simplest and a common practice. Other mercurial and non-mercurial fungicides such as Arasan, N.I. Ceresan, Tillex, Captan, Ceresan M have also been used with success. The systemic fungicides Carboxin and Bavastin etc., are better than the protectant fungicides.

Solar energy treatment of seed was also recommended. The seeds are soaked in water at ordinary temperature during summer for 4 hours in the morning and then spread out in the sun or shade to dry. This method has been also found effective. The sorghum varieties viz., T29/1, PJ23K, Nandyal, Bilichigan, CSH-9, SPV-104, SPV-102, SPV-115, SPV-297, SPV-138, SPV-245, RSV-1-R, SDM-9, CSH-7-R and CSH-5 are reported as resistant varieties to this disease.

Loose Smut of Sorghum:

The loose smut of sorghum is not as common as the grain smut in most of the sorghum growing areas. It closely resembles the grain smut in its appearance and sometimes mistaken for it. This disease is reported from China, Iran, Southern Europe, Africa and USA. In India, it occurs in Andhra Pradesh, Maharashtra, Karnataka and Tamil Nadu. The effect of this smut is not only on the grain but also on the plant growth. Thus, grain as well as fodder yield may be reduced.

Symptoms:

The affected plants become shorter than the healthy ones. The stalk becomes thinner and produces tillers. Ears come out earlier in diseased plants than that of normal plants and all the spikelets of the head are generally affected. The floral bracts tend to elongate and proliferate. Frequently, the lemma and palea as well as the ovary contain smut sori. The covering membrane of the sori ruptures early releasing the powdery mass of dark coloured spores. The columella persists after the spores have been discharged.

Fig. 7.4: Loose Smut

Causal Organism:

The disease is caused by *Sphacelotheca cruenta* (Kuhn.) Potter. The spores are formed in the ovaries and floral bracts. The covering of the sorus is made up of loosely joined rounded and grey fungal cells which are about twice the diameter of the spores. In some host varieties, the wall ruptures early while in others it persists for some time than thus showing characters of both loose and covered smuts. The spores are round or shortly elliptical, dark brown than that of *S. sorghi*, with echinulate wall and measures 5-10 microns in diameter. They germinate to form a 4-celled promycelium with laterally borne sporidia. The promycelium may also develop into branched or unbranched hyphae without forming sporidia. At lower temperatures, sporidia are formed while at higher temperatures direct germination is common. At least 3 races of the pathogen are known and the fungus can be easily cultured on potato dextrose agar and Czapek-Dox agar media.

Disease Cycle

The pathogen is externally seed-borne but may be also soil-borne in dry regions. The spores retain viability for 4 years when kept dry. Germination of spores can occur at 8^{o} C – 38^{o} C temperature but the optimum lies between 18^{o} C – 32^{o} C. Temperatures on the lower side favour the germination of sporidia. Optimum conditions for infection are same as for the *S. sorghi*. Low temperature and low soil moisture as well as deep sowing favour infection. The infection of seedlings occurs at the germination of seeds and before the emergence of seedlings. Entry into the seedlings takes place through the radicle, mesocotyl or hypocotyl. Further development of the pathogen in the host is similar to that of other systemic smuts.

Management of Disease:

The seed treatments recommended for the control of grain smut are effective in the control of loose smut also. Where the survival of spores in soils is possible, crop rotation and filed sanitation are recommended.

Long Smut of Sorghum:

In India, this long smut disease occurs in Tamil Nadu, Maharashtra, Andhra Pradesh, Karnataka, Madhya Pradesh and Uttar Pradesh. However, this disease causes little damage to the crop. It is also reported from Egypt, West Africa, Iraq and Pakistan.

Symptoms:

Individual and only a few grains are transformed into smut sori. Each sorus is surrounded by healthy grains. The sori are very prominent, long, cylindrical, slightly curved, and rupture at the apex to release the brownish-green spore balls and expose a bundle of 8-10 dark brown filaments.

Fig. 7.5: Long Smut

Causal Organism

Long smut of sorghum is caused by the fungus *Tolyposporium ehrenbergii* (Kuhn) Pat remains united in solid balls. This fungus produces spores that stick together in balls, which can survive in the soils for years and infect sorghum plants. The disease appears during the booting stage, when the fungus converts the individual spikelets of the sorghum head into long, cylindrical smut sori. The exposed surface of the spores is covered by flattened echinulations. The spores do not have a dormancy period. They germinate *in situ* by the formation of an elongated promycelium which is frequently branched. Sporidia are numerous, single or in chains.

The spore balls are soil-borne. Germination of spores can occur at any temperature from 15° to 36° C, the optimum being 28° C. Infection does not occur by either blossom or seed inoculation. The sporidia produced by soil-borne spore balls are wind-borne to buds and initiate a systemic mycelium which later expresses itself in the heads. The ovary is converted into smut sorus in the same season. Continuous cultivation of sorghum in the same field is said to increase the incidence of this smut. There is the possibility of primary inoculum being introduced from the same alternate host. Infection can also occur through flowers by wind-borne sporidia.

Since the inoculum is air-borne seed treatment is of no use. Early sowing of the crop reduces disease incidence. Crop rotation and field sanitation to keep down the buildup of inoculum should be practiced. In the south, variety Irungu, in which glumes cover the grains, is usually free from this smut.

7.5. SUMMARY:

Late blight of potato symptoms includes water-soaked spots on leaves that turn brown/black, a white mildew on the underside, stem lesions, and purplish-brown spots on tubers that rot from the surface inward. It spreads via wind and rain, requiring integrated management with fungicides, resistant varieties, and careful cultural practices to control this fungus-like pathogen. Powdery mildew caused by *Erysiphe cichoracearum* and *Sphaerotheca fuliginea* is a serious disease of various cucurbit crops. The pathogens are ecto parasites and produce white powdery patches on all aerial parts of the susceptible hosts. The growth of the pathogens on host surface appears as white powdery flour. The disease is severe in cool dry conditions of winter; the disease appears only in the post rainy season period. Sulphur dusting and spraying of fungicides like karathane are very effective in reducing the disease. Symptoms of smut of sorghum include smut sori (spore sacs) replacing kernels, distorted growth, and black spores, managed through resistant varieties, seed treatment (fungicides like Captan/Thiram), crop rotation, and removing infected heads to prevent spread.

7.6. TECHNICAL TERMS:

Late blight, dry rot, Discoloration, *Phytophthora*, Powdery mildews, *Erysiphe*, Snuts, Sorus, *Sphaecelotheae*, Grain smut, Loose smut, Long smut.

7.7. SELF ASSESSMENT QUESTIONS:

1) Write an essay on symptoms, causal pathogen and control of late blight disease of potato.

2) Explain the symptoms, causal pathogen and control of powdery mildew of cucurbits.

3) Describe the symptoms, causal pathogen and control of smut diseases of sorghum.

7.7. SUGGESTED READINGS:

1) Plant Pathology, Mehrotra, R.S. (1980), Tata McGraw-Hill Pub. Comp. Ltd.

2) Applied Plant Virology, Walkey, D.G.A. (1985), Heinemann; London.

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LESSON-8

PLANT DISEASES – TIKKA DISEASE OF GROUNDNUT, BLAST OF RICE, ANGULAR LEAF SPOT OF COTTON, TOBACCO MOSAIC DISEASE

8.0. OBJECTIVE:

- Students will acquaint with the symptoms of the plant diseases and thereby able to identify the crop diseases in the fields.

STRUCTURE:

8.1. INTRODUCTION:

Tikka disease is most important and major fungal disease affecting groundnut (and all its varieties) in India. It occurs as well in almost all groundnut-growing countries of the world. The *disease* occurs when the crop is two months old and under severe condition defoliation. Blast disease caused by *Pyricularia oryzae* is the most important disease of rice crop and the details of this disease are also explained. Angular leaf spot of cotton, also known as bacterial blight, is a significant bacterial disease (caused by *Xanthomonas citri* pv. *malvacearum*) that creates dark, angular, water-soaked spots on leaves, leading to black lesions on stems (black arm) and boll rot, causing yield losses by affecting leaves, stems, and fruit. Studies on mosaic disease of tobacco led to the discovery of viruses, which are obligately intracellular life forms without cellular structure and metabolism, but having infective ability. Some virus infections remain symptomless. The most important viral diseases of crop plants are tobacco mosaic disease

8.2. TIKKA DISEASE OF GROUNDNUT:

Tikka disease is a world-wide disease of groundnut and occurs in all groundnut growing countries like USA, many African countries, Philippines, Indonesia, Australia, India, Malaysia, Sri Lanka etc. In India, disease is frequently found in all groundnut growing states.

All groundnut varieties grown in India are susceptible to tikka disease. In Uttar Pradesh, the tikka disease is locally known as Chitwa or Haldai due to its characteristic symptoms on leaves. The disease occurs every year in Uttar Pradesh resulting in heavy losses in yield.

Symptoms:

All the above ground parts of the plant are affected, but especially lesions appear on leaves. Symptoms begin to appear when plants are at least of 2 months old and goes up to maturity of the plants. Tikka disease occurs with two distinct types of leaf spots caused by 2 different species namely *Cercospora personata* and. Symptoms caused by 2 different pathogens differ in nature and appearance and that of *C. arachidicola* are rarely seen.

Cercospora personata – symptoms appear as small, round dark lesions or spots. These spots may often enlarge to 3-8 mm diameter and coalesce to form irregular shapes. Spots are dark brown or black and usually found on both surfaces of the leaf blade, but also form on stem and petioles. Symptoms develop about one month after sowing and becomes severe between flowering and harvesting period, under favourable climatic conditions.

Cercospora arachidicola – spots or lesions are bigger and irregular to circular. Spots are surrounded by bright yellow, circular halo with dark brown center. On lower surface of leaves, inconspicuous haloes are formed.

Fig. 8.1: Late and Early Symptoms of Tikka Disease of Groundnut

Causal Organism:

Cercospora personata – fungal mycelium shows inter-and-intra cellular spread and mostly confined to the leaf spot area. The hyphae are brown, septate, branched and slender. Haustoria produced by the pathogen are branched and intracellular in nature. In sub-epidermal region, the fungal hyphae develop into hymenial layer from which the conidiophores arise in bunches. The conidiophores are either aseptate or septate (1-2 septa), geniculated and 24-54 µm long X 5-8 µm broad in size. Conidia develop terminally on geniculations of conidiophores. Conidia are cylindrical or obclavate, transversely septate (1-7), and 18-60 µm long X 6-11 µm broad.

Cercospora arachidicola - mycelium of this species shows inter cellular or intracellular spread, brown in colour, septate, branched and slender in nature and will not produce any haustoria. Conidiophores are yellowish brown in colour, aseptate or septate (1-2 septa) and 20-45 µm X 3-6 µm in size. Conidia are yellowish, obclavate, septate (3-12 septa) with 35-108 µm X 3-6 µm in size.

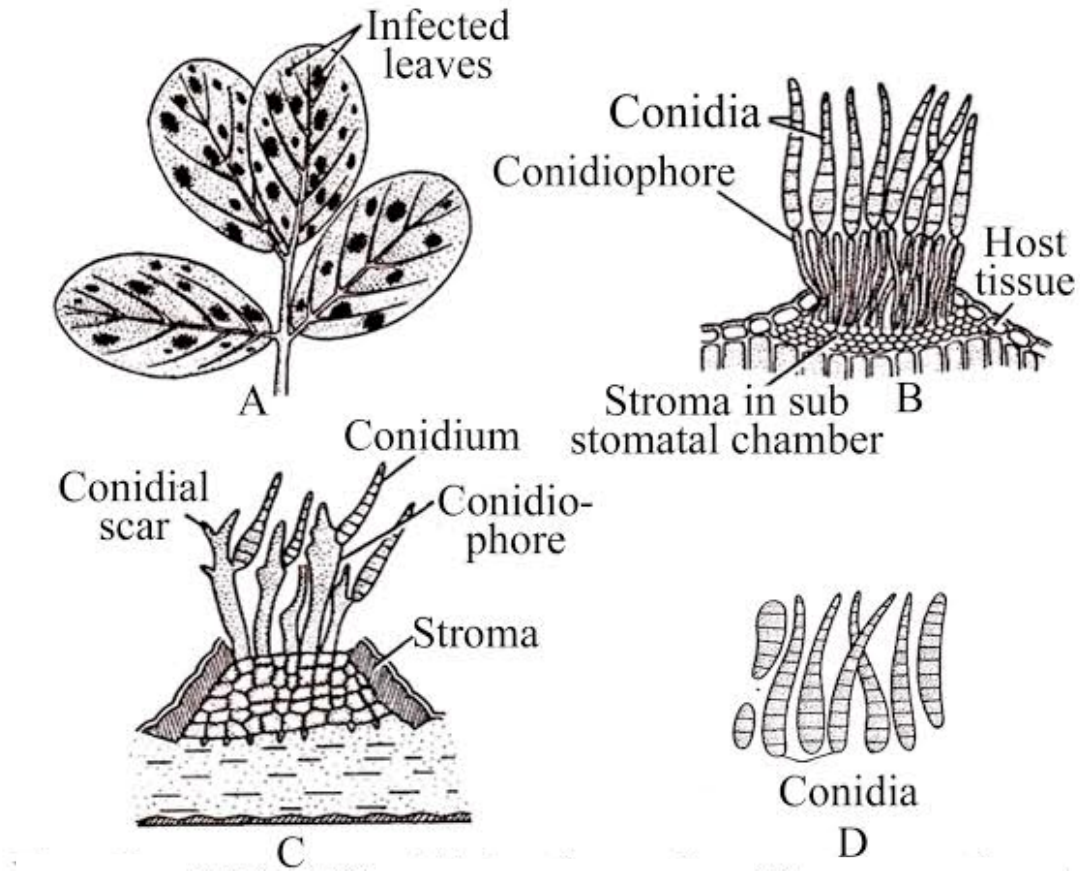

Fig. 8.2: Cercospora spp. (A) Leaf spot disease of ground nut; (B) Conidiophore bearing conidia; (C) LS of acervulus with geniculate conidiophores; (D) Conidia

Control Measures:

Spraying of 1% Bordeaux mixture or 0.2 % Ziram or Zineb or Maneb 2-3 times with weakly interval starting from 4-6 weeks after sowing is one of the best methods for controlling the disease. Spraying 0.15% Perenox and 0.15% Cupravit proved to be effective to some extent. The sulphur dusting is found effective in disease control. Crop rotation and sanitary precaution like destruction of diseased plant debris will reduce the fungal inoculum in the fields. Mixed cropping with pigeon pea will reduce the disease incidence. Sowing disease resistant varieties and early maturing varieties contribute to good results.

8.3. BLAST OF RICE:

Distribution:

Rice blast disease is one of the earliest known plant diseases. It occurs in most of the rice growing countries over the world and causes considerable reduction in yield. It is first recorded from China in 1637 and Japan in 1704. But in India, first reported from Tanjore (now Thanjavur) district of Tamil Nadu in 1918 and Maharashtra in 1923. In India, the disease is more common in southern rice growing areas and causes heavy losses in Tamil Nadu, Andhra Pradesh and Orissa in particular. Yield losses are also reported in other states viz., Maharashtra, Gujarat, Punjab, Kashmir, Bihar, West Bengal, Uttar Pradesh and Assam. In severe cases of disease, the loss may account to 70 – 80% grain yield.

Symptoms:

The pathogenic fungus attacks all the aerial plant parts at all stages of plant growth. The leaves and neck of the panicle are the most commonly affected parts. The symptoms are found on leaf blades, leaf sheaths, rachis (elongated axis of inflorescence), joints of culms, and even on glumes (leaf-like bract below spikelet). Symptoms on leaves originate as small specks that subsequently enlarge into spindle-shaped spots which vary in length from 0.5 to several cm. The lesions are broad at the center with narrow elongations on top and bottoms. The central portion of the spot is whitish grey in colour with brown margin. On the nodes, blackening extends in both ways up to about 1-2 cm. When ears emerge, neck of the panicle is affected resulting in blackening and shriveling of neck region and referred as black neck or neck blast. In these affected ears, the grain set is completely or partially inhibited. When grain set occurs, panicle breaks at the neck due to weakening of neck tissues and panicle droops.

Fig. 8.3: Blast of Rice Symptoms

Causal Organism:

Pyricularia oryzae is the causative fungal pathogen for the blast of rice. Basing on the infection types developed on different hosts, some 28 physiological races of *P. oryzae* are recognized. Fungus produces septate, branched and hyaline to olivaceous mycelium which is localized in the area of lesions. Young mycelium is hyaline and mature mycelium is olive brown in colour. The mycelium is inter-or-intra cellular within the host tissues. The conidiophores emerge from leaf through stomata or epidermal cells by rupturing the cuticle. Conidiophores usually arise as singly or in small clusters. Each conidiophore is septate (2 to 4 in number), rarely branched, slender and greyish in colour. On each conidiophore, 7 to 9 conidia borne terminally or sympodially. The zig-zag appearance of terminal portion of the conidiophores is the characteristic feature of sympodial growth. The conidia are hyaline to pale olive colour and pyriform or obclavate of top shaped with a small basal appendage. The conidia are usually septate (3-celled) and measures 14-40 µm in length and 6-15 µm in width.

Disease Cycle:

In temperate regions, the fungus overwinters in straw piles, hay stacks and seeds. The fungal mycelium survives in infected straw for 1 or 2 years under dry condition, but can be destroyed easily by moisture and microbial activities. In tropical regions, air-borne conidia are present all-round the year. The disease is also seed borne in nature as the fungus hibernates in the seeds during their storage. In certain cases, conidia are found between husk and caryopsis of the grain. Several grasses like *Panicum repens, Digitaria marginata, Brachiara mutica* etc., serve as collateral hosts for the pathogen.

Predisposing Factors:

The environmental factors like temperature, humidity and moisture greatly influence the initiation, development and infection intensity of the disease. The seedlings that grow in water covered seed beds show less infection than those grow in dry seed beds. The soil temperature of 15 – 20º C greatly responsible for the disease incidence of blast of rice. The temperature around 20 º C during night alternating with day temperature around 30 º C with day light for about 14 hours and darkness for about 10 hrs. will predispose the plants to infection. Occurrence of frequent rains, continuous spells of cloudy weather and high humidity at critical period of tillering and heading favors relatively higher incidence of disease. The fungus invades the leaves more rapidly at temperature of 24-28 º C. For conidial germination and infection, high RH (92%) and free water is required and the presence of dew drops on leaves also stimulate the spore germination. Usually, fungal spores are deposited on leaves more during night than day time. The amount of spore deposition on leaves depends on the angle of leaf with plant stem. Plants with leaves in vertical position trap fewer spores than those with slanting and horizontal leaves. The intensity of infection is also influenced by the age of plant also. For example, seedlings of 25 days old are more prone to infection than the 3-month-old seedlings. Of the seedlings, upper younger leaves are more susceptible than the middle or lower older leaves. Over doses of nitrogen fertilizers increase the susceptibility of the crop to disease.

Control Measures:

The most economic method to control the blast of rice is to develop and cultivate the resistant, high yielding varieties. Several breeding experiments has been carried out and still going on in India at Central Rice Research Institute (CRRI), Cuttack. Genes for blast disease resistance can be combined with those controlling other desired agronomic characters viz., drought tolerance, grain quality, yield, response to fertilizer application, early maturity etc. The cultivars viz., Jaya, Vani, Akashi, Rasi, Swarnadhan, IR8 and IR36 that developed at different research centres were found to be promising resistant varieties to blast disease.

Seed Treatment:

Immersion of seeds in 0.2% kalimat B solution for 24 hours before sowing control the disease and also promote the growth. Seed treatment with organomercurial compounds is effective and hot water treatment of seeds is also suggested. Seed protectants like Agrosan GN, Cerasan, Phygon, Spergon have also proved to control the disease.

Spraying and Drying:

Spraying of Bordeaux mixture 2 times before flowering and 2 times after flowering can effectively control the blast disease.

Spraying of copper fungicides like Perenox, Coppersan, Cupravit etc. are also found effective on blast disease.

Dusting with organomercurials are also suggested for controlling the blast of rice.

Few antibiotics like Blasticidin – S and Kasugamycin at 20 ppm concentration; some organophosphorous compounds like Kitazin P and Hinosan; and few bensimidazole formulations like Carbendazim, Benlate, Bavistin and Vitavax are found to be effective in treating blast of rice disease.

Sanitation and Proper Manuring:

- Plant debris and collateral hosts for pathogen should be collected and destroyed.
- Excess application of nitrogenous fertilizers should be avoided as it predisposes the plants to infection.
- Balanced application of potassic and phosphatic manures are recommended.

8.4. ANGULAR LEAF SPOT OF COTTON:

Angular leaf spot or black arm is the most serious bacterial disease of cotton. The disease was first reported from Alabama State of USA in 1891 and is now found in all major cotton growing regions of the world including South America, Egypt, Sudan and other African countries, Russia, Sri Lanka, China, Australia, etc. In India, the disease was first observed in Tamil Nadu in 1918 and is now known to occur in Maharashtra, Madhya

Pradesh, Andhra Pradesh and Uttar Pradesh. The annual yield loses of cotton vary from 5 to 25 per cent due the disease. Severe epidemics of the disease were reported in Tamil Nadu during 1948-1952 that resulted in rejection of many promising cotton varieties of four species of *Gossypium*.

Symptoms:

The bacterium can infect all aerial parts of the plant and also at different stages of plant growth. The disease has four distinct phases depending on the plant part affected – angular leaf spot (leaf infection), black arm (stem infection), boll rot (boll infection) and seedling blight (seedling infection). The earliest symptom of the disease is seen in the cotyledons of germinating seeds. Minute, water-soaked spots appear on the under surface of the cotyledons. Later, they increase in diameter, turn brown to black and form irregular patches distorting the shape of the cotyledons causing them to dry and wither. The disease spreads to new leaves formed and seedlings may ultimately collapse and die. Similar water-soaked spots appear on the underside of leaves first and later on upper surface. As disease progress, spots increase in size, become angular, bound by small veinlets of the leaf and turn brown to black. Often, the disease spreads along the edge of veins and hence called Vein blight or Black vein. Sometimes, large patches are formed due to coalescing of a number of small spots leading to death and shedding of leaves. The may also spread to petioles causing them to collapse. In the affected areas large quantities of bacterial slime are exuded which form a dry film on the brown lesions.

The lesions on stem, petioles, and fruiting branches are dark brown to sooty black which are elongate and sunken. The affected stems show cracks and gummosis and are easily broken by wind or there may be girdling and death of the affected organs. These are the black arm symptoms. The disease on bolls or fruits is characterized by the appearance of water-soaked lesions on the surface. The lesions turn brown and finally black, and are invariably sunken. Young infected bolls fall down prematurely. In case of infected mature bolls, the lint is not of much commercial value. The bacterium within the boll passes along the fibers and infects the seed externally. It may also reach the interior of the seed either through micropyle or through punctures.

Fig. 8.4: Angular Leaf Spot of Cotton

Causal Organism:

The bacterial agent that causes the angular leaf spot disease is *Xanthomonas campestris* pv. *malvacearum* (Smith) Dye. The bacterium is rod-shaped measuring 1.3-2.7 X 0.3-0.6 microns, Gram-negative, encapsulated, motile, aerobic, and non-endospore forming. The bacterial colonies on beef agar are pale yellow, round, thin, raised, smooth, and shining. Optimum temperature for growth in culture varies from 25^{o} C to 32^{o} C temperature. Bacterium is positive for gelatin liquefaction and digestion of starch and casein. There are about 32 races of the pathogen which vary in their pathogenicity on different species of *Gossypium*. In India, 26 of these races have been identified and the race 10 is reported to be the most common.

Disease Cycle:

The bacterium may remain alive in dried leaves for 17 years of period and in dry or moist soils for 8 days at 21^{o} - 33^{o} C temperature. The main source of primary inoculum is seed. The bacterium may be present as slimy mass on the fuzz or inside the seed. On germination of such seeds, the bacterium moves to cotyledons and then maintains a resident population on the first and second leaves. In favourable weather conditions, the inoculum from this source spread to new leaves and further spread continues. Infected cotton bolls, leaves and twigs present on soil surface also form an important source to carryover the bacterial inoculum. The infected seeds lying dormant in the field and germinating in the crop season prior to the main crop also serve as source of primary inoculum. Leaves are infected mainly through stomata. Secondary spread is through wind-splashed rain and dew.

High humidity and moderate temperature of 28^{o} C favour the development of the disease. Soil temperature and moisture at the time of sowing and a few days after that are very important. Primary infection is generally favoured by a temperature of 30^{o} C and secondary infection is better at 35^{o} C. Presence of moisture content is very important for secondary infection during first 48 hours. Highest progress of the disease occurs when there are frequent rains followed by cloudy weather. Dry and hot weather retards the development of disease.

Disease Management:

The best recommendation to reduce the soil-borne inoculum is the removal and destruction of the diseased plant debris. Deep ploughing after harvest buries the infected stalks and, thus, reduces survival ability of the bacterium in the soil. In some countries, pre-sowing irrigation to enable the left-over seeds to germinate, followed by ploughing and then planting of the main crop is in practice. Destruction of possible alternate of collateral hosts is also required. Crop rotation, late sowing, early thinning, good tillage, early irrigation and addition of potash to soil help in reducing the disease incidence.

Seed-borne inoculum can be eliminated by seed treatment. The external inoculum present on the seed can be destroyed by delinting of seed with concentrated sulphuric acid. In this process, seeds are immersed in acid for 10-15 minutes, then rinsed thoroughly by suspending in water to remove acid and finally dried and treated with organo-mercurial compounds such as Agrosan GN, Ceresan, etc. This treatment will not destroy the internal

seed-borne inoculum. Treatment of seeds with antibiotics like streptomycin eliminates the internal seed-borne inoculum and systemic fungicides viz., Vitavax and Plantvax are more effective in this than antibiotics. As the bacterium is known to live in the seed for about a year or so, ageing of the seed for two years before sowing has also been recommended. Hot water treatment of seed material at 56o C temperature for 10 minutes destroys the external as well as internal inoculum without affecting the seed viability.

The secondary spread can be checked by regular sprays with copper fungicides at 0.2-0.3 per cent concentration. Depending on the severity of disease, 3-6 sprays should be given at 15 days interval with the first spray on the crop of 5-6 weeks old. The seed treatment with Agrimycin (3g/40kg seed) and its spray (25 ppm) are most promising in controlling the black arm of cotton. The bacterial blight of cotton can be controlled by 3 times spray of a mixture of 0.01% Agrimycin and 0.2% Blitox at 40-50 days, 70-80 days and 85-90 days after planting. The best and most effective preventive measure is the development and cultivating the disease resistant varieties. *Gossypium herbaceium* and *Gossypium arboretum* are considered to be practically immune to the disease, whereas *G. barbadens, G. herbaceum* var. *typicum* and *G. hirsutum* are considered to be susceptible varieties. The cultivars of hirsutum group viz., 70IH-480/2, 70IH-480/9, K-4005, Badnawar-1, B-1007, Khandwa-2, DHY-286 and M-937-CTO-421are reported to be the resistant varieties.

8.5. TOBACCO MOSAIC DISEASE:

TMV disease is the first recognized viral disease of plants. It is worldwide in distribution found in all the tobacco growing tracts of the world. Most common disease on tobacco crops in India. Over 20 different viral diseases occur on tobacco, of which tobacco mosaic disease is most important.

Symptoms:

The first symptom of the disease is the light discoloration along the veins of the youngest leaves. Later (very soon), leaves develop a characteristic light and dark green pattern appear wherein dark green areas usually associated with the veins. This typical mosaic symptom sometimes accompanied by a blistered appearance of the leaf due to more rapid growth of the dark green tissues. In case of severe infections, leaves are narrowed, puckered and thin, crinkle, malformed beyond recognition. Plants that are infected early in season are very much stunted. Plants that are infected late in season shows little reduction in size. The disease causes extensive damage by way of reduction in yield and quality of the product.

Fig. 8.5: Mosaic Symptom of Tobacco

Pathogen:

The viral pathogen is a type member of large group of viruses within the genus *Tobamovirus* that infects a wide range of plants, especially tobacco and other members of the family solanaceae. Virus is rod shaped in structure with a central hollow tube and measures 300 x 18 nm in size. It is composed of a helical protein coat (capsid) consisting of 2,130 identical protein subunits. Each protein subunit consists of 150 amino acids. It surrounds a single stranded RNA genome which is coiled inside the protein shell and contains about 6,400 nucleotides. The thermal death point of virus is approximately 90^{o}C on 10 minutes exposure. Virus can survive on dry tobacco leaves in store houses even for 50 years also.

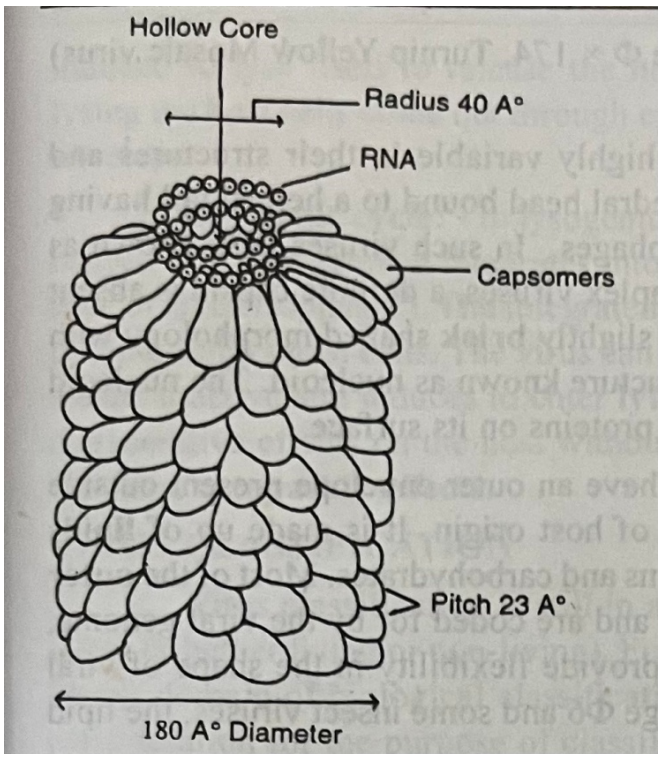

Fig. 8.6: Structure of TMV

Disease Cycle:

Virus is sap transmissible. The virus enters into host through injuries and wounds on host surfaces. Most resistant to adverse environmental and climatic conditions. So, when it reaches the field, become infective at any time the crop is sown. They can easily transmit by mechanical means, by wind, and by water. The virus can spread from diseased to healthy plants through wounds caused by different agricultural practices and cultural operations. Contaminated tools and even the workers in the field may transmit the virus from chewing tobacco and snuffing to the standing crop. Virus can also be easily transmitted when infected leaf rubs against healthy leaf. Agricultural practices such as continuous cropping may also spread the virus.

Control Measures:

1) Best method of controlling tobacco mosaic disease is growing resistant varieties like TMV-RR-2, TMV-RR-3 and TMV-RR2a which are developed at CTRI, Rajahmundry.

2) The cultivars viz., GSH-3, L-2663a, MDS-13, L series 687 to 772, V2809 to 2814 are found to be resistant to mosaic disease.

3) Field sanitation by regular weeding in field also controls the disease incidence.

4) Employing crop rotation practice of cultivating resistant plants followed by non-host crops reduces the amount of viral inoculum in the field.

5) Disinfection of hands of workers and proper cleaning with 10% house hold bleach or soap and water during agricultural operations is very much needed to control the disease spread.

6) Workers should not chew or smoke while working in nursery or field.

7) Diseased plants and seedlings should be identified, collected and destroyed. Because the dried infected leaves can be easily blown around as dust and infect other plants.

8) Spraying 0.5 to 1.0 % tannic acid at 20, 30- and 40-day intervals, after planting tobacco plants can control the disease.

9) Treatment of seeds with 10% trisodium phosphate solution for 15 minutes controls the disease.

10) Cross protection is control strategy which includes the inoculation of mild strain of virus onto young plants protecting them from subsequent infection by more severe strains of TMV.

11) Development of transgenic plants is another control measure.

8.6. SUMMARY:

Tikka disease, also known as *Cercospora* leaf spot, is a common and economically significant fungal disease of groundnut (peanut), caused by *Cercospora arachidicola* (early) and *Cercospora personata* (late), characterized by dark, circular spots on leaves, often with yellow halos, leading to defoliation and reduced yield, managed through resistant varieties, seed treatment, crop rotation, and fungicides, favored by warm, humid conditions. Blast disease of rice caused by *Pyricularia oryzae* is the most important disease of rice crop. The plant is susceptible at all stages of growth. Leaf spots develop in nursery and rapid tillering stage. Nodal infection and rotting of the neck of inflorescence occur at flowering stage. The seeds may also get infected. The spots on leaves are typically spindle shaped with greyish white centre and dark reddish-brown raised margins. They rapidly expand and coalesce during favourable weather. Neck rots result in dropping of the inflorescence. The fungus survives in the infected straw and also seed borne secondary spread of the disease is by aerial spread of the conidia. Host nutrition favouring nitrogen metabolism increases the disease while silicon uptake reduces the disease. For controlling the diseases, cultural practices, fungicidal sprays and use of resistant cultivars is suggested. Hinosan, an organophosphorus fungicide, is very effective against the disease. Some of the exotic varieties from Japan are resistant in India.

Management of angular leaf spot of cotton focuses on resistant varieties, clean cultural practices like crop rotation, avoiding wet field scouting, and seed treatments to control this pathogen, which spreads via infected seeds or wet foliage. Mosaic disease of tobacco is the most severe disease of the crop and characterized by random distribution of

light and dark green patches on the leaves. The disease is caused by a single stranded RNA virus; which is rod shaped. It survives for long periods under dry conditions, easily transmitted mechanically, have wide host range. It is very difficult to eradicate the disease once established in the field, and growing resistant varieties is only solution.

8.7. TECHNICAL TERMS:

Leaf spot, Tikka disease, *Cercospora*, Lesions, *Pyricularia*, *Xanthomonas*, Mosaic pattern, Tobacco mosaic virus

8.8. SELF ASSESSMENT QUESTIONS:

1) Write an account on symptoms, causal organism and control of tikka disease of groundnut.
2) Describe the symptoms, causal organism and control of blast disease of rice.
3) Give an account on symptoms, causal organism and control of angular leaf spot of cotton.
4) Write an essay on symptoms, causal organism and control of tobacco mosaic disease.

8.9. SUGGESTED READINGS:

1) Plant Pathology, Mehrotra, R.S. (1980), Tata McGraw-Hill Pub. Comp. Ltd.
2) Applied Plant Virology, Walkey, D.G.A. (1985), Heinemann; London.
3) Introduction to Principles of Plant Pathology, Singh, R.S. (2002), Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi.
4) Basics of Plant Virology, Verma, H.N. (2004), Oxford & IBH Pub. Co. Pvt. Ltd., New Delhi.
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LESSON-9

GENERAL PRINCIPLES OF PLANT DISEASE CONTROL

9.0. OBJECTIVE:

- The students will understand the methods by which the plant diseases can be controlled.

STRUCTURE:

9.1. INTRODUCTION:

The main objective of plant disease control is to prevent economic loss and increase the yield of the crop. For effective control, the knowledge on the pathogen and factors effecting the host-pathogen interactions are essential. Such information is available only for major diseases that cause significant yield losses, and control measures were developed against only such diseases. The aim of the control measures is not to completely annihilate the pathogen, because it is neither possible nor necessary, but to manage the diseases so that it does not cause appreciable yield loss. Further, the cost of disease control should not be more than the cost of yield loss by the disease.

The principles of disease control or management aim at attacking the pathogen in different ways. When a particular disease is not present in any given area, measures are taken so that it does not enter the area which is called as exclusion and quarantine measures can achieve it. When a particular disease is already established in an area, measures are taken to eradicate the primary source of inoculum, and it is achieved by seed treatment and cultural practices like sanitation and eradication. When a pathogen is established in the field and causing severe disease, chemical control measures are taken to reduce the severity of disease. The use of chemicals for disease control is costly, and often results in environmental pollution. Hence, the measures are taken to increase the resistance in host plants, and it is achieved by breeding for disease resistance.

9.2. PLANT QUARANTINE:

Plant quarantine can be defined as a legal restriction on the movement of agricultural commodities for the purpose of exclusion, prevention or delay in the spread of plant diseases to the new uninfected areas. Unrestricted movement of plant material in the past between countries and within the countries has resulted in the spread of some of the devastating plant diseases. Some important examples of diseases introduced into India are –

Disease	Pathogen	Year of Introduction	Introduced from
Blast of rice	*Pyricularia oryzae*	1918	South East Asia
Coffee rust	*Hemileia vastatrix*	1879	Sri Lanka
Powdery mildew of rubber	*Oidium heaveae*	1938	Malaya
Crown gall of apple	*Agrobacterium tumefaciens*	1940	England
Bunchy top of banana	*Virus*	1940	Sri Lanka
Potato wart	*Synthytrium endobioticum*	1953	Netherlands
Onion smut	*Urocystis cepulae*	1958	Europe

Realizing the importance of restricting the movement of disease, almost all countries have enacted quarantine laws. In India, Destructive Insect and Pest Act (DIP Act) was passed in 1914 and 16 quarantine stations were established at all major ports (sea, air and land) of entry. Due to extensive transport facilities, entry of pathogens to new area increases to several folds. Sometimes, it is difficult to check the movement of diseased plant material that transmits the disease. In several agriculturally advanced countries, plant quarantine legislation is followed strictly. Cultures of bacteria or fungi will not be allowed to brought into India, unless Head of Division of Mycology and Plant Pathology, IARI, New Delhi satisfied that particular organism would not become a parasite in India. Phytosanitary certificates issued by quarantine stations are necessary to import or export plant materials. Domestic quarantine is also imposed to restrict certain endemic diseases. For example, potato wart is endemic to Darjeeling district of West Bengal, and karnal Bunt of wheat is restricted to Haryana. Domestic quarantine applies to these diseases. The certification date should be not more than 14 days prior to shipment. Compulsory inspection and certification by authorities is necessary.

9.3. SEED TREATMENT:

Seeds are both victims and vectors of diseases. Any pathogen that is present in or on the seed affects its quality, viability or carries the pathogen to the field. Seed borne pathogens are mainly important in introducing the disease into new areas. Hence, seed treatment is practiced since early days. Seed treatment is mainly of three types viz., seed disinfection, seed disinfestation and seed protection.

Seed Disinfection:

Seed treatment directed towards the eradication of the pathogen in the seed is referred to as seed disinfection. Hot water treatment is devised to eradicate a number of fungal pathogens in seeds and effective in case of loose smut of wheat and barley. It involves immersing the seed in warm water (55^{o}C) for 15 minutes and then spreading out in shallow layers to dry. To eradicate the seed borne bacteria, the seeds are treated with antibiotics like streptomycin etc., at proper concentrations.

Seed Disinfestation:

Seed treatment directed against the pathogens on the surface of the seeds is known as seed disinfestation. Various organomercurial compounds like ceresan, agrosan etc. are widely used for disinfestation of seeds of millets and other crops.

Seed Protection:

Seed protection is based on the principle of surrounding the seed and the young seedling with a fungicide, which will prevent infection and damage by soil organisms. Captan, Thiram, Ziram etc. are widely used as protectant fungicides for maize, pea, bean, and other crops. These fungicides are applied to the seed by immersing the seed in fungicides made into slurry. To enhance the adherence of fungicides to the seed coat, stickers or adhesives like methocel or cellulose acetate are added to the slurry.

9.4. CULTURAL PRACTICES:

Methods employed to reduce the severity of plant disease incidence through methods other than using chemicals and disease resistant varieties are referred to as cultural practices for disease control. These mainly include eradication, sanitation, change in cropping patterns and cultivation practices.

Eradication: Eradication of the pathogens is carried out by many ways.

1) Eradication of seed borne inoculum – The pathogen may be externally or internally seed borne, and it may be eradicated by seed treatment.

2) Eradication of diseased plants – One of the most extensive eradication operations carried out so far was one that got rid of citrus canker bacterium, *Xanthomonas campestris* pv. *citri,* in USA during 1927-35. More than 4 million citrus trees were cut and burnt, and then quarantine measures were strictly implemented to eradicate the disease. Such eradication programs on field scale are often carried out for many soil borne diseases of crop plants.

3) Eradication of alternate hosts – One of the classic examples is eradication of barberry bushes in Europe and America to control wheat rust epidemics.

4) Eradication of collateral hosts – The host plants other than the principal cultivated crop, which are attacked by the same stage of the pathogen, are called collateral hosts. They help in perpetuation of the pathogen in the absence of main crop, and eradication of these collateral hosts removes the primary source of inoculum.

Field Sanitation:

Many pathogens survive as dormant structures in the left-over plant debris in the field. Collection and destruction of these structures reduce the inoculum potential. Various measures, which can be adopted to destroy the sources of perennation are – a) removal of diseased plant debris and burning b) ploughing to bury the fallen diseased leaves etc. deep into the soil and c) use of chemicals to disinfect the fallen plant debris. Frequent weeding and maintaining a clean crop are also an important measure of sanitation.

Change of Cropping Patterns:

Continuous monoculture tends to increase disease incidence and change of cropping patterns reduce the disease severity. Crop rotation and mixed cropping are the two important patterns of cultivation for disease control.

1) **Crop Rotation:** It is beneficial in eradicating soil borne diseases like wilts, root rots etc. The crop rotation should always be with unrelated hosts, which are not susceptible to the pathogen of affected crop. The rotation should be long enough to starve out the pathogen. For example, cotton may be rotated with rice for preventing *Verticillium* wilt.

2) **Mixed Cropping:** Mixed cropping such as cultivation of wheat-barley, wheat-gram, pigeon pea-sorghum etc. reduce economic loss from diseases in addition to several other advantages such as increased availability of nitrogen for crops grown along with legumes. Since the same pathogen does not attack both crops, at least one of the crops is saved, even if other is badly damaged by a disease.

Cultivation Methods:

The incidence of many diseases on crop plants can be reduced by following proper cultivation practices.

1) **Choice of Area:** Many fungal and bacterial diseases are more severe in wet areas than in dry areas. Selection of proper area for cultivation reduces the disease incidence. However, such a practice can be adopted by farmers with large acreage but not by small farmers.

2) **Date of Sowing:** For most diseases, favourable environment occur during particular part of the year, and adjusting sowing dates in such a way that major part of crop season does not fall during that favourable period helps in less exposure of the crop to diseases. E.g. Blast of ragi can be escaped by growing the crop during September-October instead during June-July.

3) **Soil Amendments:** Adjustment of soil pH, balanced use of fertilizers, correction of minor elements is important to make soil conditions favourable for the crop plant rather than to the pathogen. Use of compost increases soil organic matter and also stimulate native soil microbial populations, some of which may be antagonistic to pathogens. E.g. Club root of cabbage, favoured by acidic soils, can be controlled by liming which reduce the acidity and improve the soil fertility or O_2 levels.

4) **Soil Hygiene:** Field sanitation is an important for healthy and vigorous growth of plants. Uncleaned fields and remnants of previous crops in field are dangerous and harbor disease causing organisms. Crops grown under such conditions are seriously infected by soil borne pathogens. For example, Ratoon crops like cotton and sugarcane can get easily affected by soil borne pathogens.
5) **Rogueing:** It is an act of identifying and removing plants with undesirable characters from agricultural fields to preserve the quality of crop being grown. It is a routine method in control of virus diseased plants. Examples of diseases controlled or checked by this method are yellow mosaic of bhendi, whip smut of sugar cane, loose smut of wheat, covered smut of barley etc.

9.5. CHEMICAL CONTROL:

The use of chemicals for control of plant diseases is the most commonly practiced method. Since the fungal diseases are very common on plants, fungicides are extensively used. The fungicides that are used for control of plant diseases are mainly of two types viz., contact fungicides and systemic fungicides. The contact fungicides are effective as long as they are in contact with the plant surface. Systemic fungicides are taken up by the plants and translocated internally and give protection for long periods. Antibiotics are also systemic in action, and used mainly against bacterial diseases.

I. Contact Fungicides:

A large number of fungicidal preparations are used as contact fungicides for seed treatment, and also for dusting or spraying on standing crop.

Sulphur:

Sulphur is the earliest known fungicide. It is particularly effective against powdery mildews. Two types of inorganic sulphur preparations are used - 1. Elemental sulphur and 2. Lime sulphur.

Different formulations of elemental sulphur or sulphur powder mixed with inert materials are available. Before mixing, the sulphur is ground into fine particles. For some preparations, sulphur is roasted in a furnace and the resultant product is made into fine powder. Wettable sulphur is prepared by fusion of elemental sulphur and bentonite clay and resultant particles acquire the wettable property of bentonite. Lime sulphur is obtained by boiling hydrated lime and sulphur together. The main products of the reaction are calcium thiosulphate and calcium polysulphide. The later confers fungicidal and insecticidal properties.

Sulphur is believed to act as a fungicide in more than one way. It may be oxidized to SO_2, or it may be reduced to H_2S and both forms are toxic to fungi. Sulphur may also act directly on fungal cells by entering the cells, and membrane lipids favour the direct uptake of sulphur. The dry spores of powdery mildews contain more lipids, and it may explain their affinity and susceptibility to sulphur.

Copper:

Benedict Prevost first discovered the fungicidal activity of copper in 1807, but its large-scale use as a fungicide started in 1886 after the discovery of Bordeaux mixture by Alex Millardt. Bordeaux mixture is the only fungicide available for the control of plant

diseases over 50 years from 1886 to 1940s. It is highly effective against a number of foliar diseases, especially leaf spots and blights caused by a number of plant pathogenic fungi. Copper sulphate is the toxic compound in Bordeaux mixture. The commercial copper fungicides available now-a-days contain copper oxychloride as the toxic principle. Blitox, miltox, fytolan etc. are the commonly used copper fungicides. Because of the phytotoxicity of copper compounds, its use is declined with the advent of organic fungicides, which are better tolerated by the plants.

Mercury:

The fungicidal properties of elemental mercury are well known, but because of its toxicity to man and animals, it is used to a limited extent as a plant protectant. Organomercurials like ceresin, agrosan, mercuran, semesan, germisan etc. are used in dry or slurry form for seed treatment.

Dithiocarbamates:

Dithiocarbamates are the derivatives of dithicarbamic acid, and the usefulness of carbamates was first reported by Tisdale in 1931, but they came into wide use only after 1960s. The important dithiocarbamates are thiran, ziram, ferbam, nabam, zineb, maneb, and mancozeb. Dithane compounds are useful as foliar sprays on vegetable and fruit crops and trees to control many leaf spots, downy mildews, blight, scab, anthracnose etc. The dithiocarbamates are water soluble and their fungicidal activity is through inhibition of enzyme action in fungal cells.

Quinone Fungicides:

Quinones are naturally present in plants and animals, and are the source of coloration. They are also produced by oxidation of phenols. Quinones often exhibit antimicrobial activity and may be responsible for resistance in plants to pathogens. Chloranil and dichlone are the two well-known fungicides in this group. Chloranil is mainly used for seed treatment and also as a foliar spray. It is effective against seed rot of bean, apple scab, peach leaf curl, late blight of potato etc.

Benzene Fungicides:

Many aromatic compounds have a significant antimicrobial activity, and have been developed into fungicides by adding chlorine or other active radicals to benzene ring. The important examples of this group of fungicides are dexon, daconil, dichloran, brassicol and dinocap. Dexon is used in seed and soil treatment for the control of damping off and root rots. Daconil is effective against many diseases such as leaf spots, leaf blights, fruit spots, fruit rots etc. Dichloran is used against fruit and vegetable diseases as spry fungicide, and also in soil treatment. Brassicol is a common fungicide of this group and is effective against many soil fungi like *Sclerotium*, *Rhizoctonia* etc. Dinocap is extensively used against powdery mildews.

Heterocyclic Nitrogen Compounds:

Important fungicides of this group are captan and captafol. Captan is the most commonly used seed protectant against *Pythium*, which causes damping off and root rots. It is

also used for soil drenching and for foliar sprays. Captafol is an effective foliar spray and seed dressing fungicide against early blight of potato, apple scab etc.

Organotin Compounds:

Duter, brestan and brestanol are the three fungicides based on organiotin compounds. They are very effective against a number of plant pathogenic fungi like *Cercospora*, *Helminthosporium*, *Alternaria*, *Pythium*, *Phytophthora* and *Rhizoctonia*. They have antibacterial and anti-insect activity also and have some systemic activity. However, due to phytotoxicity to host, these finitudes have restricted from use.

Oils:

Various types of oils are capable of controlling several plant diseases. The fungicidal activity of oils came into light with their use against Sigatoka diseases of banana. Other diseases that can be controlled by using oils include greasy spot of citrus, angular leaf spot of tung tree, leaf spot of celery, wheat rust, powdery mildew of rose, downy mildew of grapes etc. The oils used in disease control are mainly mineral soils from petroleum, and to a lesser extent glyceride oils from plants and synthetic oils. The therapeutic action of oils is perhaps due to oily physical barrier which interferes with gas exchange in the leaf and alters its physiology so as to stop development of fungus. It is also likely that oils exert their therapeutic action not directly on the pathogen but rather on the physiology of the host plant.

II. Systemic Fungicides:

The introduction of systemic fungicides in 1966 is a major landmark in chemical control of plant diseases. Von Schmeling and Kulka in 1966 have shown that synthetic chemical compounds of oxathiin group have systemic activity against fungal pathogens. Later a number of systemic fungicides have been developed. A systemic fungicide is one which is taken up and translocated in the plant as a result of which the latter becomes fungitoxic. The substance may either be toxic to the pathogen concerned or converted in the plant tissues to such a fungitoxic compound. Various systemic fungicides act on pathogens either by inhibiting the fungal enzyme system, neutralization of toxins involved in the invasion or damage the semipermeable membranes of fungal hyphae or infection structures. The uptake of hitherto known systemic fungicides is passive, when it is applied to the roots and the active ingredient moves along with the sap stream. Downward translocation is insignificant. Hence, the use of systemic compounds as sprays is not always very successful, but soil application is very effective. Among the systemic fungicides, the two most important groups developed early are oxathiins and benzimidazoles. Other systemic fungicides include thiophanates, triazols, acylalanines, pyrimidines, morpholines etc.

Oxathiins:

Carboxin and oxycarboxin are the two products of oxathiin group developed by Von Schmeling and Kulka in 1966 for complete control of internally seed borne infection of loose smut in barley. They were later found to be effective against a number of other diseases,

especially those caused by basidiomycetous fungi. Vitavax is the most popular fungicide for seed treatment to control loose smut of wheat, bunt of wheat, and flag smut diseases. However, it is not effective against deuteromycetes and zoosporic fungi. Plantvax is reported to be effective against basidiomycetes pathogens and also against some deuteromycete fungi like *Helminthosporium*, *Curvularia*, *Cladosporium*, *Aspergillus* etc.

Benzimidazoles:

Benomyl, carbendazim and thiabendazoles are important systemic fungicides of benzimidazole group. Benomyl was discovered by Dupont in 1968. It is marketed as benlate. It is a wide spectrum fungicide effective against a number of fungi including powdery mildew fungi (*Erysiphae*, *Sphaerotheca*, *Podoshpaera*, *Uncinula*), *Cercospora*, *Fusarium*, *Verticillium*, *Colletotrichum*, *Gloeosporium*, *Botrytis*, *Monilia* etc. Carbendazim is a break down product of benomyl. When placed in water, benomyl readily breaks down to two compounds viz., methyl-2-benzimidazole carbamate (MBC) and butyl carbamate, which is converted into a volatile compound – butyl isothiocyanate. MBC is now exploited to develop another systemic fungicide carbendazim, marketed under the trade name bavistin. It is having the same fungitoxic properties as benomyl but is more stable. Thiobendazole (TBZ) has a similar fungitoxic spectrum as benomyl, but qualitatively less effective than benomyl.

Thiophanates:

Thiophanates are often classified under benzimidazole fungicides, although they are different in that they are based on thiourea. For its activity, it is converted to benzimidazole ring and its fungicidal spectrum also resembles that of benomyl. Two compounds namely thiophanate and thiophanate methyl are commercially available.

Acylamines:

Chemically these are acylanilides. The group includes metalaxyl, furalaxyl and banalaxyl. Of these, metalaxyl is the first developed and widely used fungicides marketed under the trade name Ridomil. It is highly effective against *Pythium*, *Phytophthora* and many downy mildew fungi.

Pyrimidine Fungicides:

This group includes diamethirimol, ethirimol, bupirimate, fenarimol etc. All these fungicides effects sterol synthesis and effect stability of fungal cells. Diamethirimol is highly effective against powdery mildews and smusts.

Triazoles:

Some of the best fungicides which inhibit sterol synthesis belongs to this group and include triadimenol, bitertanol, boutrizol etc. They show long term protective and curative activity against many powdery mildews, rusts, smuts, leaf spots and blights.

Morpholines:

Tridemorph, marketed as calixin, is a widely used fungicide of this group. It has both preventive and eradicant properties and effective against rusts, powdery mildews and leaf spots.

Organophosphates:

Kitazin and Hinosan are widely used systemic organophosphate fungicides. They are highly effective against blast disease of rice. In India, hinosan is extensively used against rice blast.

Antibiotics:

Antibiotics are defined as substances produced by microorganisms, which in very dilute concentrations have the capacity to inhibit the growth of, or even destroy, other microorganisms. Antibiotics are systemic in their action. They are used in very small concentrations as they are phytotoxic. Antibiotics can be antibacterial (streptomycin, tetracycline etc.) or antifungal (griseofulvin, aureofungin etc.). On being absorbed by foliage or roots, the antibiotics either directly kill the pathogen in tissues or check its development through altered physiology of the host. They remain effective for considerable time, thus acting both as eradicative and protective in action.

Streptomycin:

It is a product of *Streptomyces griseus* and marketed as agrimycin. It is used in ppm concentrations both as foliar sprays and for seed treatment. It is highly effective against fire blight of apple caused by *Erwinia amylovora*, citrus canker caused by *Xanthomonas citri* and other bacterial diseases. It is also mixed with tetracycline in 9:1 ratio and marketed as streptocycline.

Tetracyclines:

They are produced by species of *Streptomyces*. They are effective against diseases caused by mycoplasmas like little leaf diseases, yellows etc. The commercial formulation for plant disease control is marketed as ledermycin.

Cycloheximide:

It is produced by Streptomyces griseus. Cycloheximide is marketed under the trade name actidione. It is highly effective against a number of diseases caused by fungi as well as bacteria, and hence described as antifungal antibiotic. It is effective against powdery mildews, covered smuts, bunt of wheat etc. However, it is highly phytotoxic even at low concentrations, but limited in usage.

Griseofulvin:

It is derived from *Penicillium griseofulvum* and is quite effective against a number of fungi like powdery mildew fungi, *Alternaria* sp., *Botrytis* and also rust fungi.

Aureofungin:

It is a broad-spectrum antifungal antibiotic isolated from *Streptoverticillium cinnamomeum* var *terricola* by Tirumalachar et al in 1964. It is commercially produced by Hindustan antibiotics Co, at Pimpri, Pune. It is used as a spray fungicide, and also for seed treatment. It is highly effective against powdery mildews, downy mildews, anthracnoses etc. It is suggested for control of fruit rots of mango and tomato.

Nystatin:

It is the first antifungal antibiotic isolated from *Streptomyces noursei*. It is a polyene antibiotic and it is poorly absorbed by plant and animal tissue. Hence, it is suggested only for surface pathogens. It is highly effective against cigar end disease of banana caused by *Colletotrichum*, and post-harvest dip of bananas is recommended for control of the disease.

Antibiotics Used Against Paddy Blast:

Blasticidin-S and Kasugamycin and a number of other antibiotics were successfully used in Japan against paddy blast caused by *Pyricularia oryzae*.

Nematicides:

Nematicides are known to control several root-knot and other nematode diseases of crop plants. Carbon disulphide, an earliest chemical, as soil fumigant checks the nematode growth. But is not widely used as have some drawbacks. Chloropicrin used in limited scale in green houses and nursery beds. Methyl bromide, a soil nematicide is too phytotoxic for standing crop. So, should be applied in soil few weeks before the sowing of crop or plantation. Dichloropropene and dichloropropane (DD mixture - ovicidal) and Ethylene dibromide EDB) are the effective nematicides that came into market soon after World War II. Nematicides in vapour or gaseous forms are most effective as the penetration to the desired depth into the soil is possible. Liquid and solid forms of nematicides are less effective. Generally, the most common nematicides in use are of volatile liquids.

9.6. DEVELOPMENT OF DISEASE RESISTANT VARIETIES:

Growing resistant varieties is the only method to control plant diseases in some cases, and perhaps the ideal one. It not only reduces the burden of carrying out expensive control measures using chemicals but also reduces the environmental pollution.

The important methods of obtaining the disease resistant varieties of crop plants are:

1) Selection; 2) Introduction; 3) Breeding.

Selection:

In the early days, the resistant varieties were often obtained by selection from the existing crop populations. Seed was taken from the plants which survived within severely infected crops, and by continued planting and selection under conditions favouring disease, resistant varieties are obtained. In some instances, this method alone sufficed to produce acceptable resistant varieties. Orton (1900) obtained lines of Sea Island and upland cotton in this way that were resistant to wilt. The advantage of the process is that all the commercial qualities are retained in the resistant cultivars. Two types of selections are followed –

1) **Pure Line Selection:** In this method, seed from each selected plant is collected and raised separately. The stock thus produced is genetically pure.

2) **Mass Selection:** A group of plants which are apparently similar, are selected and harvested and the seed is composited.

Farmers commonly follow the method of selection in raising the crops from traditional or indigenous varieties. However, in case of high yielding varieties the method of selection is not generally useful.

Introduction:

The introduction of exotic varieties which showed resistance often solved the problem of development of resistant varieties is some cases. The examples of introduction of exotic varieties of rice and cotton reveal the importance of introduction in obtaining disease resistant varieties.

Rice:

Indica varieties of rice though susceptible to blast disease caused by *Pyricularia oryzae* in India were found to be resistant in Japan. Similarly, japonica varieties of rice though susceptible to blast in Japan are resistant to blast in India.

Cotton:

The indigenous cotton varieties viz., herbaceium and arboretum cottons are highly susceptible to wilt disease caused by *Fusarium* in India, however, found be resistant to wilt when tested in USA, not only to US strains of the pathogen but also to Indian strains. The long-stapled cottons of New World viz., hirsutum and barbadense cottons are highly susceptible to wilt in USA, but when introduced to India, they are found to be resistant to wilt under Indian conditions. Thus, the problem of cotton wilt in India was solved by introducing the high yielding exotic varieties. However, with it came the problem of bacterial disease of cotton.

Breeding for Disease Resistance:

Biffen (1905) first demonstrated with wheat and *Puccinia striiformis* that disease resistance was inherited in Mendelian fashion. Flor (1956) through studies on flax rust showed that there was a gene for gene relationship between host resistance and pathogenicity. With the realization that the resistance to diseases was genetically inherited, the programs for breeding for disease resistance started. In breeding for disease resistance, two important aspects are the sources of resistance and breeding methods.

1) **Sources of Resistance:** For successful breeding program, the sources of resistance must be first identified. Different ways of obtaining sources of disease resistance are a) germplasm collection, b) wild varieties and c) induced mutations.

Germplasm Collection: With the introduction of National and International centers of research, it is becoming very common. The scientists of the research centers are collecting cultivated varieties of the crop from various sources and maintaining the germplasm banks for future use.

Wild Varieties: Non-cultivated relatives of the crop plants are called as wild varieties. They are usually found in the centers of origin of the crop plant concerned. The wild varieties in many cases possess resistance but no other agronomically acceptable characters. Hence, they cannot replace the cultivated varieties but can be used in breeding programs to transfer resistance to cultivated varieties.

Induced Mutations: When natural sources of resistance are not available, it may be induced in the cultivated plants by subjecting the seeds to physical or chemical mutagens. Radiations such as X-rays, Ultraviolet rays, and chemicals like colchicine and ethylmethane sulphonate (EMS) are commonly used for inducing mutations in plants. The mutant lines may be useful or useless depending upon chance. The useful mutant lines are selected for further study.

2) **Breeding Methods:** The technique of breeding varies with the type of plants involved.

Self-Pollinated Crops: The commercially acceptable but susceptible variety of the host is crossed with varieties showing stable genetic resistance, and plants in which desirable features of parents combined are selected from the segregating progeny.

Cross-Pollinated Crops: Recurrent selection is used with cross-pollinated crops to concentrate genes for disease resistance without marked loss of genetic variability.

Back Cross Method: This method is used for the first time by Briggs (1938) as a means of adding disease resistance to wheat and barley varieties. It entails the crossing of a recurrent parent with hybrid progeny for the purpose of recovering its characters, except for addition of disease resistance from the non-recurrent parent. The ease with which this method can be carried out depends upon whether the character to be transferred is oligogenic or polygenic and dominant or recessive.

When scientists try to breed the cultivated varieties of crop plants for disease resistance in laboratories, the pathogens breed for virulence in nature. Hence, it is an unending and ever going program.

9.7. SUMMARY:

Exclusion, eradication, sanitation, developing disease resistance varieties are some of the important methods to control the plant diseases or manage the disease incidence in such a way that it does not because serious yield loses. Quarantine is a legal restriction on the movement of diseased material and it is imposed by the Governmental laws. Eradication aims at destroying the inoculum before it reaches the field, and seed treatment is a measure of eradicating primary sources, since seeds are vectors of many diseases. Destroying collateral hosts, alternate hosts etc. also come under eradication. Maintaining the fields without weeds,

and roguing of infected parts of the plants comes under sanitation measures. Cultural practices for disease control involve crop rotation, mixed cropping, selection of fields, sowing methods etc. Chemical control is commonly practiced to manage many crop diseases. Various chemicals used for disease control can be categorized into contact fungicides, systemic fungicides and antibiotics. Contact fungicides are effective as long as they are in contact with the host surface, and include compounds of Sulphur, Copper, Mercury, dithiocarbamates, quinines, benzene compounds, heterocyclic nitrogen compounds, oils etc. Systemic fungicides are taken up by the plant and translocated within the host tissue and they act for a long time. Oxanthiins, benzimidazoles, thiophanates, acylamines and others are important systemic fungicides. Antibiotics are useful mainly against bacterial diseases and against a few fungal diseases also. Agronomically useful antibiotics are streptomycin, tetracyclines, cycloheximide, griseofulvin, aureofungin, nystatin etc. Use of genetically resistant varieties is the best method to control plant disease epidemics. Selection, introduction and breeding are important methods of developing disease resistant varieties.

9.8. TECHNICAL TERMS:

Plant Quarantine, Seed Disinfection, Seed Disinfestation, Cultural Practices, Cropping Patterns, Contact Fungicides, Systemic Fungicides, Antibiotics, Nematicides, Disease resistance, Breeding.

9.9. SELF ASSESSMENT QUESTIONS:

1) Discuss the Principles of Plant Disease Control
2) Give an account of range of chemicals used in control of plant diseases
3) Discuss the non-chemical methods of managing the plant diseases
4) Write an account on contact and systemic fungicides.

9.10. SUGGESTED READINGS:

1) Singh, R.S. 1998. Plant Diseases 7th Edn., Oxford & IBH Publishing Co., Pvt., Ltd., New Delhi.
2) Rangaswami, G. and Mahadevan, A. 1999. Diseases of Crop Plants in India. 4th Edn., Prentice-Hall of India Private Ltd., New Delhi.
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LESSON-10

BIOLOGICAL CONTROL OF PLANT DISEASES

10.0. OBJECTIVE:

- Students will understand how that plant diseases can be controlled by using bio-agents in the place of chemical compounds and their significance.

STRUCTURE:

10.1. INTRODUCTION:

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 bio-control agent (antagonist), the microbial community on and around the plant and the physical environment. G.B. Sanford in 1926 reported that potato scab, caused by Streptomyces scabies, was suppressed under field conditions by green manuring through the activity of antagonistic microorganisms, and suggested the scientific possibility of using antagonistic microorganisms for the control of plant diseases. Since then, much data accumulated to show that biological control is a feasible proposition for plant management.

Cook and Baker (1983) defined biological control of plant diseases as “reducing the amount of inoculum or disease producing activity of a pathogen accomplished by one or more organisms, other than man”. It is now receiving much attention because it is cheaper, safer to use and ecofriendly when compared to the use of chemical pesticides which are costly, hazardous and cause environmental pollution.

10.2. APPROACHES TO BIOLOGICAL CONTROL:

There are three main approaches to biological control of plant pathogens: 1) Biological control of inoculum, 2) Biological protection of plant surfaces and 3) Cross protection and induced resistance. More than one approach may be adopted for control of a particular plant disease.

1) **Biological Control of Inoculum:** It is achieved by direct destruction of inoculum formation and reducing the vigour or virulence of the pathogen. This approach normally does not involve the host plant. The antagonistic microorganism attacks the resting structures or actively growing mycelium of fungal pathogens surviving in soil. For example, sclerotia of pathogenic fungi are destroyed by parasitism of *Sprodesmium sclerotivorum*, actively growing mycelium of *Rhizoctonia solani* is destroyed by parasitism of *Trichoderma harzianum*.

2) **Biological Protection of Plant Surfaces:** It involves establishment of an antagonist in or around the site of infection, so as to provide protection of the area against a pathogen. The host is not involved in the interaction between the pathogen and the antagonist, though it occurs on host plant surface. Best example for such protection is ectomycorrhizae. The ectomycorrhizal fungi form a thick sheath of mycelium of mantle on the surface of the host root, and acts as a mechanical barrier to infection of root by soil borne pathogens. Many rhizosphere bacteria also occupy the root surface, which are normal points of entry of pathogens, thus excluding the infection.

3) **Cross Protection and Induced Resistance:** In this method, the host plays a major role. A host plant colonized by an avirulent or mild strain of the pathogen becomes resistant to attack by the virulent strains of the pathogen. It is because the avirulent strain might have induced formation of certain chemical substances in the host which gives protection against attack by the virulent strains of the pathogens. It is very common in virus infections and in case of a few soil borne fungal diseases like wilts.

10.3. BIOCONTROL AGENTS:

A number of viruses, bacteria and fungi are recognized as potential biocontrol agents, and some of them are used for biocontrol of diseases at field level. The viruses that attack plant pathogenic fungi either completely destroy them or reduce their virulence. A few soil fungi like species of *Trichoderma* effectively reduce infection by *Sclerotium rolfsii*, *Rhizoctonia solani*, *Pythium aphanidermatum* on their host plants. Other fungi that have gained prominence as biocontrol agents are *Myrothecium*, *Verrucaria*, *Penicillium vermiclatum*, *Gliocladium roseum*, *Coniothyrium minitans* etc. Among the soil bacteria, species of *Pseudomonas* and *Bacillus* are receiving much attention as biocontrol agents. *Agrobacterium radiobacter* is exploited for biocontrol of crown gall disease caused by *Agrobacterium tumefaciens.*

Diseases Controlled Through the Use of Microorganisms:

Antagonistic activity of a large number of viruses, bacteria and fungi against plant pathogenic organisms has been reported by many workers. But, only a relatively few diseases of crop plants were successfully controlled through biological means at field level. Most of

these success stories of biological control concern with soil borne diseases. Though pathogens of aerial plant parts are also having antagonistic microorganisms, very few plant diseases of aerial plant parts were successfully controlled by biological means. This is mainly because the antagonistic microorganisms have sufficient time and means of attack soil borne plant pathogens in their natural environment than antagonists of aerial pathogens. Some of the important plant diseases are controlled by biological means are –

1) **Take-All Disease of Wheat:** Take-all disease of wheat caused by *Gaeumannomyces graminis* is a very serious disease of wheat crop and infected plants are completely destroyed and no yields come from it, and hence the name. It is the most researched case of biological control strategy. Fluorescent *Pseudomonas* species isolated from the rhizosphere of the plants have found to effectively control the disease. The strains of *Pseudomonas fluorescens* produce antifungal metabolites like phenazine, 2, 4-diacetylphloroglucinol etc. and effectively control the disease. Another approach found successful was cross protection with avirulent fungi.

2) **Crown Gall of Apple:** *Agrobacterium tumefaciens* is a very common soil borne bacterium that infects a large number of plants spread over 93 families of flowering plants causing crown gall disease. It is particularly severe in nurseries of fruit crops like apple, pome fruit, stone fruit, peaches etc. The bacterial strains with biocontrol potential were isolated from symptomless plants growing in diseased fields and these are identified as belonging to *Agrobacterium radiobacter*. These are non-pathogenic and produce a bacteriocin that effectively inhibits *Agrobacterium tumefaciens*. The *A. radiobacter* strain K-84 was commercialized in 1973 in Australia under the trade name Goltrol, Norback and Diegall. A derivative strain *A. radiobacter* strain K-1026 was later developed and marketed in 1991 as Nogall.

3) **Stump Rot of Pines:** Freshly cut pine stumps are infected by *Heterobasidion annosum* causing severe rotting and spread to roots. It was particularly serious in USA and Europe. The pinus stump rot was susccessfully controlled by inoculating the freshly cut surfaces with the spores of *Peniophora gigantea*, another basidiomycete fungus. *Peniophora* spreads into lateral roots and competes with the pathogen by hyphal interference. The spores of *Peniophora* are applied to the cut surfaces as a water suspension or as powder. It is one of the success stories of biological control in forest pathology.

4) **Damping Off of Vegetable Crops:** Damping off is a serious disease of germinating seeds and young seedlings of vegetable and other crops and is caused by species of *Pythium, Rhizoctonia solani* and other soil borne fungal pathogens. Actively growing mycelium of these fungi are attacked and destroyed by species of another soil fungus *Trichoderma*. Hence, the *Trichoderma* species, especially *T. harzianum* and *T. viridae* are used for control of damping off pathogens. Preparations of *Trichoderma* containing active mycelium and spores are commercially available. The preparation is applied to the seeds of many crop plants before sowing for control of damping off disease.

5) **Wilt of Flax:** Wilt disease of flax (*Linum usitatissimum*) caused by *Fusarium oxysporum* f.sp. *lini* is a very serious disease affecting seedling viability as well as established plants. Seed bacterization with *Pseudomonas fluorescens* increase seedling viability and reduce the wilt in standing crop. The bacterial biocontrol agent produces siderophores and reduce the growth of fungal pathogen.

6) **Fire Blight of Apple and Pears:** Fire blight caused by *Erwinia amylovora* is a devastating plant disease of rosaceous plants like apple and pears. Strains of *Erwinia herbericola* and *Pseudomonas fluorescens* isolated from the phylloplane of the plant are antagonistic to the pathogen. Spraying of these bacteria on healthy leaves effectively control the incidence of fire blight. *Pseudomonas fluorescens* strain-506 was commercialized as 'blight ban' for biocontrol of fire blight of apples and pears.

10.4. VIRUSES AS BIOCONTROL AGENTS:

The viruses act as biocontrol agents by reducing the virulence of pathogenic fungi. Loss of infectivity of many fungi is attributed to the presence of virus like particles or double stranded RNA mycoviruses. This was reported in case of *Rhizoctonia solani* due to which damping-off of sugar beet was significantly reduced. Association of mycoviruses with hypovirulent strains of *Gaeumannomyces graminis* var *tritici* has been reported, and these are responsible for loss of virulence of the pathogen. These observations raised the hope of using mycoviruses as biocontrol agents. However, the enthusiasm for using mycoviruses as biocontrol agents is now diminishing mainly because there is no effective method to transfer viruses mechanically to fresh fungal mycelium of the pathogens.

10.5. FUNGI AS BIOCONTROL AGENTS:

The fungi act as biocontrol agents by more than one way. The mechanisms include hyper parasitism, cross protection and formation of mechanical barrier.

1) **Hyperparasitism:** *Trichoderma* species are parasitic on a large number of soils borne fungi and they have been reported to control several soils borne plant diseases. Four species of *Trichoderma* are commonly used as biocontrol agents viz., *T. harzianum, T. viridae, T. konigii* and *T. hamatum*. *T. harzianum* is a mycoparasite of *Rhizoctonia solani* and *Sclerotium rolfsii*, and both these fungi are omnivorous pathogens having wide host range causing damping off and root rots. *T. harzianum* cultures are recommended for application to seeds before sowing, for control of damping off and root rots. The culture preparations are available commercially. The pathogenic fungi that attack aerial plant parts like rusts and powdery mildews are attacked by a number of hyperparasites. *Sphaerellopsis filum* and *Verticillium lecanii* are hyperparasites on rusts on many hosts. Several powdery mildews are parasitized by *Ampelomyces quisqualis*. Experimentally, it is shown that the hyperparasites of rusts and powdery mildews reduce the incidence of these diseases by reducing the vigour of mycelium and its sporulation. However, these are not used at field level.
2) **Cross Protection:** Cross protection from soil borne plant pathogens by using avirulent but closely related strains of soil borne plant pathogens has been demonstrated with many diseases. This phenomenon involves prior colonization of infection court by antagonists or avirulents. Biological control of take-all disease of wheat caused by *Gaeumannomyces graminis* var *tritici* has been extensively worked out. Avirulent fungi used in control of take-all disease include *Phialophora radicicola* var *graminicola* and *Gaeumannomyces graminis* var *graminis* etc. Resistance against soil borne diseases induced by prior colonization of roots with other fungi has also been demonstrated in case of *Verticillium* wilt of tomato by use of *Fusarium oxysporum* f.sp. *lycoperscici*. The draw backs of this method are that 1) the avirulent strains of the pathogens may have negative effect on plant health and vigour and 2) cross protecting strains may have pathogenic effect on other plants grown in the same field.

3) **Mechanical Barrier:** Mycorrhizal fungi, especially ectomycorrhiza, are reported to be useful in biological control of soil borne pathogens. As the ectomycorrhizal fungi form a thick sheath or mantle of fungal hyphae on the surface of the roots, they provide mechanical barrier to penetration of root cortical cells by the pathogen. The ectomycorrhizal fungi may also protect the plants by other mechanisms such as 1) using available nutrients at plant surface, thus depleting nutrients for the pathogens, 2) by the production of volatile and non-volatile inhibitors and 3) supporting the growth of antagonists in the rhizosphere. Ectomycorrhizal fungi like *Boletus variegatus*, *Pisolithus tinctorius* and *Cenococcum graniforme* produce volatile substances in pine roots which are inhibitory to *Phytophthora cinnamomi*.

10.6. BACTERIA AS BIOCONTROL AGENTS:

Plant root exudates had a highly positive effect on the bacterial populations in the rhizosphere soil. The bacterial populations in the rhizosphere zone are 10-20 times more than those in non-rhizosphere soil. These bacteria in the rhizosphere zone are called as rhizobacteria, and among rhizosphere bacteria species of *Pseudomonas* and *Bacillus* are predominant. *Pseudomonas* and *Bacillus* species exhibit biocontrol properties and are receiving much attention. These are now considered as plant growth promoting rhizobacteria (PGPR). About 10% of the bacteria in the rhizosphere appear to be aggressive in reducing the population of deleterious rhizosphere microorganisms and the possible mechanisms responsible for biocontrolling activity of PGPR include one or more of the following: a) Competition for substrate and niche exclusion, b) Competition for nutrients, c) Production of antifungal metabolites and antibiotics and e) Induced systemic resistance (ISR).

a) Competition for Substrate and Niche Exclusion:

Pseudomonas and other bacteria are aggressive colonizers of rhizosphere and rhizoplane. About 4-10% of root surface is colonized by bacteria and that much area is excluded for colonization by pathogenic microorganisms. Biocontrol of root rot of sugar beet seedlings by *Pythium ultimum* has been reported by the seed bacterization with *Pseudomonas* species. These species compete for the substrate with the pathogen and thus protects the sugar beet seedlings from infection.

b) Competition for Nutrients:

Competition for the nutrients supplied by root and seed exudates occurs between rhizosphere bacteria and plant pathogens. The fast growing rhizobacteria effectively compete with relatively slow growing plant pathogens, thus adversely affecting the pathogens. Elad and Chet (1987) reported that root colonizing populations of *Arthrobacter* sp. reduce the amount of available carbon and nitrogen required to stimulate the germination of oospores of *Pythium aphanidermatum*, the causal organism of damping off and root rot of various crop plants, thus reducing the disease incidence.

c) Production of Siderophores:

Iron is one of the most abundant minerals on the earth and is an essential micronutrient for the living organisms. However, iron in the soil is unavailable for the direct assimilation by microorganisms because ferric iron, the predominant form in nature, is sparingly soluble in water. This amount of soluble iron is much too low to support microbial growth. Consequently, to survive in this environment, soil microorganisms synthesize and secrete low molecular weight iron binding proteins known as 'siderophores'. The siderophores bind to ferric iron with a very high affinity and transport back to the microbial cell, where it is taken by cellular receptors. Once inside the cell, the iron is released to ferrous form and utilized by the microorganisms.

Many fluorescent *Pseudomonas* species secrete a yellow green fluorescent siderophore called psudobactin which has a very high affinity for binding with ferric iron. Several investigators have shown that various strains of *Pseudomonas fluorescens,* especially strain B-10, produce pseudobactin that inhibit the growth of *Erwinia carotovora* which causes soft rot of potatoes. Similar results were also reported with flax and wheat. Flax was susceptible to Fusarium oxysporum f.sp. lini which cause wilt of flax. Seed bacterization with *Pseudomonas* increase seedling viability in soils infested with *Fusarium oxysporum* f.sp. *lini*. Similarly, wheat is susceptible to *Gaeumannomyces graminis* which cause take-all disease and seed treatment with *P. fluorescens* strain B-10 effectively reduce the disease and increase the growth.

d) Production of Antifungal Metabolites and Antibiotics:

Most of the *Pseudomonas* strains, used as biocontrol agents, produce antifungal metabolites with include phenazines, pyrrolnitrin, pyoluteorin, viscosinamide, tensin, 2,4-diacetylphloroglucinol, oomycin etc. Seed treatment with purified pyoluteorin and pyrrolnitrin provide protection of cotton seedlings against damping off disease caused by *Pythium ultimum* and *Rhizoctonia solani*, respectively. The suppression of black root rot of tobacco and take-all disease of wheat by application of *Pseudomonas fluorescens* strain CHAO involves production of 2,4 diacetylphloroglucinol. *Agrobacterium radiobacter* strain k84 produce an antibiotic agrocin-84 which control the crown gall disease caused by *Agrobactrium tumefaciens*. Agrocin-84 is considered as a bacteriocin. The bacteriocins are low molecular weight proteins produced by bacteria and are lethal to other strains of closely related bacteria.

e) Induced Systemic Resistance (ISR):

A number of *Pseudomonas* species have ability to induce a state of systemic resistance in plants which provides protection against a broad spectrum of phytopathogenic fungi, bacteria and viruses. Some examples of induced systemic resistance in plants by *Pseudomonas* species –

***Pseudomonas* Strain**	**Bacterized Host Plant**	**Challenge Pathogen**
P. fluorescens WCS417r	Carnation (roots) *Arabidopsis* (roots)	*Fusarium oxysporum* *Xanthomonas campestris*
P. fluorescens WCS374r	Radish (roots)	*Fusarium oxysporum* *Xanthomonas campestris*
P. fluorescens CHAO	Tobacco (roots)	Tobacco necrosis virus
P. fluorescens S97	Bean (seeds)	*Pseudomonas phaseolicola*
P. aeruginosa 7NSK2	Bean (seeds)	*Botrytis cinerea*
P. putida 89B27	Cucumber roots	*Fusarium oxysporum* Cucumber mosaic virus

The basis of mechanism for induced resistance seems to be multi component. It is postulated that ISR may be due to production of low molecular weight proteins called pathogenesis related (PR) proteins, salicylic acid etc.

PR-Proteins:

The systemic accumulation of PR proteins is found to be associated with ISR to some plant pathogens. These PR proteins are of two types viz., acidic and basic PR proteins. The acidic PR proteins include acidic β-1, 3-glucanses and chitinases. Usually these PR proteins are secreted into intercellular spaces where they would be encountered by and act against fungal and bacterial plant pathogens at an early stage of the infection process. The basic PR proteins include basic β-1, 3-glucanses and chitinases. They are generally accumulated intracellularly in the vacuoles. These PR proteins may interact with plant pathogens at a later stage of infection during host cell deterioration. Defago et. al. (1995) demonstrated induced systemic resistance and the accumulation of PR proteins, β-1, 3-glucanses and chitinases in tobacco leaves against tobacco necrosis virus by *P. fluorescens* CHAO.

Salicylic Acid:

Pseudomonas species produce salicylic acid in very small amounts and it is also implicated in induction of systemic resistance. DeMeyer et. al. (1999) reported that nanogram amounts of salicylic acid produced by the *Pseudomonas aeruginosa* 7NSK2 activate the systemic acquired resistance in bean against *Botrytis cinerea*.

Some other compounds like cytokinins and inhibitors of viral replication have also been implicated in ISR to localized tobacco virus infection.

Biological Control at Field Scale:

- Potato scab caused by *Streptomyces scabies* can be controlled by *Bacillus subtilis.*
- Cereal cyst caused by nematode, *Heterodera avenae* can be controlled by *Nematophtora gynophila* and/or *Verticillium chlamydosporum* which parasitize the nematode pathogen and inhibit the cyst formation.
- Damping off and seedling blight caused by *Pythium, Rhizoctonia* and *Fusarium* species can be controlled by *Trichoderma, Penicillium,* and *Bacillus* effectively when coated the seeds with these antagonistic microorganisms.
- Root knot disease caused by *Meloidogyne* species can be controlled at field level at some places by *Bacillus penetrans* and *Dactylella oviparasitica.*
- *Armillaria* root rot can be controlled by fumigation of soil with carbon disulphide or methyl bromide, which weakens pathogen, followed by the attack by *Trichoderma viridae.*
- Crown gall caused by *Agrobacterium tumefaciens* can be controlled by *Agrobacterium radiobacter* pv. *radiobacter* strain k84.

Commercialization of Biocontrol Agents:

Though there are thousands of examples in literature where it has been demonstrated that microbes parasitize or antagonize or compete in any other way with pathogens, they are very few cases where this potential has been exploited on commercial scale. Some important examples of commercially available biocontrol agents are –

Biocontrol Agent / Trade Name	Disease Controlled	Pathogen
Agrobacterium radiobacter (Galltrol, Diegall, Nogall)	Crown gall of apple	*Agrobacterium tumefaciens*
Peniophora gigantea	Stump rot of pines	*Heterobasidion annosum*
Pseudomonas fluorescens strain 506 (Blight ban)	Fire blight of apple and pears	*Erwinia amylovora*
Trichoderma harzianum	Damping off of crop plants	*Pythium* sp., *Rhizoctonia solani, Sclerotium rolfsii*
Bacillus subtilis (Quantum 4000)	Wilt of cereals and vegetables	*Fusarium* sp., *Verticillium* sp.

Advantages and Disadvantages of Biological Control of Diseases:

Advantages of Biocontrol Agents:

1) Biological control is less costly and cheaper than any other methods.
2) Biocontrol agents give protection to the crop throughout the crop period.
3) Biocontrol agents 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) They 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 phosphorus and makes it available to the plant.
9) They are very easy to handle and apply to the target.
10) They can be combined with the biofertilizers.
11) They are easy to manufacture.

Disadvantages of Biocontrol Agents:

1) They can be used only against specific diseases.
2) They are less effective than the fungicides.
3) They have slow effect in the control of plant diseases.
4) At present, only few biocontrol agents are available for use and are available in few places.
5) They are unavailable in larger quantities at present.
6) This method is only a preventive measure and not a curative measure.
7) They should be multiplied and supplied without contamination which requires skill.
8) The shelf life of biocontrol agents is short. Antagonists, *Trichoderma viridae* is viable for four months and *Pseudomonas fluorescens* is viable for 3 months only.
9) The required amount of population of biocontrol agents should be checked at periodical interval and should be maintained at required level for effective use.
10) The efficiency of biocontrol agents is mainly decided by environmental conditions.
11) A biocontrol agent under certain circumstances may become a pathogen.

10.7. SUMMARY:

Biological control of plant diseases may be defined as "reducing the amount of inoculum or disease-reducing- activity of a pathogen accomplished by one or more organisms other than man. There are three main approaches to biological control of plant pathogens viz., Control of inoculum outside the host by either parasitism, producing antimicrobial substances, control of pathogen on the surface of plant by competition and antagonism; and by inducing resistance and cross protection in which host plant is also involved. The major biocontrol agents are fungi and bacteria; viruses also control some diseases by reducing vigour of pathogens but no proper technology was developed for use of viruses. Mycoparasites like *Trichoderma* and mycorrhizal fungi are important biocontrol agents among fungi. Among bacteria, species of *Pseudomonas, Bacillus* and *Agrobacteria* are receiving much attention as biocontrol agents.

The important diseases controlled through biological methods include take-all disease of wheat, crown gall of apple, stump rot of pines, damping off of vegetable crops, wilt of flax, fire blight of apple etc. The mechanisms of action of viruses, fungi and bacteria as biocontrol are explained. Among commercially exploited biocontrol-agents, *Agrobacterium radiobacter* (as Diegall), *Pseudomonas fluorescens* strain 506 (Blight ban) *Bacillus subtilis* (QUANTUM 4000), *Trichoderma harzianum, Peniophora gigantea* are important.

10.8. TECHNICAL TERMS:

Biocontrol agents, Viruses, Fungi, Bacteria, PGPR, Siderophores, Induced systemic resistance, PR-proteins.

10.9. SELF ASSESSMENT QUESTIONS:

1) Discuss the Biological Control of Plant Diseases.
2) Discuss the Mechanisms of Biocontrol of Plant Diseases giving suitable examples.
3) Give an Account of Microorganisms used for Biological Control of Plant Diseases and Diseases they Control.
4) Describe the Fungi used for Biocontrol of Plant Diseases.

10.10. SUGGESTED READINGS:

1) Singh, R.S.1998. Plant Diseases 7th Edn. Oxford- & IBH Publishing Co. Pvt. Ltd; New Delhi.
2) Rangaswami, G. and Mahadevan, A. 1999. - Lliseases of Crop Plants in India 4th Edition, Prentice Hall of India Publications, New Delhi.
3) Subba Rao, N.S. 1999. Soil Microbiology 4th Edn. Oxford and IBH Publishing Co. Pvt. Ltd; New Delhi.
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LESSON-11

BIOPESTICIDES

11.0. OBJECTIVE:

- Students will understand how that plant diseases can be controlled by using bio-agents in the place of chemical compounds and their significance.

STRUCTURE:

11.1. INTRODUCTION:

Biopesticides are the pest management tools that are based on beneficial microorganisms (bacteria, viruses, fungi and protozoa), beneficial nematodes or other safe, biologically based active ingredients. The benefits of biopesticides include effective control of insects, plant diseases and weeds, as well as human and environmental safety. Biopesticides also play an important role in providing pest management tools in areas where pesticide resistance, niche markets and environmental concerns limit the use of chemical pesticide products. Biopesticides include naturally occurring substances that control pests (biochemical pesticides), microorganisms that control pests (microbial pesticides), and pesticidal substances produced by plants containing added genetic PIP (Plant-Incorporated Protectants) materials.

The efficacy of many of the biopesticides can equal that of conventional chemical pesticides. However, the mode of action will be different. With many of the biopesticides, the time from exposure to morbidity and death of the target insect may be 2 to 10 days. Understanding the fundamental differences in the mode of action of biopesticides and traditional pesticides is important, since the usage patterns of a biopesticide may be different from traditional pesticides to control a particular pest species. The use of an Integrated Pest Management Program (IPM) is important to ensure success. Biopesticides are an important group of pesticides that can reduce the risk of pesticides.

11.2. BIOPESTICIDES:

Characteristics of Biopesticides

- They have a narrow target range.
- They have a specific mode of action.
- They are slow acting and have relatively critical application times.
- They can suppress, rather than eliminate a pest population.
- They have limited field persistence and a short shelf life.
- They are safer to humans and the environment than conventional pesticides.
- They have a short residual effect.

Types of Pesticides:

Microbial Pesticides:

Microbial pesticides are those pesticides, which contain a microorganism like bacterium, fungus, virus, protozoan as active ingredient. Microbial insecticides are another kind of biopesticide. They come from naturally-occurring or genetically altered bacteria, fungi, algae, viruses and protozoans. They suppress pests by producing a toxin specific to the pest, causing a disease and preventing establishment of other microorganisms through competition or other modes of action.

Plant Pesticides:

Plant pesticides are those pesticides which have been derived from genetic material produced by the plants that have pesticidal properties. Pesticidal substances that plants produce from genetic material are added to the plants. For example, scientists can take the gene of the Bt pesticidal protein and introduce the gene into the genetic material of plants. Then the plant instead of the Bt bacterium, manufactures the substance that destroys the pests. Plant-Incorporated-Protectants (PIPs) are pesticidal substances that plants produce from genetic material that has been added to them.

Natural Pesticides:

Natural pesticides are environment friendly, non-toxic, completely biodegradable natural products with comprehensive insect-pest control properties, effective on more than 300 types of insect-pest based on Neem (*Azadirachta indica*). Neemhit is for diverse biological effects such as insecticidal, antifeedant, repellent, nematicidal, antibacterial, antiviral and deterrent. Neemaura is an environmentally friendly pesticide, which has the natural bitterness of neem. It is non-toxic and has a safe biodegradable urea coating agent containing neem triterpenes, which inhibits the growth of nitrifying bacteria, resulting into the delayed transformation of ammonical nitrogen into nitrite nitrogen. This ensures slow and continuous availability of nitrogen, matching the requirements of crop plants during their life cycle and effectively retards the nitrification of urea.

Nematode Biopesticides:

The infective stages of insect-killing or entomopathogenic nematodes (ENs) are primitive microscopic round worms that can seek out and kill a wide range of insect pests but are harmless to other animals, plants and the environment. ENs currently account for most of the World's biopesticide market, in comparison to all other organisms apart from Bt. However, further expansion of this market is limited by expense of production, short shelf life, under developed formulation and application technology and limited strain collection and evaluation.

Applications of Biopesticides:

Microbial agents, effective control requires appropriate formulation and application. Biopesticides have established themselves on a variety of crops for use against crop disease. For example, biopesticides help control downy mildew diseases. Their benefits include: a 0-day pre-harvest interval, success under moderate to severe disease pressure, and the ability to use as a tank mix or in a rotational program with other fungicides. Because some market studies estimate that as much as 20% of global fungicide sales are directed at downy mildew diseases, the integration of biofungicides into grape production has substantial benefits by extending the useful life of other fungicides, especially those in the reduced-risk category.

A major growth area for biopesticides is in the area of seed treatments and soil amendments. Fungicidal and biofungicidal seed treatments are used to control soil-borne fungal pathogens that cause seed rot, damping-off, root rot and seedling blights. They can also be used to control internal seed-borne fungal pathogens as well as fungal pathogens on the seed surface. Many biofungicidal products show capacities to stimulate plant host defence and other physiological processes that can make treated crops more resistant to stresses.

Importance of Biopesticides:

Most farmers attempting to establish a sustainable farming system know that the chemical shed is not their first line of defence against unwanted pests. A "softer" biopesticide or a conventional, synthetic treatment is the farmer's two options when a pest infestation gets too serious, and a chemical application is required. The Integrated Pest Management (IPM) program combines cultural measures, biological controls (such as predatory insects), and chemical control to keep pest populations under control.

Biopesticides are more environmentally friendly and do not harm the soil, water supply, or wildlife, including beneficial insects, which is one of the main advantages of introducing them into a sustainable agriculture system.

Biopesticides are typically used in rotation with conventional products rather than as a replacement, which reduces the amount of synthetic chemicals used. Insects and diseases develop resilience to synthetic chemicals over time. The effectiveness of the synthetic chemical is increased by alternating it with biopesticides.

Despite the potential benefits of using biopesticides, their usage has not been as popular as expected for the following reasons:

1) Expenses associated with creating, testing, and obtaining regulatory approval for new biological agents contribute to the high cost of pesticide manufacture.

2) Due to regional and climatic changes in humidity, temperature, soil conditions, etc., there is limited field effectiveness.

3) Farmers are hesitant about biopesticides because of their high specificity, which means that they only work against specific pathogens and pests.

4) Multiple biological agents are used in biopesticides to manage a variety of insects and pests in the field. These treatments are difficult to use, expensive, and inconvenient, and they are not suitable for many pests and pathogens.

5) Because biopesticides are sensitive to changes in temperature and humidity, they have a short shelf life.

11.3. *BACILLUS THURINGIENSIS* AS BIOPESTICIDE:

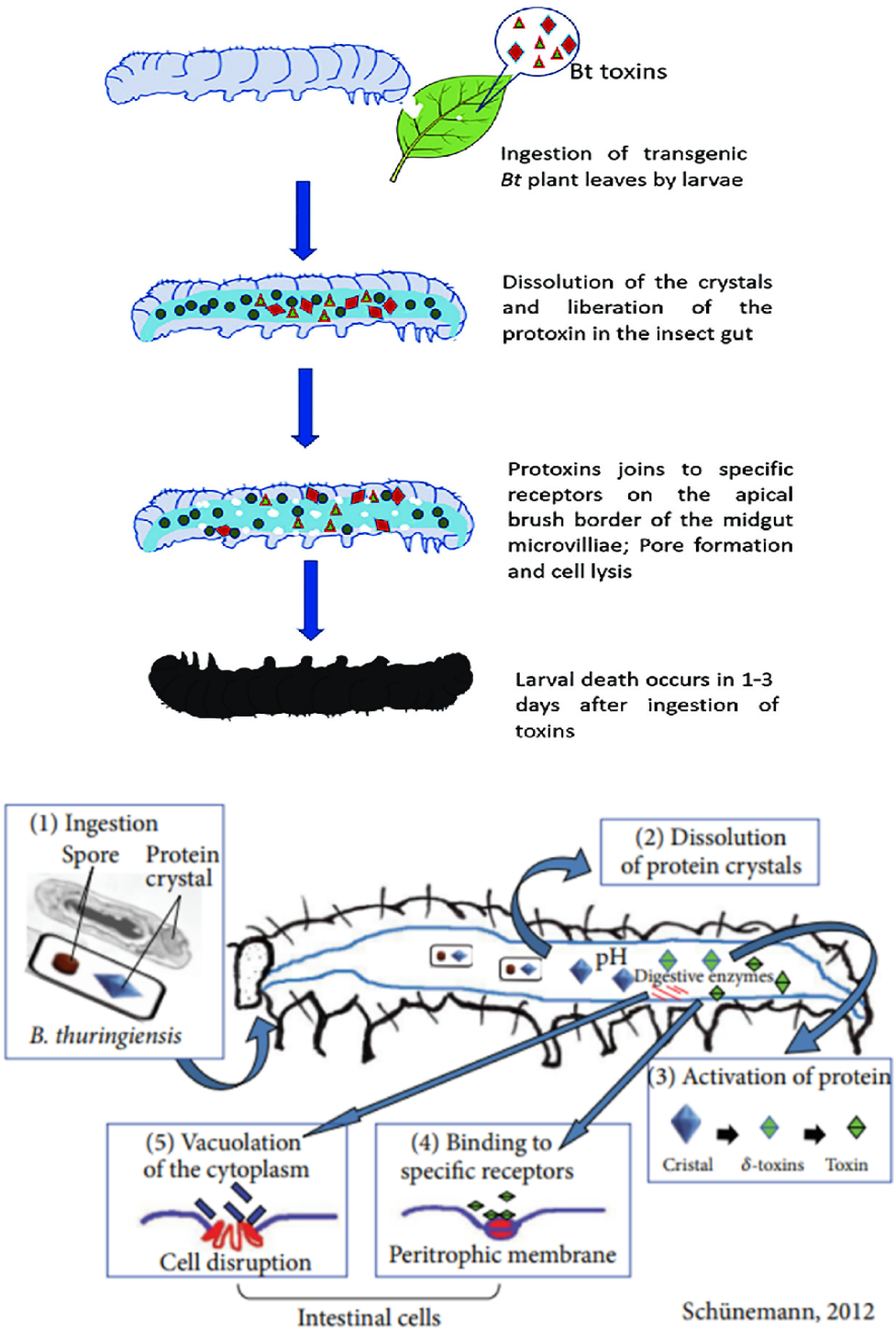
Bacillus thuringiensis (Bt) is a Gram-positive, rod-shaped, spore-forming soil bacterium that is widely used as a microbial biopesticide for the control of insect pests. It belongs to the *Bacillus cereus* group and is naturally distributed in soil, water, plant surfaces, and insect habitats. The pesticidal property of Bt is due to its ability to produce crystalline inclusion bodies (δ-endotoxins) during the sporulation phase. These crystal proteins, known as Cry (crystal) and Cyt (cytolytic) toxins, which are toxic to specific groups of insects and form the basis of Bt-based biopesticides used worldwide. Bt is regarded as one of the most successful and environmentally safe biopesticides because of its high specificity, biodegradability, and lack of toxicity to non-target organisms, including humans, livestock, birds, fish, and beneficial insects.

Classification and Types of Bt:

Bt strains are classified based on their toxin profile and insect host specificity. *Bacillus thuringiensis* subsp. *kurstaki* (Bt-k) is effective against lepidopteran larvae such as bollworms, armyworms, and caterpillars. *Bacillus thuringiensis* subsp. *israelensis* (Bt-i) targets dipteran larvae, particularly mosquitoes and black flies. *Bacillus thuringiensis* subsp. *tenebrionis* (Bt-t) is effective against coleopteran pests such as beetles. This diversity of Bt strains allows selective pest control without disturbing ecological balance.

Mode of Action of Bt Toxins:

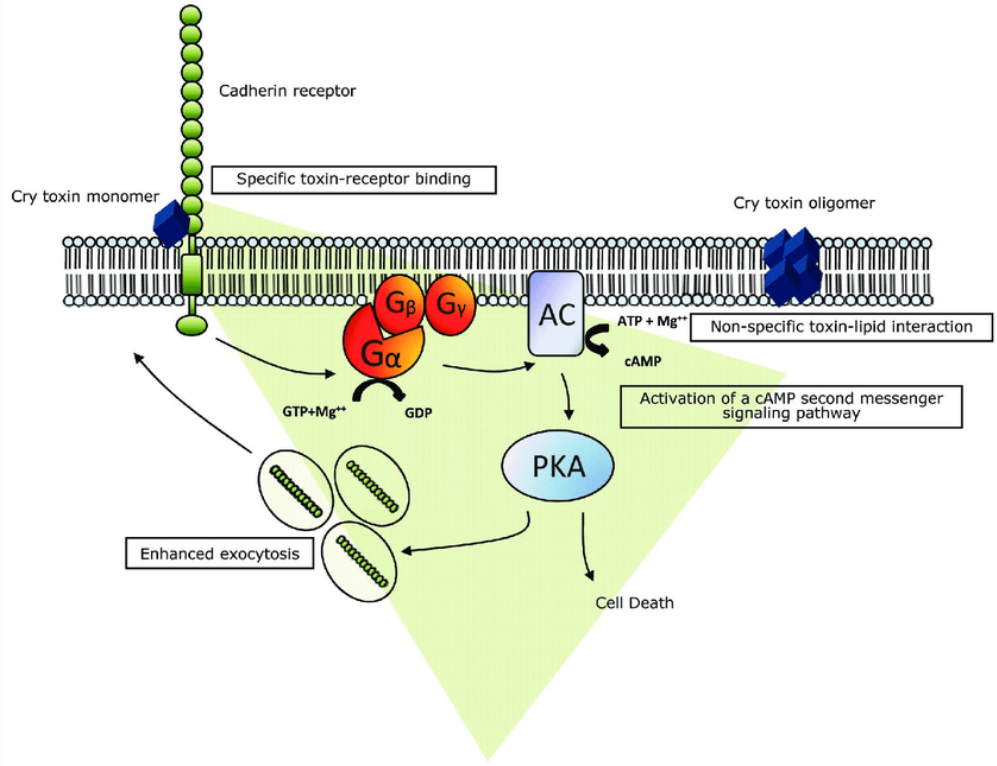
The mode of action of Bt is a multi-step physiological and biochemical process. Susceptible insect larvae ingest Bt spores and crystal proteins while feeding on treated plant surfaces. In the alkaline environment of the insect midgut, the crystal proteins dissolve and are converted from inactive protoxins into active toxins by gut proteases. These activated toxins bind to specific receptors on the epithelial cells of the midgut, leading to pore formation in the cell membrane. The pores cause osmotic imbalance, cell lysis, disruption of gut integrity, and paralysis of the digestive system. Eventually, the insect stops feeding and dies due to starvation and septicemia.



Mechanism of Bt Toxin Action

Cellular Events during Bt Action:

At the cellular level, Cry toxins bind to cadherin-like and aminopeptidase-N receptors present only in susceptible insects. This receptor-mediated specificity explains why Bt does not affect non-target organisms. Following receptor binding, the toxins oligomerize and insert into the membrane, forming ion-conducting pores that lead to irreversible cellular damage.



Production and Formulation of Bt Biopesticides:

Bt biopesticides are produced by large-scale fermentation of selected Bt strains under controlled conditions. After sporulation, the bacterial culture containing spores and crystal toxins is harvested, concentrated, and formulated into products such as wettable powders, granules, dusts, suspension concentrates, and flowable formulations. These formulations are applied as foliar sprays or soil treatments using conventional agricultural equipment.

Application and Field Use:

Bt formulations are most effective when applied against early larval stages, as younger larvae ingest higher quantities of toxin relative to body weight. Because Bt toxins degrade rapidly under ultraviolet (UV) radiation and adverse environmental conditions, applications are often recommended during evening hours. Bt is widely used in integrated pest management (IPM) programs and is compatible with biological control agents, botanicals, and reduced doses of chemical pesticides.

Bt Transgenic Crops:

A major advancement in Bt technology is the development of Bt transgenic crops, such as Bt cotton, Bt maize, and Bt brinjal. In these crops, Cry genes are inserted into the plant genome, enabling continuous expression of the Bt toxin in plant tissues. This provides season-long protection against insect pests, reduces pesticide usage, and enhances crop yield. However, resistance management strategies such as refuge planting are essential to delay the evolution of resistant insect populations.

Advantages of Bt as a Biopesticide:

Bt offers numerous advantages, in cluding high target specificity, environmental safety, biodegradability, absence of toxic residues, and compatibility with sustainable agriculture. It significantly reduces dependence on synthetic insecticides and helps preserve biodiversity in agro-ecosystems.

Limitations of Bt:

Despite its benefits, Bt has certain limitations. Its effectiveness is reduced by UV degradation, it has a narrow host range, and resistance development has been reported in some insect populations. Continuous monitoring and integration with other pest management strategies are therefore necessary.

Role of Bt in Sustainable Agriculture:

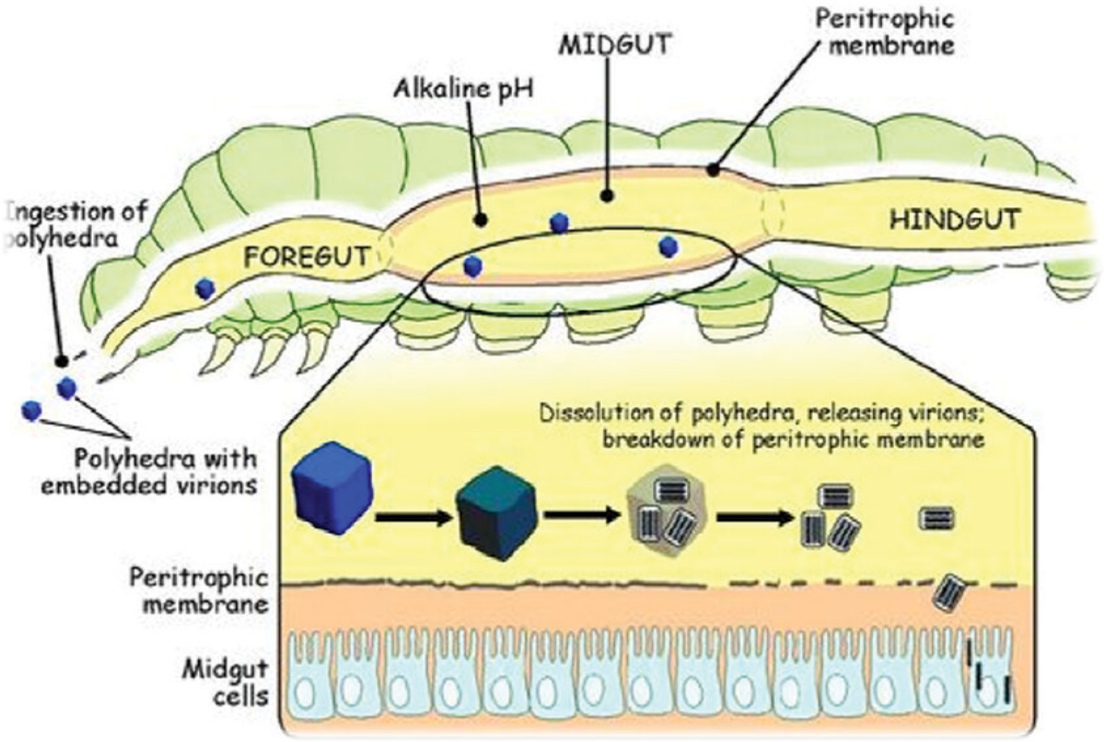
Bt plays a vital role in promoting **eco-friendly pest control** and sustainable agriculture. Advances in molecular biology, formulation technology, and toxin discovery are expanding the scope of Bt-based pest management. Bt remains a cornerstone of biological control strategies aimed at achieving food security with minimal environmental impact.

11.4. NUCLEO POLYHEDRO VIRUS AS BIOPESTICIDE:

Nucleopolyhedroviruses (NPVs) are a group of insect-specific viruses belonging to the family Baculoviridae, which are widely used as viral biopesticides for the management of major agricultural insect pests. NPVs are double-stranded DNA viruses characterized by the presence of large proteinaceous occlusion bodies known as polyhedral inclusion bodies (PIBs) that protect viral particles in the external environment. These viruses are highly host-specific and primarily infect larval stages of lepidopteran pests, making them an environmentally safe and target-specific alternative to chemical insecticides.

NPVs occur naturally in insect populations and play a significant role in regulating pest outbreaks in agro-ecosystems. Each NPV strain is usually specific to a single insect species or a narrow group of closely related species. For example, Helicoverpa armigera NPV (HaNPV) is used against cotton bollworm, Spodoptera litura NPV (SlNPV) against tobacco caterpillar, and Spodoptera frugiperda NPV (SfNPV) against fall armyworm. This host specificity minimizes risks to non-target organisms, including beneficial insects, pollinators, humans, and other vertebrates.

Mode of Action of NPV:



The infection process of NPV begins when susceptible insect larvae ingest foliage contaminated with viral polyhedral inclusion bodies. In the alkaline environment of the insect midgut, the protein matrix of the polyhedra dissolves, releasing occlusion-derived virions (ODVs). These virions infect midgut epithelial cells and initiate viral replication in the nucleus. Newly produced virions spread systemically to other tissues, including fat body, tracheae, hemocytes, and epidermal cells. As infection progresses, massive viral replication occurs, leading to cellular disintegration, tissue liquefaction, and eventual death of the larva. The dead insect typically hangs from plant parts and ruptures, releasing millions of polyhedra into the environment, facilitating horizontal transmission to healthy larvae.

Symptoms of NPV Infection:

Larvae infected with NPV exhibit characteristic symptoms such as reduced feeding, sluggish movement, discoloration, and swelling of the body. In advanced stages, the integument becomes fragile, and the larva often hangs in an inverted “V” shape before death. After death, the body liquefies due to enzymatic degradation, releasing viral particles. These visual symptoms are important diagnostic features in the field for identifying NPV-induced epizootics.

Production and Formulation of NPV:

NPVs are mass-produced in vivo using laboratory-reared host insect larvae. Healthy larvae are inoculated with a known concentration of NPV suspension and maintained under controlled conditions. After death, the larvae are collected, homogenized, filtered, and purified to obtain polyhedral inclusion bodies. The purified virus is formulated as aqueous suspensions, wettable powders, or oil-based formulations to improve field stability and adherence to foliage. The viral concentration is commonly expressed as polyhedral occlusion bodies (POB) per millilitre, which serves as a standard measure for dosage.

Application and Field Use:

NPV formulations are applied as foliar sprays during early larval stages, when insects are most susceptible. Applications are generally recommended during evening hours to minimize ultraviolet (UV) degradation of viral particles. Because NPVs act relatively slowly compared to chemical insecticides, visible mortality may take 5–7 days; however, infected larvae stop feeding within 24–48 hours, effectively reducing crop damage. NPVs are highly compatible with integrated pest management (IPM) programs and can be combined with botanical pesticides, pheromone traps, and biological control agents.

Advantages of NPV as a Biopesticide:

NPVs offer several advantages over conventional pesticides. They are highly specific, environmentally benign, biodegradable, and safe to humans and non-target organisms. There is no issue of chemical residues in harvested produce, making them suitable for organic and export-oriented agriculture. NPVs also have the ability to persist in the environment through recycling in host populations, providing long-term pest suppression.

Limitations of NPV:

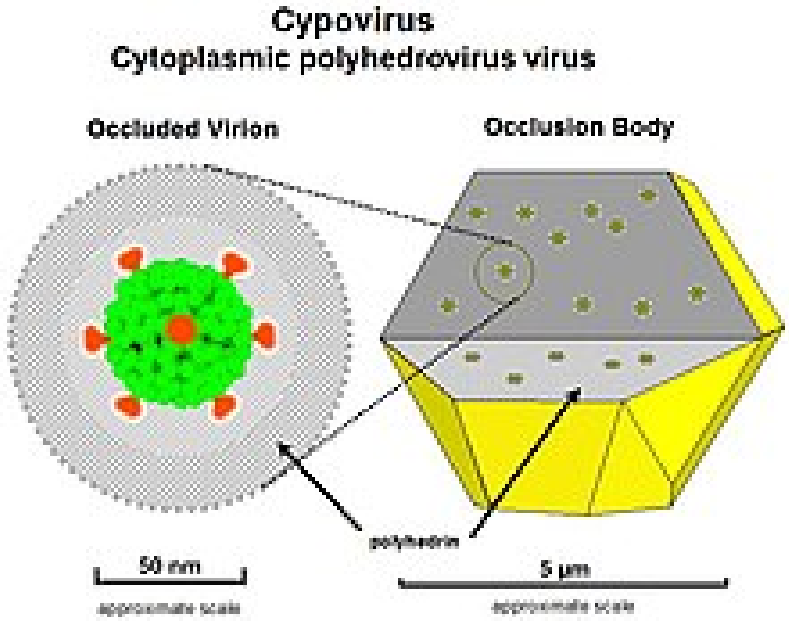
Despite their benefits, NPVs have certain limitations. Their slow speed of action compared to chemical insecticides may limit farmer acceptance. Viral particles are sensitive to UV radiation and high temperatures, reducing field persistence. Large-scale production is labor-intensive, and host specificity limits their use against multiple pest species. Resistance development is rare but possible under prolonged and improper usage.

Role of NPV in Sustainable Agriculture:

NPVs play a crucial role in promoting sustainable and eco-friendly pest management by reducing dependence on synthetic insecticides and preserving ecological balance. Advances in formulation technology, molecular characterization, and recombinant baculovirus development are enhancing the effectiveness and stability of NPVs. As agriculture moves toward reduced chemical inputs, NPVs represent a vital component of next-generation biological pest control strategies.

11.5. CYTOPLASMIC POLYHEDROSIS VIRUS AS BIOPESTICIDE:

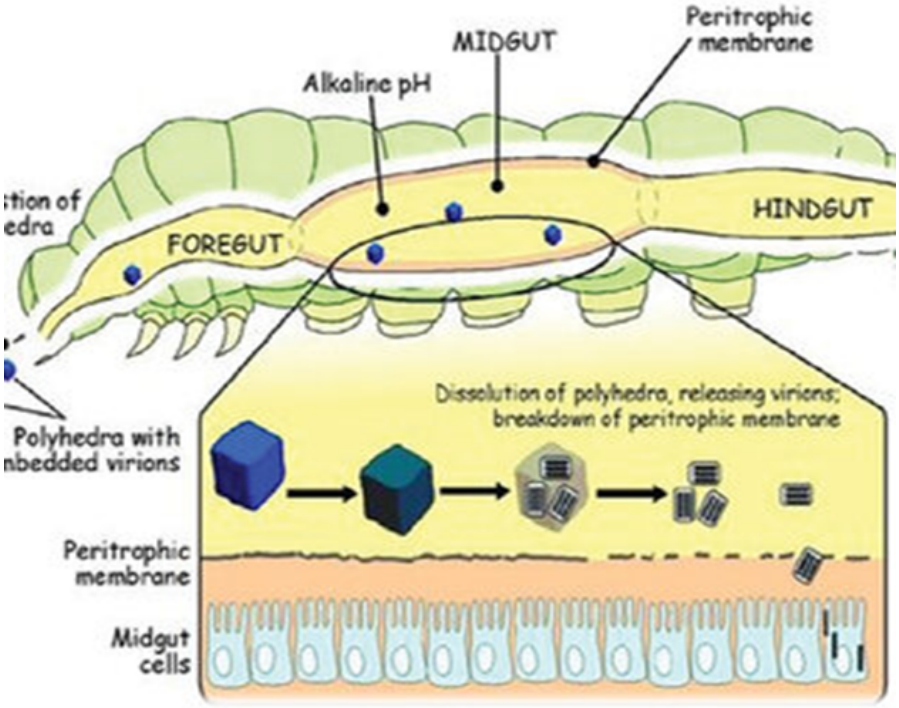
Cytoplasmic polyhedrosis viruses (CPVs) are a group of insect-pathogenic viruses belonging to the family Reoviridae and genus Cypovirus. CPVs are obligate intracellular pathogens that primarily infect the larval stages of lepidopteran insects and are widely recognized for their potential as viral biopesticides. Unlike nucleopolyhedroviruses (NPVs), which replicate in the nucleus, CPVs replicate exclusively in the cytoplasm of host cells, a distinctive feature reflected in their name. These viruses produce proteinaceous inclusion bodies called polyhedra, which protect viral particles outside the host and facilitate transmission under natural field conditions.

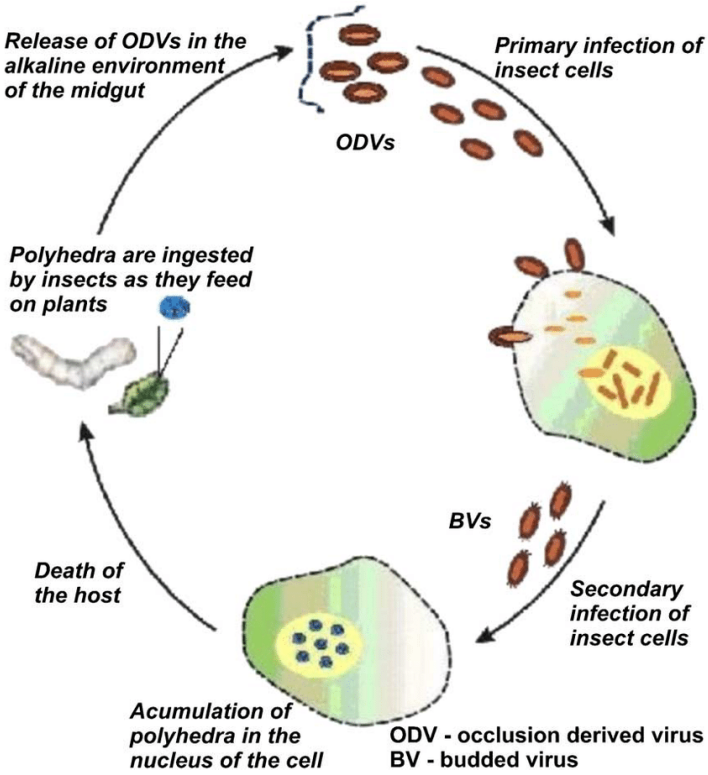


CPVs occur naturally in insect populations and contribute to the regulation of pest outbreaks, particularly in forest and agricultural ecosystems. Each CPV strain is highly host-specific, usually infecting a single insect species or a small group of closely related species. Important CPVs include *Bombyx mori* CPV (BmCPV), *Spodoptera litura* CPV (SlCPV), and *Helicoverpa armigera* CPV (HaCPV). This high degree of specificity makes CPVs environmentally safe and ideal for incorporation into integrated pest management (IPM) programs.

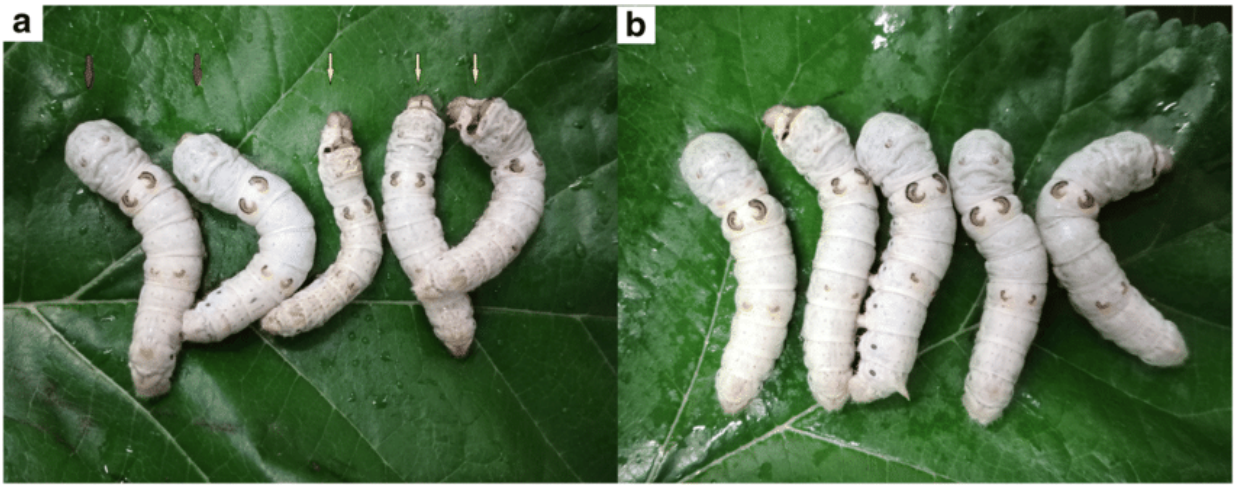
Mode of Action of CPV:

The infection cycle of CPV begins when susceptible insect larvae ingest foliage contaminated with viral polyhedra. In the alkaline environment of the larval midgut, the polyhedral matrix dissolves, releasing virions into the gut lumen. These virions infect midgut epithelial cells, where viral replication occurs entirely within the cytoplasm. CPV replication interferes with normal cellular metabolism, disrupts nutrient absorption, and leads to degeneration of midgut epithelial cells. As infection progresses, larval feeding is severely reduced, resulting in starvation and eventual death. Upon death, polyhedra are released into the environment through fecal matter or decomposition, allowing further spread of the virus among larval populations.


MIDGUT
Peritrophic membrane
Alkaline pH
HINDGUT
FOREGUT
Dissolution of polyhedra, releasing virions; breakdown of peritrophic membrane
Polyhedra with
Peritrophic membrane
Midgut cells


Release of ODVs in the alkaline environment of the midgut
Primary infection of insect cells
ODVs
Polyhedra are ingested by insects as they feed on plants
BVs
Death of the host
Secondary infection of insect cells
Acumulation of polyhedra in the nucleus of the cell
ODV - occlusion derived virus
BV - budded virus

Symptoms of CPV Infection:



Larvae infected with CPV show distinct symptoms including reduced feeding, sluggish behavior, and pale or milky discoloration of the body. A characteristic symptom is diarrhea with white fecal matter, caused by massive shedding of polyhedra from infected midgut cells. Infected larvae usually die slowly, often remaining attached to plant surfaces. Unlike NPV infection, CPV-infected larvae do not undergo complete body liquefaction, making field diagnosis possible based on midgut symptoms and fecal appearance.

Production and Formulation of CPV:

CPVs are produced **in vivo** by infecting laboratory-reared host larvae with a standardized virus suspension. Infected larvae are harvested before death, homogenized, and filtered to recover polyhedral inclusion bodies. These are further purified and formulated as aqueous suspensions or wettable powders. The virus concentration is expressed as polyhedral inclusion bodies (PIBs) per millilitre. Research is on-going to develop improved formulations with enhanced stability against ultraviolet radiation and environmental degradation.

Application and Field Use:

CPV formulations are applied as foliar sprays during early larval instars, when insects are most susceptible. Application during cooler hours of the day is recommended to reduce UV inactivation. CPVs act more slowly than chemical insecticides, but infected larvae show rapid feeding cessation, resulting in reduced crop damage. CPVs are compatible with other biocontrol agents and botanical pesticides and are particularly useful in organic and sustainable farming systems.

Advantages of CPV as a Biopesticide:

CPVs offer several advantages including high host specificity, environmental safety, biodegradability, and absence of chemical residues. They do not harm beneficial insects, pollinators, vertebrates, or humans. CPVs can persist in the environment through natural

recycling within insect populations, providing long-term suppression of pest outbreaks. Their specificity makes them valuable tools for targeted pest management strategies.

Limitations of CPV:

Despite their advantages, CPVs have certain limitations. They exhibit slow speed of action, are sensitive to ultraviolet radiation, and require precise timing for effective application. Large-scale production is labor-intensive due to reliance on live insect hosts. Additionally, their narrow host range restricts their use to specific pest species, necessitating careful pest identification prior to application.

Role of CPV in Sustainable Agriculture:

CPVs contribute significantly to eco-friendly pest control by reducing dependence on synthetic insecticides and preserving biodiversity. Advances in molecular virology and formulation technology are enhancing the efficacy and stability of CPVs. Their integration into IPM programs supports sustainable agriculture and aligns with global efforts to reduce chemical pesticide usage.

11.6. ADVANTAGES AND DISADVANTAGES OF BIOPESTICIDES:

Advantages:

- Biopesticides are generally have lower intrinsic toxicity and inherently less harmful in comparison to conventional pesticides.
- Contrary to broad-spectrum, conventional pesticides, which can impact a variety of organisms, including birds, insects, and mammals, biopesticides often only affect the target pest and closely related organisms.
- Biopesticides are effective in very small quantities and often decompose quickly, thereby resulting in lower exposures and largely avoiding the pollution problems caused by conventional pesticides.
- Biopesticides can significantly reduce the use of chemical pesticides while maintaining excellent crop yields when utilised as a part of Integrated Pest Management (IPM) programs.

Disadvantages:

- High specificity: which may require an exact identification of the pest/pathogen and the use of multiple products used; although this can also be an advantage in that the biopesticide is less likely to harm non-target species.
- Slow action speed (thus making them unsuitable if a pest outbreak is an immediate threat).

- Variable efficacy due to the influences of various factors (since some biopesticides are living organisms, which bring about pest/pathogen control by multiplying within or nearby the target pest/pathogen).
- Living organisms evolve and increase their tolerance to control. If the target population is not exterminated or rendered incapable of reproduction, the surviving population can acquire tolerance of whatever pressures are brought to bear, resulting in an evolutionary arms race.
- Unintended consequences: Studies have found broad spectrum biopesticides have lethal and nonlethal risks for non-target native pollinators such as *Melipona quadrifasciata* in Brazil

However, users must be highly knowledgeable about managing pests and strictly adhere to all label instructions to use biopesticides efficiently and safely.

11.7. SUMMARY:

The word "biopesticides" refers to compounds that, as opposed to general chemical pesticides, are used to control agricultural pests through specialised biological effects. Used to manage pests, biopesticides refer to products containing biocontrol agents, natural entities or chemicals produced from natural materials (such as animals, plants, bacteria, or specific minerals). The FAO defines biopesticides as passive biocontrol agents, compared to those that actively seek out the pest, such as parasitoids, predators, and numerous types of entomopathogenic nematodes.

Biopesticides provide a good alternative to chemical crop protection. By responding concurrently to the interests of farming, forestry, and the industrial sector, biopesticides offer a considerable potential for utilization in sustainable agriculture. When all the features are added, the advantages of biopesticides in crop protection suggest that utilization of this class of pesticides can be a highly attractive proposition.

11.8. TECHNICAL TERMS:

Biopesticides, *Bacillus thuringiensis*, Nucleo polyhedral virus, Cytoplasmic polyhedrosis virus, Formulation.

11.9. SELF ASSESSMENT QUESTIONS:

1) What are the Biopesticides? Explain their Importance, Advantages and Disadvantages.
2) Give a detail account of *Bacillus Thuringiensis* as a Biopesticide.
3) Explain the Role of NPV as a Biopesticide.
4) Describe the CPV from Biopesticide Point of View.

11.10. SUGGESTED READINGS:

1) Singh, R.S.1998. Plant Diseases 7th Edn. Oxford- & IBH Publishing Co. Pvt. Ltd; New Delhi.

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4) Thakur I.S. 2006-Environmental Biotechnology: Basic Concepts and Applications, I.K. International Pvt. Ltd., New Delhi.

5) Murali Baskaran R.K. et. al., 2023-Microbial Biopesticides in India, NIPA GENX Electronic Resourses & Solutions Pvt. Ltd., New Delhi.

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LESSON-12

BIOSTATISTICS-BASIC PRINCIPLES

12.0. OBJECTIVE:

- Students are able to know what is statistics and significance of statistics in biological sciences to determine the data validity.

STRUCTURE:

12.1. INTRODUCTION:

Statistics is a scientific discipline that focuses on the collection, organization, analysis, interpretation, and presentation of data. It provides systematic methods to understand variability, identify patterns, and make reliable conclusions based on quantitative evidence. In scientific research especially in plant, biological, environmental, and agricultural sciences natural variations arise from genetic differences, environmental conditions, experimental treatments, and measurement errors. Statistics helps researchers manage these variations by summarizing data through descriptive measures and graphical tools, and by applying inferential techniques such as hypothesis testing, confidence intervals, ANOVA, and regression to draw meaningful conclusions about populations from sample observations. Through proper statistical analysis, scientists can design robust experiments, validate hypotheses, evaluate treatment effects, and enhance the accuracy, reliability, and reproducibility of research findings.

12.2. BIOSTATISTICS:

Statistics, as a discipline, began not with complex calculations but with the need to collect and manage information for the state, hence the name, derived from the Latin *statisticum* (of the state). The 18th century formalized the Theory of Probability, linking statistics to mathematics. Key figures include Pierre-Simon Laplace (1749–1827) and Carl Friedrich Gauss (1777–1855), who developed the Least Squares Method and the Normal Distribution (the bell curve), which is foundational to statistical inference. The 19th century focused on social statistics and the rise of Descriptive Statistics (mean, variance, etc.) to summarize vast datasets. Karl Pearson (1857–1936), a key founder of mathematical statistics,

established the discipline's first department at University College London. He developed the Pearson correlation coefficient and the Chi-squared test, methods used to measure relationships and independence. Sir Ronald A. Fisher (1890-1962) transformed statistics into a tool for scientific research, formalizing experimental design, Analysis of Variance (ANOVA), and the concept of Maximum Likelihood Estimation.

Biostatistics (or Biometry) is the application of statistical methods to biological and medical problems. Its history is inseparable from the study of inherited traits, public health, and agricultural productivity. The term "Biometry" was coined in the late 19th century. Early biostatisticians were primarily concerned with evolution, genetics, and heredity. Perhaps the earliest important figure in Biostatistics thought was Adolphe Quetelet (1796-1874), a Belgian astronomer and mathematician, who in his work combined the theory and practical methods of statistics and applied them to the problems of biology, medicine and sociology. Sir Francis Galton (1822–1911), a cousin of Charles Darwin, pioneered the study of regression and correlation to analyse inherited characteristics like height. John Snow (1813-1858) is considered the father of modern epidemiology. His 1854 work mapping cholera cases in London to a contaminated water pump demonstrated a clear statistical link between environment and disease, leading to a public health intervention.

After the rediscovery of Mendel's work, there were conceptual gaps in understanding between genetics and evolutionary Darwinism. Statisticians and models that exploited statistical reasoning helped to bridge this gap. Work at the intersection of genetics, evolution, and populations lead to a foundational advance in the history of biological thought, called the Neo-Darwinian Modem evolutionary synthesis. The most significant development was the adoption of Randomized Controlled Trials (RCTs). The Medical Research Council (MRC) trial on streptomycin for pulmonary tuberculosis (1946) is a landmark event, formalizing the use of randomization and blinding to ensure unbiased comparisons between treatment groups. In the latter half of the 20th century, biostatistics matured with the development of techniques like Survival Analysis (to study time-to-event data, like patient survival) and advanced longitudinal data analysis methods, becoming indispensable to drug development, genetics, and public health policy worldwide.

Application of Biostatistics:

Biostatistics is the application of statistical reasoning and methods to data derived from biological, medical, and public health settings. Its applications are vast and essential across nearly every domain of modern life sciences and healthcare.

I. **Medicine and Clinical Research:** Biostatistics is the backbone of clinical evidence, determining the efficacy and safety of new treatments. It can be useful in the following ways.
   a) **Clinical Trials Design and Analysis:** This is the most visible application. Biostatisticians design trials (Phase I, II, III), determine the necessary sample size to detect a clinically meaningful difference, establish randomization methods, and analyse outcomes.

i) **Efficacy and Safety:** Calculating endpoints like treatment effect size, p-values, and confidence intervals to determine if a new drug or procedure is better than a placebo or standard care.

ii) **Survival Analysis:** Using methods like the Kaplan-Meier curve and Cox proportional hazards model to analyse time-to-event data, such as patient survival after a diagnosis or treatment.

b) **Diagnostics and Screening:** Assessing the quality of diagnostic tests.

i) **Sensitivity and Specificity:** Quantifying a test's ability to correctly identify diseased and non-diseased individuals, respectively.

ii) **ROC Curves (Receiver Operating Characteristic):** Used to evaluate the performance of diagnostic markers and determine optimal cut-off points.

c) **Pharmacokinetics and Pharmacodynamics (PK/PD):** Modelling how drugs are absorbed, distributed, metabolized, and excreted (PK) and how they affect the body (PD).

II. Public Health and Epidemiology: Biostatistics tracks disease patterns, identifies risk factors, and informs public health policy.

a) Disease Surveillance and Monitoring: Using time-series analysis and spatial statistics to track the incidence and prevalence of infectious and chronic diseases (e.g., influenza, COVID-19, cancer).

b) Identifying Risk Factors: Analysing case-control and cohort studies to determine the association between exposures (e.g., smoking, diet, pollution) and health outcomes.

i) Measures of Association: Calculating metrics like Odds Ratios (OR) and Relative Risks (RR) to quantify the strength of these associations.

c) Program Evaluation: Assessing the impact of health interventions and campaigns (e.g., vaccination programs, nutrition education) on a population level.

d) Health Economics: Analysing cost-effectiveness and cost-benefit ratios of various healthcare strategies to guide resource allocation.

III. Genomics, Proteomics, and Bioinformatics: Biostatistics is essential for managing and interpreting the massive datasets generated by modern molecular biology.

a) Genome-Wide Association Studies (GWAS): Employing complex statistical models to identify specific genetic markers (SNPs) associated with diseases. This involves handling millions of data points and addressing the problem of multiple comparisons.

b) Microarray and Sequencing Data Analysis: Identifying genes that are significantly **up- or down-regulated** under different conditions (e.g., normal vs. cancerous tissue).

c) **Phylogenetics:** Using statistical models to construct and test evolutionary relationships (phylogenetic trees) between species, genes, or populations.

IV. **Ecology and Environmental Sciences:** In this domain, biostatistics helps understand population dynamics and environmental impacts.

a) **Population Ecology:** Using capture-recapture models (e.g., Lincoln-Petersen method) and spatial statistics to estimate the size, density, and movement patterns of animal and plant populations.

b) **Environmental Risk Assessment:** Modelling the impact of pollutants (e.g., heavy metals, pesticides) on ecological systems and human health, often involving complex regression models.

c) **Climate Change Impact:** Analysing long-term ecological data (e.g., species distribution, blooming times) to quantify and model the effects of changing climate patterns.

V. **Agriculture and Animal Husbandry:** Biostatistics optimizes crop yields, animal breeding, and disease resistance.

a) **Experimental Design:** Designing agricultural field trials to test the efficacy of different fertilizers, pesticides, or irrigation methods while controlling for soil variability.

b) **Genetic Selection:** Using **quantitative genetics** to analyse complex traits (like milk yield, growth rate, or drought resistance) and predict the success of breeding programs.

c) **Disease Management:** Modelling the spread of plant or animal diseases (epizootics) to develop effective quarantine and control strategies.

VI. **Psychology and Behavioural Science:** Biostatistics is used to analyse human and animal behaviour, perception, and cognitive processes.

a) **Psychometrics:** Developing and validating scales and questionnaires (e.g., depression scales, IQ tests) using techniques like Factor Analysis and Reliability Analysis.

b) **Cognitive Science:** Analysing reaction times, error rates, and neuroimaging data (fMRI, EEG) using ANOVA and mixed-effects models to draw inferences about brain function.

Role of Biostatistics:

1) Conduct of original research on important biostatistics problems across the spectrum: foundations B methodology B applications.

2) Leadership of biostatistics education for public health/biomedical scientists and professionals.
3) Participation in other current and future educational programs involving substantial statistical reasoning. Such as quantitative genetics, bioinformatics and clinical investigations and information technology.
4) Facilitation of biomedical and public health research that depends on statistical collaboration or consultation.
5) Given continued growth and decentralization of biostatistics research and applications across the country, we have to discuss how best to organise this discipline and to make it maximally useful in advancing health.

Principles of Statistics:

Statistics is the study of methods and procedures for collecting, classifying, summarizing and analysing data and for making scientific interferences from such data. Statistics breaks naturally into two reasonably distinct subcategories I) Descriptive statistics and II) Inferential statistics.

I) Descriptive Statistics: Descriptive statistics serve as devices for organising data and bringing into focus their essential characteristics for the purpose of reaching conclusions at a later stage. Descriptive statistics is a crucial branch of statistics that focuses on summarizing, organizing, and presenting data in an informative way. It involves interpreting the implications of distribution shape and selecting the most appropriate measures for complex biological or social data. It can be categorised into 1. Data characterization and Preliminaries 2. Measures of Central tendency 3. Measures of Dispersion (Variability) 4. Measures of Shape (Distributional Characteristics) 5. Advanced Data Visualization

1) **Data Characterization and Preliminaries:** Before any calculation, the nature of the data must be understood. It is of different types A) Classification of variables B) Frequency distribution

A. Classification of Variables:

The appropriate descriptive technique depends entirely on the variable type:

The classification of variables in biostatistics is fundamental, as it dictates the appropriate statistical tests and methods for analysis. Variables are broadly categorized into i. Qualitative (Categorical) and ii. Quantitative (Numerical) variables, with further subdivisions (Table 1).

i) **Qualitative (Categorical) Variables:** These variables represent categories or groups and cannot be meaningfully measured numerically. These variable of following types

Nominal Variables: Categories are distinct and unordered. Eg: Gender (Male, Female, Other), Blood Type (A, B, AB, O), Race/Ethnicity, Presence of a Disease (Yes/No).

Ordinal Variables: Categories are distinct and have a meaningful order or rank, but the difference between the ranks is not measurable or consistent. Eg: Disease severity (Mild, Moderate, Severe), Pain scale (Low, Medium, High), Education level (High School, College, Post-Graduate).

ii) Quantitative (Numerical) Variables: These variables are measured on a numerical scale, where the values have true mathematical meaning. They are further classified based on the nature of their values.

Discrete Variables: Can only take on a finite or countable infinite number of values, typically integers. They result from counting. E.g.: Number of Children, Number of Hospital Visits, and Number of Bacteria Colonies on a plate.

Continuous Variables: Can take any value within a given range, including fractions and decimals. They result from measuring. E.g.: Height, Weight, Blood Pressure, Temperature, and Time.

TABLE 12.1: CLASSIFICATION OF VARIABLES

Type	Subtype	Characteristics	Appropriate Measures
Categorical (Qualitative)	**Nominal**	Categories with **no** inherent order (e.g., Blood type, Genotype).	Mode, Frequencies, Proportions.
	Ordinal	Categories with a defined order or rank (e.g., Disease stage: I, II, III; Likert scale).	Median, Quartiles, Frequencies.
Numerical (Quantitative)	**Discrete**	Values are countable and finite (e.g., Number of hospital admissions, number of colonies).	Mean, SD, all measures.
	Continuous	Values can take any value within a given range (e.g., Temperature, drug concentration).	Mean, SD, all measures.

B. Frequency Distribution:

A frequency distribution in statistics is an organized, tabular, or graphical representation of data that shows the number of observations (or frequency) for each possible value or range of values in a dataset. Its primary purpose is to simplify large datasets by presenting them in a structured way, allowing researchers to quickly visualize patterns, identify trends, and understand the central tendency and spread of the data.

Types of Frequency Distributions: Frequency distributions are categorized based on how the data values are presented:

a) Ungrouped Frequency Distribution: Used for discrete data or small datasets where the number of distinct values is small (e.g., categorical or low count numerical data). Each distinct individual value is listed alongside its corresponding frequency (the number of times it occurs). *Example*: Number of absences per student (0, 1, 2, 3...) and the count of students for each number.

b) Grouped Frequency Distribution: Used for continuous data or very large datasets with many different values (like measurements). Data is organized into non-overlapping class intervals (or bins), and the frequency is the count of observations that fall within each interval. *Example*: Age of participants (0-10, 11-20, 21-30, etc.) and the count of participants in each range.

Components and Variations:

Apart from the above a standard frequency distribution table often includes these variations:

Absolute Frequency (or Count):

The raw number of times a value or class interval occurs.

Relative Frequency:

The proportion of the total observations that fall into a category or interval. It is calculated as:

$$\text{Relative Frequency} = \frac{\text{Frequency}}{\text{Total Number of Observations}}$$

Percentage Frequency:

The relative frequency expressed as a percentage.

Cumulative Frequency:

The running total of frequencies. It shows the number of observations that are less than or equal to a particular value or the upper limit of a class interval.

Graphical Representations:

Further the frequency distributions are commonly displayed using various graphs for visualization. The graphical representations are of different types.

Histogram:

Used for **grouped frequency distributions** of **continuous data**. The bars are typically continuous and touch, with the area of the bar representing the frequency.

Bar Chart:

Used for **ungrouped frequency distributions** of **categorical or discrete data**. Bars are separated by a space.

Frequency Polygon:

A line graph created by plotting the midpoints of the class intervals (or categories) against their frequencies and connecting the points with straight lines.

Cumulative Frequency Graph:

A graph that displays the cumulative frequency, useful for finding percentiles and medians. This is the organization of data into classes/intervals and counting the number of observations (f) that falls into each class. This forms the basis for initial graphical analysis. This is of two types a) Relative frequency b) Cumulative frequency.

a) **Relative Frequency:** The proportion of observations in a category (Σff).

b) **Cumulative Frequency:** The running total of frequencies, essential for finding percentiles and the median in grouped data.

2) Measures of Central Tendency:

A measure of central tendency is a single value that attempts to describe a set of data by identifying the central position within that data set. These measures are often referred to as averages or measures of central location and their primary goal is to summarize a large amount of data into one typical or representative number.

The three most common measures of central tendency are the i) Mean, ii) Median, and iii) Mode.

i) **The Arithmetic Mean ($\bar{x}$):** The **mean** is the most widely used measure of central tendency. It is simply the arithmetic average of a dataset. The mean is calculated by summing all the values in a dataset and then dividing by the total number of values.

$$\bar{x} = \frac{\sum X}{n}$$

Where:

$\sum X$ = is the sum of all observations.

N = the total number of observations

ii) **The Median (M):** The median is the middle value in a dataset when the values are arranged in ascending or descending order. It effectively divides the data into two equal halves (50% of the data falls below it, and 50% falls above it).

iii) **The Mode (Mo):** The **mode** is the value that appears most frequently in a dataset. The mode is found by simply counting the frequency of each value and identifying the one with the highest count. A dataset can have **No Mode** (if all values appear only once), **One Mode** (unimodal), **Two Modes** (bimodal) and **More than two Modes** (multimodal).

3) Measures of Dispersion (Variability):

The Measures of Dispersion (or Variability) are statistical metrics used to quantify the extent to which data points in a distribution are spread out from the average value. They are crucial because they provide context for the measures of central tendency (mean, median, and mode). If a measure of dispersion is small, the data points are clustered closely together. If it is large, the data points are widely scattered. It is of two types

a) **Absolute Measures of Dispersion:** Absolute measures express the variability in the same units as the original data (or in squared units, as with variance). It is again divided into following types.

Range (R):

The simplest measure of dispersion. It is the difference between the largest and the smallest observation in a data set.

Interquartile Range (IQR):

The range of the middle 50% of the data, which is the difference between the first quartile (Q1) and the third quartile (Q3).

Variance (σ2 or s2):

Variance is the average of the squared deviations from the mean. It uses every data point and is fundamental to advanced statistical analysis.

Standard Deviation (SD) (σ or s):

The **Standard Deviation** is the most widely used measure of dispersion. It is simply the square root of the variance. The standard deviation is the preferred measure of dispersion when the mean is used as the measure of central tendency (i.e., for symmetric data). A shorter, wider curve has a higher standard deviation, indicating greater variability, while a taller, narrower curve has a lower standard deviation, indicating less variability.

b) Relative Measures of Dispersion:

Relative measures are unitless ratios (usually expressed as a percentage) used for comparing the variability of two or more data sets that have different units or different means. It is studied as coefficient of variation.

Coefficient of Variation (CV):

The Coefficient of Variation expresses the standard deviation as a percentage of the mean.

Dispersion helps in understanding how much variability exists in the data, allow for comparison between different datasets or groups and measures like IQR can help identify outliers in the data.

4) Measures of Shape (Distributional Characteristics):

The Measures of Shape (or distributional characteristics) in statistics describe how the data is distributed around the mean and are used to assess whether a distribution is similar to a normal distribution (the bell curve). The two primary measures of shape are Skewness and Kurtosis.

a) Skewness: Skewness is a measure of the asymmetry of the probability distribution of a dataset. It indicates the extent to which the data values are clustered or skewed toward one end of the distribution. Skewness is of different types as mentioned in the Table 12.2 and Figure 12.1.

TABLE 12.2: TYPES OF SKEWNESS

Type	Coefficient Value	Description	Relationship of Central Tendency
Zero/Symmetric	Skewness = 0	The distribution is perfectly symmetrical, like a Normal Distribution (bell curve). The left and right sides are mirror images.	Mean = Median = Mode
Positive Skewness (Right-Skewed)	Skewness > 0	The tail is longer on the right side. Most of the data is concentrated on the left side (smaller values). The mean is pulled to the right by a few large extreme values (outliers).	Mode < edian < Mean
Negative Skewness (Left-Skewed)	Skewness < 0	The tail is longer on the left side. Most of the data is concentrated on the right side (larger values). The mean is pulled to the left by a few small extreme values (outliers).	Mean < Median < Mode

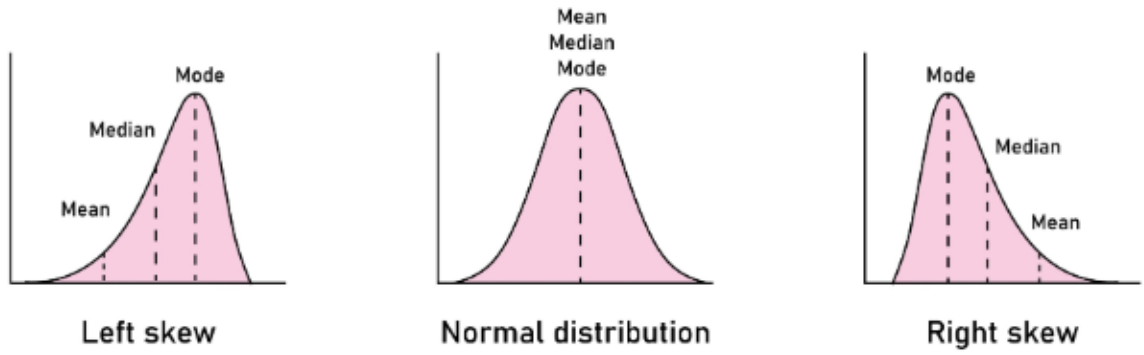

Figure 12.1: Skewness of Mean, Median and Mode

b) Kurtosis: Kurtosis is a measure that describes the "tailedness" of a distribution. It indicates the weight of the tails (the frequency of extreme values or outliers) relative to the tails of a normal distribution. While often incorrectly associated only with the peak's sharpness, it primarily describes the movement of probability from the shoulders of a distribution into the tails and peak. The kurtosis value of a standard normal distribution is 3. Statisticians often use Excess Kurtosis (Kurtosis - 3), where the normal distribution has a value of 0. The various types of Kurtosis were mentioned in the table 12.3.

TABLE 12.3: TYPES OF KURTOSIS

Type (Based on Excess Kurtosis)	Excess Kurtosis Value	Tailedness (Outliers)	Peak
Mesokurtic	Excess Kurtosis = 0 (Kurtosis = 3)	Moderate tails; the same as a Normal Distribution.	Normal peak.
Leptokurtic	Excess Kurtosis > 0 (Kurtosis > 3)	Heavy tails (more outliers). A distribution is more peaked than a normal distribution.	Sharper/higher peak.
Platykurtic	Excess Kurtosis < 0 (Kurtosis < 3)	Light tails (fewer outliers). A distribution is flatter than a normal distribution.	Flatter/lower peak.

5) Advanced Data Visualization:

Advanced visualization transforms complex datasets into insightful graphics, allowing for a deeper understanding of multi-variable relationships, patterns, and outliers that simple measures or tables cannot capture. The basic principles of advanced data visualization include 1. Data-to-Ink Ratio: Maximize the use of graphical "ink" to represent data, minimizing non-data ink (chart junk). 2. Telling a Story: Organizing visuals to lead the viewer to the main conclusion. 3. Interactivity: Allowing users to filter, zoom, and drill down into the data. The main goals of data visualization are a) Exploratory Data Analysis (EDA): Discovering unknown patterns and testing hypotheses. b) Explanatory Visualization: Communicating findings clearly to an audience. c) Handling High Dimensionality: Visualizing data with many variables.

Tools and Libraries:

The creation of advanced visualizations is typically done using specialized libraries and software (Table 12.4).

TABLE 12.4: TOOLS AND LIBRARIES OF ADVANCED VISUALIZATIONS

Tool Category	Examples	Purpose
Python Libraries	Matplotlib, Seaborn, Plotly, Altair, Bokeh	Powerful, highly customizable, and scriptable for statistical analysis and web-based interactive plots.
R Packages	ggplot2, Shiny, Leaflet	A standard for statistical graphics; Shiny for web-based interactive dashboards.
BI Tools	Tableau, Power BI	Drag-and-drop interfaces for rapid, interactive visualization and dashboard creation.

II) Inferential Statistics:

Inferential statistics is the use of probability theory to draw conclusions or make predictions (inferences) about a larger population based on observations made from a representative **sample**. It is the theoretical bridge between observed data and generalizable knowledge. Inferential statistics can be studied under the following headings 1. Foundation; 2. Estimation; 3. Hypothesis testing; 4. Common inferential tests

1. Foundation: Population, Sample, and Sampling Distributions:

The foundation of inferential statistics rests on the principles and tools that allow researchers to make reliable generalizations about a large population based on observations from a smaller, representative sample. It is of two types A. Core concepts B. The Central Limit Theorem.

A) Core Concepts:

The core concepts are the fundamental ideas and methods used to draw conclusions about a larger population based on data collected from a sample.

Population (P): The entire group of individuals or objects about which the inference is to be made (e.g., all adults with Type 2 diabetes). Population characteristics are called parameters (e.g., population mean μ, population standard deviation σ).

Sample (S): A subset of the population selected for study. Sample characteristics are called **statistics** (e.g., sample mean $\bar{x}$, sample standard deviation s).

Sampling Error: The natural, unavoidable difference between a sample statistic and the true population parameter. Inferential statistics aims to quantify this error.

B) The Central Limit Theorem (CLT):

The CLT is the most critical principle underlying inferential statistics. Regardless of the shape of the population distribution, if the sample size (n) is sufficiently large (typically $n \geq 30$), the distribution of the sample means (the Sampling Distribution of the Mean) will be approximately Normally Distributed.

- Mean of the Sampling Distribution ($\mu_{\bar{x}}$): Equals the population mean (μ).
- Standard Deviation of the Sampling Distribution ($\sigma_{\bar{x}}$): Known as the Standard Error of the Mean (SEM), calculated as:

$$\text{SEM} = \sigma_{\bar{x}} = \frac{\sigma}{\sqrt{n}}$$

`The SEM quantifies the precision of the sample mean as an estimate of the population mean. A smaller SEM (achieved by larger n) indicates greater precision.

2) Estimation: Confidence Intervals (CIs)

Estimation involves using sample data to determine a range of possible values for an unknown population parameter. It is of two types A. Points B. Interpretation of the confidence level

A. Point vs. Interval Estimates

i) **Point Estimate:** A single, best-guess value for a parameter (e.g., the sample mean $\bar{x}$ is the point estimate for the population mean μ).

ii) **Interval Estimate (CI):** A range of values constructed around the point estimate, within which the population parameter is expected to lie with a specified degree of certainty (confidence level).

B. Interpretation of the Confidence Interval:

The 95% Confidence Interval is the most common. It means that if the process of sampling and constructing the CI were repeated many times, 95% of the resulting intervals would contain the true population parameter. The general formula for a CI is:

CI = Point Estimate ± (Critical Value × Standard Error)

For a 95% CI for the population mean (using the Z-distribution for large n or known σ):

$$CI_{95\%} = \bar{x} \pm (1.96 \times SEM)$$

3. Hypothesis Testing (Significance Testing):

Hypothesis testing is a formal, four-step procedure used to determine if a set of data provides sufficient evidence to reject a null hypothesis (H0) in favor of an alternative hypothesis (H1).

A. The Four Steps:

1. **State Hypotheses:**
    a. **Null Hypothesis (H0):** A statement of no effect or no difference (e.g., μ treatment = μ control). It is the hypothesis tested statistically.
    b. **Alternative Hypothesis (H1):** A statement that there is an effect or difference (e.g., μtreatment $\neq$ μcontrol). This can be **one-tailed** (directional) or **two-tailed** (non-directional).
2. **Set Significance Level (α):** The maximum risk willing to be accepted for rejecting a true null hypothesis. Commonly set at α=0.05 (or 5%).

3. **Compute Test Statistic:** Calculate a value (e.g., t, Z, F, or $\chi 2$) based on the sample data. This statistic measures how many standard errors the sample result is from the value stated in the null hypothesis.
4. **Make Decision (Compare P-value to α):**
    a. **P-value:** The probability of observing a test statistic as extreme as, or more extreme than, the one calculated, assuming the null hypothesis is true.
    b. **Decision Rule:**
        - If P-value ≤ α: **Reject H0** (The result is statistically significant).
        - If P-value > α: **Fail to Reject H0** (There is not enough evidence to support the alternative hypothesis).

B. Types of Errors:

i) **Type I Error (α):** Incorrectly rejecting a true null hypothesis (False Positive). Controlled by the significance level.

ii) **Type II Error (β):** Incorrectly failing to reject a false null hypothesis (False Negative).

iii) **Statistical Power (1−β):** The probability of correctly rejecting a false null hypothesis. Higher power is desired and is typically ensured through adequate sample size calculation.

4. Common Inferential Tests:

The choice of test depends on the data type, number of samples, and underlying distribution assumptions.

A. Parametric Tests:

These tests assume the data is drawn from a population that follows a specific distribution (usually Normal) and require numerical data.

i) **T-tests:** Used to compare means.
    a) **One-Sample T-test:** Compares a sample mean to a known population mean or hypothesized value.
    b) **Independent Samples T-test:** Compares the means of two independent groups (e.g., Treatment A vs. Treatment B).
    c) **Paired Samples T-test:** Compares the means of the same group measured at two different times (e.g., Before vs. After).

ii) **ANOVA (Analysis of Variance):** Used to compare the means of three or more independent groups. It tests the ratio of the variance between groups to the variance within groups (F-statistic).

a. Post-hoc tests (e.g., Tukey's HSD) are used to determine which specific pairs of groups differ significantly after a significant F-test.

iii) **Linear Regression:** Models the linear relationship between one or more independent variables (predictors) and a continuous dependent variable.

B. Non-Parametric Tests:

Used when data does not meet the assumptions of parametric tests (e.g., highly skewed data, ordinal data, small sample size). They test hypotheses about the **median** or **rankings** rather than the mean.

i) **Mann-Whitney U Test:** Non-parametric alternative to the Independent Samples T-test.

ii) **Wilcoxon Signed-Rank Test:** Non-parametric alternative to the Paired Samples T-test.

iii) **Kruskal-Wallis H Test:** Non-parametric alternative to One-way ANOVA.

C. Categorical Tests:

Used when the data is categorical (nominal or ordinal).

i. **Chi-Square ($\chi 2$) Test:**

a. Goodness of Fit: Tests if a sample distribution matches a hypothesized population distribution.

b. Test of Independence: Tests whether two categorical variables are associated or independent (e.g., is gender independent of voting preference?).

12.3. SUMMARY:

Statistics is the scientific discipline that deals with the systematic collection, organization, analysis, and interpretation of data, allowing researchers to draw meaningful conclusions in the presence of uncertainty and natural variation. In scientific fields such as biology, plant sciences, agriculture, and environmental studies, data often vary due to genetic differences, environmental conditions, and measurement errors. Statistics provides tools to summarize this variability through descriptive measures such as mean, median, variance, and standard deviation, as well as graphical methods that help visualizes data patterns. Beyond description, inferential statistics allow researchers to generalize findings from a sample to a larger population using hypothesis testing, confidence intervals, chi-square tests, ANOVA, correlation, and regression.

The basic principles of statistics guide how data should be collected and analysed to ensure accuracy and reliability. **Descriptive statistics** refers to the methods used to summarize, organize, and present data in a meaningful way. It focuses on describing the main features of a dataset through numerical measures such as mean, median, mode, range, variance, and standard deviation, as well as graphical tools like histograms, bar charts, pie charts, box plots, and scatter diagrams. Descriptive statistics does not attempt to draw

conclusions beyond the data at hand; instead, it provides a clear picture of the distribution, central tendency, and variability within a dataset. It is the first step in data analysis, helping researchers understand patterns, detect outliers, and prepare the data for more advanced analyses. **Inferential statistics**, on the other hand, involves using information from a sample to generalize or predictions about a larger population. It relies heavily on probability theory to account for uncertainty and sampling variability. Through techniques such as hypothesis testing, confidence intervals, correlation, regression, chi-square tests, and analysis of variance (ANOVA), inferential statistics allows researchers to test scientific hypotheses, identify significant differences or relationships, and estimate population parameters based on sample data. Unlike descriptive statistics, which only describes what is observed, inferential statistics extends beyond the sample, enabling valid and evidence-based conclusions about broader populations.

12.4. TECHNICAL TERMS:

Population, Sample, Variable, Data Types, Frequency Distribution, Central Tendency, Skewness, Kurtosis Outlier, Scatter Plot, Correlation, Regression.

12.5. SELF ASSESSMENT QUESTIONS:

1) Define biostatistics and explain its major branches.
2) Explain in detail the meaning, scope, and importance of statistics in scientific research.
3) Describe the major components of descriptive statistics.
4) Describe in detail the various measures of central tendency and dispersion. Illustrate how they help in understanding data structure.

12.6. SUGGESTED READINGS:

1) **Rosner, B. (2015).** *Fundamentals of Biostatistics* **(8th ed.). Cengage Learning.**
2) **Suresh, K.P. (2014).** *An introduction to Biostatistics* **(2nd ed.). IK International Publishing House.**
3) **Kothari, C.R., & Garg, G. (2019).** *Research Methodology: Methods and Techniques* **(4th ed.). New Age International.**
4) **Pandey, M. (2022).** *Biostatistics: Basic and Advanced.* **Scientific Publishers.**
5) **Prasad, S., & Sinha, B.K. (2010).** *Biostatistics.* **New Age International Publishers.**
6) P.N. Arora and P.K. Malhan (2010). Biostatistics. Mrs. Meena Pandey. Branch Offices for Himalaya Publishing House, "Rmndnot", Dr Bhalerao Marg, Girgaon, Mumbai-400 004.

Dr. Kakumanu Babu

LESSON-13

MEASURES OF CENTRAL TENDENCY, STANDARD DEVIATION AND STANDARD ERROR

13.0. OBJECTIVE:

- Students are able to know what are the measures of central tendency in statistics and its significance in biological sciences to determine the data validity.

STRUCTURE:

13.1. INTRODUCTION:

In statistical analysis, understanding how data behave, how they cluster, and how much they vary is essential for making accurate scientific interpretations. Three fundamental concepts measure of central tendency, standard deviation, and standard error form the core of descriptive and inferential statistics, particularly in biological, agricultural, and environmental research. Measures of central tendency are statistical tools used to identify the central or typical value around which data points in a dataset tend to cluster. They provide a single, representative figure that summarizes an entire distribution, making it easier to understand and compare large sets of data. The three primary measures are the mean**,** median, and mode. The mean represents the arithmetic average and is useful when data are evenly distributed. The median is the middle value when data are ordered and is especially valuable for skewed distributions and the mode is the most frequently occurring value, helpful for categorical or discrete data. Together, these measures help researchers quickly grasp the overall pattern of data, making them essential components of descriptive statistics and a foundational step in any scientific analysis.

Standard deviation is a key statistical measure that describes the amount of variability or dispersion within a dataset. It indicates how far individual data points tend to deviate from the mean (average) value. A small standard deviation means that the values are closely clustered around the mean, showing consistency in the data; whereas a large standard deviation indicates that the values are widely spread out, reflecting greater variability. This measure is crucial in scientific research because it helps evaluate the reliability of data,

compare the stability of different treatments or groups, and understand natural variations within biological, environmental, or experimental systems. Standard error is a statistical measure that reflects the precision of a sample estimate, most commonly the sample mean. While standard deviation describes the variability among individual data points, the standard error indicates how much the sample mean is expected to vary from the true population mean. It is calculated by dividing the standard deviation by the square root of the sample size, which means that larger samples produce smaller standard errors and more reliable estimates. In research and data analysis, the standard error is essential for constructing confidence intervals, performing hypothesis tests, and assessing how accurately sample results represent the population. Thus, it plays a critical role in inferential statistics by quantifying the uncertainty associated with sample-based estimates.

13.2. MEASURES OF CENTRAL TENDENCY:

Measures of Central Tendency are descriptive statistics that summarize a dataset by identifying the single, central value that best represents the entire distribution. They are often called averages. The purpose of these measures is to provide a central or typical value around which the rest of the data cluster, helping to understand the characteristic score of the data. The measure of central tendency is defined as: *"It is a sort of average or typical value of the items in the series and its function is to summarise the series in terms of this average value"*.

The Most Common Measures of Central Tendency are:

1) Arithmetic mean or Mean
2) Median
3) Mode.

Characteristics of an Ideal Measure of Central Tendency:

According to Professor G.V. Yule, a Good Average must have the Following Characteristics:

1) It should be rigidly defined so that different persons may not interpret it differently.
2) It should be easy 10 understand and easy to calculate.
3) II should be based on all the observations of the data.
4) It should be easily subjected to further mathematical calculations.
5) It should be least affected by the fluctuations of the sampling.
6) It should not be unduly affected by the extreme values.
7) It should be easy to interpret.

1. Mean (Arithmetic Average):

Definition: The mean is the sum of all values divided by the total number of values.

Types of Mean: It is of Three Types

1) **Arithmetic Mean:** The arithmetic mean is calculated by adding up all the values in a dataset and then dividing by the total number of values.
2) **Geometric Mean:** The arithmetic mean is calculated by adding up all the values in a dataset and then dividing by the total number of values.
3) **Harmonic Mean:** The arithmetic mean is calculated by adding up all the values in a dataset and then dividing by the total number of values.

Formula:

$$\text{Mean} = \frac{\sum x_i}{n}$$

Where:

∑xi is the sum of all observations.

n is the total number of observations.

Example 1: Height of Tomato Plants:

A biologist measures the height (in cm) of 5 tomato plants: **65, 70, 68, 72, 75**

$$\textbf{Mean} = \frac{65+70+68+72+75}{5} = \frac{350}{5} = \textbf{70 cm}$$

Interpretation: The average height of the tomato plants is 70 cm.

The average height of tomato plant is 70 cm

Example 2: Average Height of Sunflower Plants

A botanist grows 5 sunflower plants under the same conditions and measures their heights (in cm) after 30 days:

Heights: 120, 125, 130, 128, 127

$$\textbf{Mean} = \frac{120+125+130+128+127}{5} = \frac{630}{5} = \textbf{126 cm}$$

The average height of sun flower plants is 126 cm

Example 3. Average Number of Stomata per mm² on a Leaf

A plant physiologist counts the number of stomata per mm² on 5 samples of a leaf surface:

Stomatal counts: 85, 90, 88, 92, 95

$$\text{Mean} = \frac{85+90+88+92+95}{5} = \frac{450}{5} = 90 \text{ stomata/mm}^2$$

The average number of stomata per mm^2 is 90

Example 4: Average Enzyme Activity (U/mL)

A biochemist measures the activity of an enzyme in 4 different samples:

Enzyme activity values: 2.5, 2.7, 2.6, 2.8 U/mL

$$\text{Mean} = \frac{2.5+2.7+2.6+2.8}{4} = \frac{10.6}{5} = 2.65 \text{ U/mL}.$$

The Average Enzyme Activity Across the Samples is 2.65 U/mL.

Biological Applications:

1) **Comparison:** The mean allows us to compare different populations or groups. For example, we could compare the average height of giraffes in different regions to see if there are any differences.
2) **Trends:** By tracking the mean over time, we can identify trends or patterns. For instance, we could observe the average temperature of a lake over several years to see if there's a warming trend.
3) **Outliers:** The mean can be influenced by extreme values (outliers). For example, if one giraffe in our population is exceptionally tall, it will pull the mean height up. In these cases, the median might be a better measure of central tendency.
4) **Population Dynamics:** The geometric mean can be used to estimate average population growth rates.
5) **Gene Expression:** In genomics, the geometric mean is used to calculate the average expression level of genes.
6) **Environmental Monitoring:** The geometric mean can be used to analyze data on pollutants or other environmental factors that change multiplicatively over time.
7) **Metabolic Rates:** The harmonic mean can be used to calculate the average metabolic rate of a population.
8) **Gene Frequency:** In population genetics, the harmonic mean can be used to calculate the average frequency of a gene in a population.

9) **Environmental Studies:** The harmonic mean can be used to analyze data on pollution levels or other environmental factors that change as rates.

2. Median:

Definition: The median is the middle value in a dataset when arranged in ascending or descending order.

If n is odd: median = middle value

If n is even: median = average of the two middle values

Example 1: White Blood Cells Count

White blood cell (WBC) counts (in $10^3/\mu L$) from 7 patients: **5.2, 4.8, 6.0, 5.5, 5.0, 4.9, 5.4**

Sorted: 4.8, 4.9, 5.0, **5.2**, 5.4, 5.5, 6.0

Median = **5.2**

Example 2: Median Root Length

A researcher measures the **root lengths** (in cm) of 7 bean seedlings:

Data (cm): 4.8, 5.1, 4.9, 5.2, 5.4, 8.1, 5.3

Step 1: Arrange in order: 4.8, 4.9, 5.1, **5.2**, 5.3, 5.4, 8.1

Step 2: Find the middle value (odd number of values): Median = **5.2 cm**

Despite the outlier (8.1), the median root length is 5.2 cm, giving a better central tendency than the mean.

Example 3: Median Number of LEAVES PER PLANT

A study counts the number of leaves on each of 6 sunflower plants:

Data: 22, 25, 24, 26, 23, 45

Step 1: Arrange in order: 22, 23, 24, 25, 26, 45

Step 2: Even number → Take average of two middle values (24, 25):

$$\text{Median} = \frac{24+25}{2} = 24.5$$

The median is 24.5 leaves. The high value (45) doesn't distort the median as much as it would the mean.

Example 4: Median Chlorophyll Content (SPAD Values)

A SPAD meter gives the following readings for chlorophyll content in 9 maize leaves:

SPAD values: 42, 44, 45, 45, 47, 48, 50, 52, 60

Step 1: Already sorted

Step 2: Middle value (9 readings):

Median = **47**

47 is the central value, unaffected by the higher outlier (60).

Biological Applications:

1) **Gene Frequency:** When studying the distribution of alleles (gene variants) in a population, the median can be used to represent the typical allele frequency. Outliers, such as rare alleles, might skew the mean, making the median a more reliable measure of central tendency.
2) **Species Abundance:** Ecologists often use the median to describe the typical abundance of a species in a community. For example, the median abundance of a particular bird species in a forest might be more informative than the mean abundance if there are a few exceptionally abundant individuals.
3) **Blood Pressure:** In clinical settings, the median blood pressure reading is often used to assess a patient's cardiovascular health. This is because extreme blood pressure readings can be influenced by factors like stress or anxiety, making the median a more stable measure.
4) **Enzyme Activity:** The median enzyme activity can be used to represent the typical activity of an enzyme in a sample. Outliers, such as samples with unusually high or low enzyme activity, might be due to experimental error or other factors, making the median a more robust measure.
5) **Bacterial Growth Rates:** When studying bacterial growth, the median growth rate can be used to represent the typical rate of growth in a culture. This is particularly useful when dealing with populations that have a wide range of growth rates.

3. Mode:

Definition: The **mode** is the value that appears **most frequently** in a dataset.

A Dataset can have:

1. No mode
2. One mode (unimodal)
3. Two modes (bimodal)
4. More than two (multimodal)

Example 1: Mode of Petal Number

A botanist observes the number of petals in a group of wildflowers.

Petal counts: 5, 5, 6, 5, 4, 6, 5, 4, 5, 6

Most frequent number = 5

Mode = 5 petals

The most common flower form in the population has **5 petals**.

Example 2: Mode of Leaf Shapes

A researcher categorizes leaf shapes in a population of plants:

Leaf shapes recorded: ovate, lanceolate, ovate, cordate, ovate, lanceolate, ovate

Mode = ovate (appears 4 times)

Ovate is the most frequent leaf shape in this population.

Example 3: Mode of Plant Height Class (in cm)

Heights of wheat plants are grouped into classes:

- 90–100: 3 plants
- 100–110: 6 plants
- 110–120: 10 plants
- 120–130: 7 plants
- 130–140: 4 plants

Mode = 110–120 cm class (most plants fall here)

Most common plant height falls within **110–120 cm** range.

Example 4: Mode of Seed Color

In a genetic cross, you observe the seed colors:

Seed Colors: yellow, yellow, green, yellow, green, yellow, yellow

Mode = Yellow

Yellow is the most common seed color in the progeny.

Biological Applications:

1) **Genotype Frequencies:** In population genetics, the mode can be used to identify the most common genotype (combination of alleles) in a population. For example, if a particular genotype is the mode, it suggests that it's well-adapted to the environment or that it's being selected for.
2) **Dominant Species:** Ecologists use the mode to identify the dominant species in a community. The species with the highest abundance (the mode) is often considered the most influential species in the community.
3) **Peak Enzyme Activity:** In biochemistry, the mode can be used to identify the pH or temperature at which an enzyme exhibits its peak activity. This information is crucial for understanding the optimal conditions for enzyme function.
4) **Bacterial Colony Size:** When studying bacterial growth, the mode can be used to identify the most common colony size. This can provide insights into the growth patterns of bacteria and their susceptibility to antibiotics.
5) **Optimal Phenotype:** The mode can be used to identify the most common phenotype (observable characteristic) in a population. This can provide clues about the selective pressures that are shaping the evolution of the population.

13.3. STANDARD DEVIATION:

Standard deviation (SD) is a statistical measure quantifying the dispersion of data points around the mean. It reflects variability within a dataset, with higher SD indicating greater spread. In plant biology, SD is crucial for understanding natural variation, environmental responses, and agricultural performance.

$$s = \sqrt{\frac{\sum(x_i - \bar{x})^2}{n-1}}$$

Where:

s = sample standard deviation

x_i = individual data points

$\bar{x}$ = sample mean

n = total number of data points in the sample

Example 1: Plant Height Variation

Let’s say you measured the height (in cm) of 5 sunflower plants grown under the same conditions:

Plant No.	Height (cm)
1	120
2	122
3	119
4	121
5	118

Step 1: Find the Mean ($\bar{x}$)

$$\bar{x} = \frac{120 + 122 + 119 + 121 + 118}{5} = \frac{600}{5} = 120$$

Step 2: Find Each Deviation from the Mean ($x_i - \bar{x}$)

Plant No.	Height (x_1)	$x_i - \bar{x}$	$(x_i - \bar{x})^2$
1	120	0	0
2	122	2	4
3	119	-1	1
4	121	1	1
5	118	-2	4

Step 3: Find the Sum of Squared Deviations

$$\sum (x_i - \bar{x})^2 = 0 + 4 + 1 + 1 + 4 = 10$$

Step 4: Apply the Formula for Sample SD

$$SD = \sqrt{\frac{\sum (x_i - \bar{x})^2}{n - 1}} = \sqrt{\frac{10}{4}} = \sqrt{2.5} \approx 1.58 \text{ cm}$$

The plant heights vary by approximately ±1.58 cm from the mean.

Example 2: Leaf Area in Different Fertilizers

Suppose you test 4 fertilizers and measure leaf area (in cm²) of bean plants:

Fertilizer	Leaf Area (cm²)
A	40
B	55
C	50
D	45

Step 1: Mean

$$\bar{x} = \frac{40 + 55 + 50 + 45}{4} = \frac{190}{4} = 47.5$$

Step 2: Deviation and Squared Deviation

Fertilizer	x	$x_i - \bar{x}$	$(x_i - \bar{x})^2$
A	40	-7.5	56.25
B	55	7.5	56.25
C	50	2.5	6.25
D	45	-2.5	6.25

Step 3: Sum of Squared Deviations

$$\sum (x_i - \bar{x})^2 = 56.25 + 56.25 + 6.25 + 6.25 = 125$$

Step 4: Sample SD

$$SD = \sqrt{\frac{125}{3}} = \sqrt{41.67} \approx 6.45 \text{ cm}^2$$

The leaf area shows more variability between treatments, with a deviation of ±6.45 cm².

Applications in Plant Growth Studies:

Comparing Treatments: Standard deviation helps determine if different treatments (e.g., fertilizers, watering regimes) significantly affect plant growth consistency. A smaller standard deviation in a treatment group suggests more predictable growth responses.

Identifying Outliers: Plants with unusually high or low growth compared to the average (as indicated by a high standard deviation) might warrant further investigation to identify underlying factors.

Genotype Analysis: Standard deviation can highlight the genetic variability within a plant population. High standard deviation could indicate a diverse gene pool, potentially beneficial for adaptation to various environmental conditions.

Environmental Impact Assessment: Standard deviation helps assess the impact of environmental factors (e.g., drought, nutrient deficiency) on plant growth uniformity. High standard deviation under stress conditions could signify a lack of resilience in the plant population.

Statistical Significance: While standard deviation describes variability, it doesn't directly indicate statistical significance. To determine if differences in growth between groups are statistically significant, researchers use statistical tests such as t-tests or ANOVA, which incorporate both the means and standard deviations of the datasets.

13.4. STANDARD ERROR:

Standard Error (SE) is a statistical term that measures how much the sample mean (average) of the data is expected to vary from the true population mean. It gives an idea of the reliability of the sample mean.

Lower SE → more reliable average (less variation)

Higher SE → less reliable average (more variation)

$$SE = \frac{SD}{\sqrt{n}}$$

Where:

SD = Standard deviation of the sample

n = Number of observations (sample size)

Example 1: A botanist is measuring the height of sunflower plants grown under two different fertilizers (Fertilizer A and B). They take 5 samples from each group.

Fertilizer A Heights (cm)	Fertilizer B Heights (cm)
150	140
155	138
152	142
149	137
153	135

Step 1: Calculate the mean and standard deviation for each group.

Let's say:

Fertilizer A: Mean = 151.8 cm, SD = 2.39

Fertilizer B: Mean = 138.4 cm, SD = 2.41

Sample size (n) = 5

Step 2: Calculate Standard Error for each group.

$$SE_A = \frac{2.39}{\sqrt{5}} \approx 1.07$$

$$SE_B = \frac{2.41}{\sqrt{5}} \approx 1.08$$

The SE tells us how much the sample mean might differ from the actual population mean. Smaller SEs (as in the example) suggest that the sample means (151.8 and 138.4 cm) are close estimates of the true population mean.

Biological Applications:

The standard error (SE) is a crucial statistical concept in biological research, providing insights into the reliability and variability of sample estimates. Here are some key biological applications of standard error:

Estimating Population Parameters:

The standard error helps estimate how well a sample mean represents the population mean. A smaller SE indicates that the sample mean is a more accurate reflection of the population mean.

Confidence Intervals:

SE is used to construct confidence intervals around sample means. This interval gives a range within which the true population mean is likely to fall, enhancing the reliability of the findings.

Comparing Groups:

In experiments comparing different groups (e.g., treatment vs. control), SE allows researchers to assess the significance of differences between means. A larger SE may indicate more variability and less confidence in the observed differences.

Effect Size Estimation:

When effect size statistics are not available, SE can help estimate the precision of the effect observed in a study, guiding researchers in interpreting the biological significance of their results.

Meta-Analysis:

In meta-analyses, SE is used to combine results from multiple studies, allowing for a more comprehensive understanding of biological phenomena across different research contexts.

Quality Control in Experiments:

Monitoring the SE during experiments can help identify issues with data collection or sample variability, ensuring that the results are robust and reliable.

13.5. SUMMARY:

Measures of central tendency are statistical tools used to identify the central or typical value within a dataset, providing a simplified summary of complex information. The three main measures are the mean, median, and mode. The mean is the arithmetic average and is calculated by dividing the total of all observations by the number of values, but it is sensitive to extreme scores. The median represents the middle value when data are arranged in order and is more reliable for skewed distributions because it is not influenced by outliers. The mode is the value that appears most frequently and is especially useful for categorical or discrete data. Together, these measures help describe the overall pattern of data, making interpretation and comparison easier in statistical analysis.

Standard deviation is a widely used measure of dispersion that indicates how much individual values in a dataset deviate, on average, from the mean. It reflects the degree of variability or spread within the data. A low standard deviation shows that the values cluster closely around the mean, while a high standard deviation indicates that the values are widely scattered. Calculated as the square root of the variance, it provides a more interpretable measure because it is expressed in the same units as the original data. Standard deviation is essential in statistics for understanding data consistency, comparing variability between datasets, and assessing reliability in scientific experiments and research.

Standard error is a statistical measure that indicates how much the sample mean is expected to vary from the true population mean. It reflects the precision of an estimate. A smaller standard error means the sample mean is a more accurate and reliable representation of the population mean, while a larger standard error indicates greater variability and less certainty. Standard error decreases as the sample size increases because larger samples tend to provide more stable estimates. Calculated by dividing the standard deviation by the square root of the sample size, it is widely used in constructing confidence intervals, performing hypothesis tests, and evaluating the reliability of sample-based estimates in research.

13.6. TECHNICAL TERMS:

Arithmetic Mean, Central Value, Skewness, Outliers, Dispersion, Variability, Variance, Normal Distribution, Inferential Statistics

13.7. SELF-ASSESSMENT QUESTIONS:

1) What is the difference between standard deviation and standard error?
2) Describe the mean, median, and mode in detail. Discuss their merits, demerits, and applications.
3) Explain the different types of means, arithmetic, geometric, and harmonic. Provide suitable examples for each.
4) Discuss the role of central tendency in statistical data analysis.
5) Compare and contrast mean, median, and mode with examples from symmetrical and skewed distributions.
6) Explain the concept of standard deviation. Describe the steps involved in calculating it for raw data and grouped data.
7) Define standard error and explain its significance in statistical inference.
8) Explain the difference between standard deviation and standard error with examples.

13.8. SUGGESTED READINGS:

1) Rosner, B. (2015). Fundamentals of Biostatistics (8th ed.). Cengage Learning.
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4) Pandey, M. (2022). Biostatistics: Basic and Advanced. Scientific Publishers.
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Dr. Kakumanu Babu

LESSON-14

STUDENT T - TEST, F - TEST AND CHI - SQUARE TEST

14.0. OBJECTIVE:

- Students are able to know the differences among student T-test, F-test and Chi-square test for statistical significance.

STRUCTURE:

14.1. INTRODUCTION:

The Student's t-test is a statistical test used to determine whether the means of two groups differ significantly when the sample size is small and the population standard deviation is unknown. It evaluates the difference between samples means relative to the variability within the data, helping researchers infer whether an observed difference is due to chance or reflects a real effect. Common forms include the one-sample**,** independent two-sample**,** and paired t-tests, each suited for different research designs. The t-test is widely used in scientific experiments, biological studies, and social science research where comparing group averages is essential. The F-test is a statistical test used to compare variances between two or more groups to determine whether they differ significantly. It evaluates the ratio of two variances and helps assess the spread or variability within datasets. The F-test is most commonly applied in Analysis of Variance (ANOVA), where it determines whether the means of multiple groups are significantly different by comparing the variance between groups to the variance within groups. It is widely used in scientific experiments to test model significance, compare treatments, and analyse variability in measured data. The chi-square test is a non-parametric statistical test used to examine whether there is a significant association between categorical variables or whether the observed frequencies in a dataset differ from the expected frequencies. It is commonly used in contingency tables to test independence between variables and in goodness-of-fit tests to determine how well a theoretical distribution matches the observed data. Because it does not rely on assumptions about population parameters, the chi-square test is widely used in biological, social, and behavioural research involving categorical data.

14.2. STUDENT T-TEST:

The Student's t-test is a powerful statistical tool widely used in biological research to analyze data and draw meaningful conclusions. Let's explore its types and biological

applications. The Student's t-test is used to compare the means of two groups. There are several types, each suited to different experimental designs:

a) **One-Sample t-test:** This test compares the mean of a single group to a known or hypothesized value. Biological Application: A researcher might use this to determine if the average weight of a specific plant species grown under certain conditions differs significantly from a known average weight.

b) **Two-Sample t-test (Independent Samples):** This test compares the means of two independent groups. Biological Application: Comparing the average lifespan of two different strains of mice exposed to a particular drug, to see if one strain exhibits significantly increased longevity.

c) **Paired t-test:** This test compares the means of two related groups, often involving repeated measurements on the same subjects. Biological Application: Measuring the blood pressure of patients before and after administering a new medication. The paired t-test analyses whether the blood pressure changed significantly after treatment.

Key Features to the T-Test

a) **Choosing the Right t-test:** The selection of the appropriate t-test depends on the experimental design and the nature of the data. The key distinctions are whether you have one or two groups and whether the groups are independent or paired (repeated measures).

b) **Assumptions of the t-test:** It's crucial to understand the assumptions underlying the t-test to ensure its valid application. These assumptions include:

c) **Normality:** The data should be approximately normally distributed within each group. While the t-test is relatively robust to violations of normality, particularly with larger sample sizes, significant deviations might warrant using non-parametric alternatives like the Wilcoxon test.

d) **Independence:** Observations within and between groups should be independent of each other. This means that one observation does not influence another.

e) **Homogeneity of variances (for independent samples t-test):** The variances of the two groups being compared should be approximately equal. Tests like Levene's test can assess this assumption. If the assumption is violated, a modified version of the t-test, such as Welch's t-test, can be used.

The t-test produces a p-value, which represents the probability of observing the obtained results (or more extreme results) if there were no real difference between the groups. A p-value below a pre-determined significance level (typically 0.05) indicates statistically significant differences between the group means. However, statistical significance doesn't always equate to practical significance, and effect sizes should also be considered.

Problem 1: Two-Sample t-test – Effect of Fertilizer on Plant Height

Scenario: A researcher wants to know whether a new fertilizer affects the height of tomato plants. Two groups of 6 plants are measured:

Group	Mean Height (cm)	SD	n
Control	35.2	2.5	6
Treated	39.1	3.1	6

Step 1: State the hypotheses

- **H_0 (Null)**: There is no difference in plant height.
- **H_1 (Alternative)**: There is a significant difference in height between the two groups.

Step 2: Use the formula for independent t-test

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

Step 3: Plug in the values

$$\bar{X}_1 = 35.2, \quad \bar{X}_2 = 39.1$$
$$s_1 = 2.5, \quad s_2 = 3.1$$
$$n_1 = n_2 = 6$$

$$t = \frac{35.2 - 39.1}{\sqrt{\frac{2.5^2}{6} + \frac{3.1^2}{6}}} = \frac{-3.9}{\sqrt{\frac{6.25}{6} + \frac{9.61}{6}}} = \frac{-3.9}{\sqrt{1.04 + 1.60}} = \frac{-3.9}{\sqrt{2.64}} \approx \frac{-3.9}{1.62} \approx -2.41$$

Step 4: Degrees of freedom

$$df = n_1 + n_2 - 2 = 6 + 6 - 2 = 10$$

Step 5: Check critical t-value at α = 0.05

From t-table for df = 10, two-tailed test:

$$t_{crit} \approx 2.228$$

Step 6: Conclusion

$$|t| = 2.41 > 2.228 \Rightarrow \text{Reject } H_0$$

Conclusion: The new fertilizer significantly increased tomato plant height.

Problem 2: Paired t-test – Effect of Drought on Chlorophyll

Scenario: You measure chlorophyll content of leaves before and after drought stress on 5 plants.

Plant	Control	Stress
1	2.9	2.1
2	3.1	2.4
3	2.7	2.0
4	3	2.5
5	2.8	2.2

Step-by-Step Solution:

Step 1: State Hypotheses

H_0: No change in chlorophyll.

H_1: Chlorophyll content changes after drought.

Step 2: Find the difference for Each Pair

Plant	Before	After	Difference (d)
1	2.9	2.1	0.8
2	3.1	2.4	0.7
3	2.7	2	0.7
4	3	2.5	0.5
5	2.8	2.2	0.6

$$\bar{d} = \frac{0.8 + 0.7 + 0.7 + 0.5 + 0.6}{5} = 0.66$$

Step 3: Calculate standard deviation (SD) of differences

$$s_d = \sqrt{\frac{\sum (d_i - \bar{d})^2}{n-1}} = \sqrt{\frac{(0.8-0.66)^2 + \ldots + (0.6-0.66)^2}{4}} = 0.1$$

Step 4: Calculate t

$$t = \frac{\bar{d}}{s_d/\sqrt{n}} = \frac{0.66}{0.1/\sqrt{5}} = \frac{0.66}{0.0447} \approx 14.76$$

Step 5: Degrees of freedom

$$df = n - 1 = 5 - 1 = 4$$

Step 6: Compare with critical t (α = 0.05, two-tailed)

$$t_{crit} \approx 2.776$$

Conclusion: 14.76 > 2.776 → significant drop in chlorophyll after drought.

Biological Applications:

a) The t-test is a powerful statistical tool widely used in biological research to compare means and assess whether differences between groups are statistically significant. Here are some key biological applications of the t-test:

b) **Comparing Treatment Effects:** Researchers often use the t-test to determine if a new treatment has a significant effect compared to a control group. For example, comparing the blood pressure of patients before and after a specific medication.

c) **Analysing Experimental Data:** In experiments involving plant biology, scientists might compare the heights of plants grown under different conditions (e.g., varying light levels) to see if the differences are significant.

d) **Genetic Studies:** The t-test can be applied to compare the means of gene expression levels between two different groups, such as mutant vs. wild-type organisms, to identify significant changes due to genetic modifications.

e) **Ecological Research:** Ecologists might use the t-test to compare the sizes of animals in different habitats, helping to understand how environmental factors influence growth and development.

f) **Clinical Trials:** In clinical research, the t-test is crucial for comparing outcomes (like recovery rates or side effects) between groups receiving different treatments or interventions.

g) **Behavioral Studies:** In studies examining animal behaviour, researchers may use the t-test to compare the average time spent on a task by two different groups of animals, such as those raised in enriched vs. standard environments.

14.3. F- TEST:

The F-test is a statistical test used to compare the variances of two or more groups. It's particularly useful in the context of ANOVA (Analysis of Variance), and we can determine the significant differences between the means of multiple groups.

Principle:

The F-test is based on the F-distribution, which is a probability distribution that arises when comparing two variances. The test statistic, denoted as F, is the ratio of two variance estimates:

Numerator:

Variance between groups (measures how much the group means differ from the overall mean)

Denominator:

Variance within groups (measures the variability within each group)

Types of F-tests:

a) **One-way ANOVA:** Compares the means of three or more groups. The null hypothesis is that all group means are equal.

b) **Two-way ANOVA:** Examines the effects of two or more independent variables on a dependent variable. It can also assess the interaction between these independent variables.

c) **Tests for Equality of Variances:** Specifically used to check if the variances of two or more groups are equal (a key assumption for many other statistical tests).

Key Concepts of F- Test:

a) **F-statistic:** F-test calculated as the ratio of two sample variances.

$$F = \frac{\text{Variance between groups}}{\text{Variance within groups}}$$

b) **Null Hypothesis (H_0):** States that there is no significant difference between the variances being compared.

c) **Alternative Hypothesis (H_1):** States that there is a significant difference between the variances.

d) **Degrees of Freedom:** The number of independent values or quantities that can vary in an analysis. For the F-test:

df_1 = the degrees of freedom between groups.

df_2 = the degrees of freedom within groups.

Mechanism of F-Test:

a) **Collect Data:** In the above plant examples, data is collected from plants exposed to various treatments or conditions (fertilizers, watering schedules, light intensities).

b) **Calculate Group Means and Variances:** The mean and variance of the measurements for each group are calculated.

c) **Compute the F-statistic:** The F-statistic is computed by dividing the variance between the groups by the variance within the groups.

d) **Interpret the F-statistic:** The F-statistic is compared to the critical value from the F-distribution table (based on the degrees of freedom and the chosen significance level, e.g., 0.05). If the F-statistic exceeds the critical value, the null hypothesis is rejected, indicating a significant difference between the variances.

The F-test produces a p-value. If the p-value is less than a pre-determined significance level (e.g., 0.05), we reject the null hypothesis and conclude that there is a statistically significant difference between the group means (or variances, depending on the type of F-test). If the p-value is greater than the significance level, we fail to reject the null hypothesis.

Problem 1: A researcher measures the leaf length (cm) of *Variety A* and *Variety B* of a medicinal plant to check whether the two varieties differ in variability. Test at 5% significance whether the two varieties differ significantly in their variances.

Variety A	**Variety B**
12, 14, 13, 15, 16	10, 18, 12, 20, 17

Answer:

Step 1: Compute sample variances

Variety A

Mean = (12+14+13+15+16)/5 = 14

Variance = $\Sigma(x - \text{mean})^2 / (n-1)$

= $[(12-14)^2 + (14-14)^2 + (13-14)^2 + (15-14)^2 + (16-14)^2]/4$

= $(4 + 0 + 1 + 1 + 4) / 4 = 10/4 =$ **2.5**

Variety B

Mean = (10+18+12+20+17)/5 = 14.4

Variance = $[(10–14.4)^2 + ... + (17–14.4)^2]/4$

= (29.16 + 6.76 + 11.56 + 20.16 + 2.56)/4

= 70.2/4 = **17.55**

Step 2: Compute F statistic

Take larger variance as numerator:

$$F = \frac{17.55}{2.5} = 7.02$$

Step 3: Degrees of freedom

$df_1 = n_1–1 = 4$

$df_2 = n_2–1 = 4$

Step 4: Critical value (α = 0.05)

For $df_1 = 4$, $df_2 = 4 \rightarrow F_{0.05} \approx 6.39$

Step 5: Decision

Since F = 7.02 > 6.39

∴ Reject H_0.

Conclusion: The two plant varieties show significantly different variance in leaf length.

Problem 2: A scientist tests whether salicylic acid (SA) affects the variability in germination percentage of soybean seeds. At **1% level**, test if the variances differ.

Control germination (%): 82, 85, 80, 90

SA-treated germination (%): 75, 70, 78, 65

Answer:

Step 1: Compute variances

Control group

Mean = (82+85+80+90)/4 = 84.25

Variance = $[(82–84.25)^2 + ... + (90–84.25)^2] / 3$

= (5.06 + 0.56 + 18.06 + 33.06) / 3

= 56.74/3 = **18.91**

SA treatment

Mean = (75+70+78+65)/4 = 72

Variance = (0 + 4 + 36 + 49) / 3 = 89/3 = **29.67**

Step 2: F statistic

Larger variance on top:

$$F = \frac{29.67}{18.91} = 1.56$$

Step 3: Degrees of freedom

$df_1 = 3$

$df_2 = 3$

Step 4: Critical value (α = 0.01)

F{0.01}(3, 3) ≈ **29.46**

Step 5: Decision

Since **1.56 < 29.46**,

∴ **Fail to reject H_0.**

Conclusion: There is no significant difference in variance of germination rate between control and SA-treated seeds at 1% level.

Problem 3: A researcher measures the **height (cm)** of a salt-tolerant grass grown at: **0 mM NaCl (Control) and 100 mM NaCl (Stress).** Test whether the stress treatment increases variability in height at 5% significance.

Control: 22, 25, 24, 26, 23

Stress: 15, 18, 12, 20, 17

Answer:

Step 1: Compute variances

Control

Mean = 24

Variance = $[(22–24)^2 + ... + (23–24)^2]/4$

= (4 + 1 + 0 + 4 + 1)/4 = 10/4 = **2.5**

Stress

Mean = 16.4

Variance = (1.96 + 2.56 + 19.36 + 12.96 + 0.36)/4

= 37.2/4 = **9.3**

Step 2: Compute F

$$F = \frac{9.3}{2.5} = 3.72$$

Step 3: df

$df_1 = df_2 = 4$

Step 4: Critical F (α = 0.05)

F(4,4) = **6.39**

Step 5: Decision

3.72 < 6.39 → **Fail to reject H_0**

Conclusion: Salt stress **does not significantly increase variance** in plant height at 5% significance.

Problem 4: Comparing Variability in Chlorophyll Content Between Two Fertilizers

Chlorophyll-a content (mg/g FW):

Fertilizer X: 1.8, 1.5, 1.9, 1.6

Fertilizer Y: 1.2, 1.4, 1.3, 1.1

Test if the variances are equal at **5%** significance.

Answer:

Step 1: Variances

Fertilizer X

Mean = 1.7

Variance = (0.01 + 0.04 + 0.04 + 0.01)/3 = 0.10/3 = **0.033**

Fertilizer Y

Mean = 1.25

Variance = (0.0025 + 0.0225 + 0.0025 + 0.0225)/3

= 0.05/3 = **0.017**

Step 2: F value

$$F = \frac{0.033}{0.017} = 1.94$$

Step 3: df

$df_1 = df_2 = 3$

Step 4: F critical (α = 0.05)

F(3,3) = **9.28**

Step 5: Decision

1.94 < 9.28 → **Fail to reject H_0**

Conclusion: Variances do not differ significantly between the two fertilizers.

Biological Applications:

The F-test is a powerful statistical tool widely used in biological research to compare variances and assess the significance of differences between groups. Here are some key biological applications of the F-test:

a) **Comparing Variances:** The F-test is often used to determine if there are significant differences in the variances of two or more populations. This is crucial in biological experiments where variability can affect the interpretation of results.

b) **ANOVA (Analysis of Variance):** One of the most common applications of the F-test is in ANOVA, which is used to compare the means of three or more groups. For example, researchers might use ANOVA to assess the effects of different treatments on plant growth or animal behavior.

c) **Testing Hypotheses:** The F-test helps in testing hypotheses about the equality of means across different groups. For instance, it can be used to evaluate whether different diets lead to different weight gains in animal studies.

d) **Genetic Studies:** In genetics, the F-test can be applied to compare the variances of traits among different genetic lines or populations, helping researchers understand the heritability of traits.

e) **Clinical Trials:** In clinical research, the F-test is used to analyze the effectiveness of treatments by comparing the variances in outcomes between treatment and control groups.

f) **Ecological Studies:** Ecologists may use the F-test to compare the variances of species diversity indices across different habitats, helping to understand ecological dynamics.

14.4. CHI-SQUARE (X^2) TEST:

The Chi-square test is a non-parametric statistical test used to compare observed frequencies with expected frequencies to determine whether 1. Differences are due to chance,

or 2. There is a significant association or deviation from expectations. It is widely used in genetics, ecology, plant breeding, and taxonomy. It is of two types 1. Chi-square Goodness-of-Fit Test 2. Chi-square Test of Independence.

1) **Chi-square Goodness-of-Fit Test:** Used to check whether an observed distribution fits a theoretical (expected) ratio.

   E.g.: Checking Mendelian ratios in seed colour or flower colour, Testing viability of seeds across expected survival percentages.

2) **Chi-Square Test of Independence:** Used to check whether two categorical variables are associated.

   E.g.: Association between disease resistance and plant variety, Relationship between leaf shape and habitat type.

Formula for Chi-Square:

$$\chi^2 = \sum \frac{(O - E)^2}{E}$$

Where:

O = Observed frequency

E = Expected frequency

Problem 1: A plant breeder self-pollinates a heterozygous purple flowered plant (Pp) expecting a 3:1 ratio of purple: white.

He obtains the following:

Phenotype	Observed
Purple	290
White	110
Total	400

Test whether the observed ratio fits the 3:1 model at 5% significance.

Answer:

Step 1: Expected Frequencies

3:1 ratio → Expected purple = 3/4 × 400 = **300**

Expected white = 1/4 × 400 = **100**

Step 2: Chi-square

$$\chi^2 = \frac{(290-300)^2}{300} + \frac{(110-100)^2}{100}$$
$$= \frac{100}{300} + \frac{100}{100}$$
$$= 0.33 + 1 = 1.33$$

Step 3: Degrees of freedom

df = number of categories − 1 = 2 − 1 = **1**

Step 4: Critical value (α = 0.05)

$\chi^2(0.05, 1)$ = **3.84**

Step 5: Decision

1.33 < 3.84 → **Accept H_0**

Conclusion: The observed flower-color ratio fits the expected 3:1 Mendelian ratio.

Problem 2: In a pea plant experiment, seeds are classified into round and wrinkled. The expected ratio is 9:3:3:1 for a dihybrid cross (RrYy × RrYy).

Observed:

Phenotype	Observed
Round-yellow	315
Round-green	108
Wrinkled-yellow	101
Wrinkled-green	26
Total	550

Test goodness-of-fit at **5% significance**.

Answer:

Step 1: Expected frequencies

Ratio = 9:3:3:1 → Sum = 16

Expected values = (ratio/16 × total)

RY = 9/16 × 550 = **309.4**

Rg = 3/16 × 550 = **103.1**

rY = 3/16 × 550 = **103.1**

rg = 1/16 × 550 = **34.4**

Step 2: Apply χ^2 formula

$$\chi^2 = \sum \frac{(O-E)^2}{E}$$

Compute each:

(315–309.4)² / 309.4 = 0.10

(108–103.1)² / 103.1 = 0.23

(101–103.1)² / 103.1 = 0.04

(26–34.4)² / 34.4 = 2.06

Sum: $\chi^2 = 0.10 + 0.23 + 0.04 + 2.06 = 2.43$

Step 3: Degrees of freedom

df = 4 − 1 = **3**

Step 4: χ^2 critical value at 5%

$\chi^2(0.05, 3)$ = **7.82**

Step 5: Decision

2.43 < 7.82 → Accept H_0

Conclusion: The observed seed phenotypes fit the 9:3:3:1 Mendelian ratio.

Problem 3: A researcher studies whether fungal disease resistance is associated with plant variety.

A	**40**	**20**	**60**
B	**30**	**50**	**80**

Test whether variety and resistance are independent at 5% level.

Answer:

Step 1: Comp

Data:

Variety	Resistant	Susceptible	Total

Total resistant = 40 + 30 = **70**

Total susceptible = 20 + 50 = **70**

Overall total = 140

Step 2: Expected frequencies (row × column / total)

For Variety A, Resistant:

$$E = \frac{60 \times 70}{140} = 30$$

For A, Susceptible:

$$E = \frac{60 \times 70}{140} = 30$$

For B, Resistant:

$$E = \frac{80 \times 70}{140} = 40$$

For B, Susceptible:

$$E = \frac{80 \times 70}{140} = 40$$

Step 3: Chi-Square

$$\chi^2 = \sum \frac{(O - E)^2}{E}$$

Compute each:

$(40-30)^2/30 = 3.33$

$(20-30)^2/30 = 3.33$

$(30-40)^2/40 = 2.50$

$(50-40)^2/40 = 2.50$

Sum: $\chi 2 = 11.66$

Step 4: Degrees of freedom

df = (rows−1)(columns−1) = 1×1 = **1**

Step 5: χ^2 critical value at 5%

= **3.84**

Step 6: Decision

11.66 > 3.84 → Reject H_0

Conclusion: There is a significant association between plant variety and disease resistance.

Problem 4: A scientist expects 80% germination in a seed batch. He tests 100 seeds and finds that out of 100 germinated seeds are 72 and 28 seeds are not germinated. Is the germination percentage different from the expected value?

Answer:

Step 1: Expected

Expected germinated = 80
Expected not germinated = 20

Step 2: χ^2

$$\chi^2 = \frac{(72-80)^2}{80} + \frac{(28-20)^2}{20}$$
$$= \frac{64}{80} + \frac{64}{20} = 0.8 + 3.2 = 4.0$$

Step 3: df = 1

Critical value at 5% = 3.84

Step 4: Decision

4.0 > 3.84 → Reject H_0

Conclusion: The germination rate does not match the expected 80%.

Biological Applications of the Chi-square Test:

1) **Mendelian Genetics:** Used to compare observed and expected ratios in monohybrid and dihybrid crosses to test whether inheritance follows Mendelian laws.
2) **Testing Genetic Linkage:** Helps identify whether two genes are independently assorting or linked by comparing expected and observed recombination frequencies.
3) **Population Genetics:** Applied to test deviations from **Hardy–Weinberg equilibrium**, indicating factors like selection, mutation, migration, or genetic drift.

4) **Disease Incidence Studies:** Used to assess whether the occurrence of a disease differs significantly between groups (e.g., exposed vs. non-exposed populations).
5) **Phenotypic Classification Studies:** Helps evaluate whether observed phenotypic counts match expected distributions in breeding or morphological studies.
6) **Ecology and Environmental Biology:** Applied to test associations between species distribution and environmental factors using contingency tables.
7) **Behavioral Biology:** Used to analyze frequency-based behavioral responses in animals under different experimental conditions.
8) **Microbiology and Epidemiology:** Helps examine associations between microbial strains, infection rates, host types, or resistance patterns.
9) **Clinical and Biomedical Research:** Applied to test the association between treatment groups and categorical outcomes such as recovery, improvement, or survival.

14.5. SUMMARY:

The Student's t-test is a statistical method used to determine whether the means of two groups differ significantly, especially when sample sizes are small and the population variance is unknown. It compares the difference between sample means relative to the variability within the data, helping assess whether an observed difference is due to random chance or reflects a true effect. Common types of t-tests include the one-sample t-test**,** independent two-sample t-test, and paired t-test, each suited for different experimental designs. The t-test is widely used in scientific research to compare treatments, evaluate experimental outcomes, and make inferences about population means. The F-test is a critical statistical hypothesis test that compares variances and utilizes the F-distribution, with its most common application being the basis for Analysis of Variance (ANOVA). It calculates an F-statistic as the ratio of two variance estimates, and when used in ANOVA, it determines if there is a significant difference between the means of three or more groups by comparing the variation *between* the groups to the variation *within* the groups. A large calculated F-statistic suggests that the differences among the group means are substantial enough to reject the null hypothesis that all population means are equal, indicating that at least one group mean is different from the others. The chi-square test is a non-parametric statistical method used to determine whether there is a significant association between categorical variables or whether the observed frequencies in a dataset differ from the expected frequencies. It is commonly applied in contingency tables to test relationships between variables and in goodness-of-fit tests to assess how well theoretical distributions match actual data. Because it does not assume normality and works with frequency counts, the chi-square test is especially useful in biological, social, and behavioral sciences where data are often categorical. A large chi-square value indicates that the observed differences are unlikely due to chance, suggesting a meaningful pattern or association in the data.

14.6. TECHNICAL TERMS:

Degrees of Freedom (df), Null Hypothesis (H_0), Alternative Hypothesis (H_1), Significance Level (α), p-value, ANOVA (Analysis of Variance), Critical F-value, Homogeneity of variances, Factor and levels, One-way & Two-way ANOVA

14.7. SELF ASSESSMENT QUESTIONS:

1) What is the difference between independent and paired t-tests?
2) What is the chi-square test used for?
3) Describe the different types of t-tests (one-sample, independent two-sample and paired t-test) with suitable examples.
4) Discuss the applications, merits, and limitations of the Student's t-test in scientific research.
5) Explain the concept of the F-test and describe its importance in ANOVA.
6) Compare chi-square, t-test, and F-test in terms of assumptions, applications and data requirements.

14.8. SUGGESTED READINGS:

1) Rosner, B. (2015). Fundamentals of Biostatistics (8^{th} ed.). Cengage Learning.
2) Suresh, K.P. (2014). An Introduction to Biostatistics (2^{nd} ed.). IK International Publishing House.
3) Kothari, C.R., & Garg, G. (2019). Research Methodology: Methods and techniques (4th ed.). New Age International.
4) Pandey, M. (2022). Biostatistics: Basic and Advanced. Scientific Publishers.
5) Prasad, S., & Sinha, B. K. (2010). Biostatistics. New Age International Publishers.
6) P.N. Arora and P.K. Malhan (2010). Biostatistics. Mrs. Meena Pandey. Branch Offices for Himalaya Publishing House, "Rmndnot", Dr Bhalerao Marg, Girgaon, Mumbai-400 004.

Dr. Kakumanu Babu

LESSON-15

ANALYSIS OF VARIANCE, CORRELATION, LINER REGRESSION AND EXPERIMENTAL DESIGNS

15.0. OBJECTIVE:

- Students are able to measure the degree and direction of the relationship between two quantitative variables, linear regression and also understand the experimental designs.

STRUCTURE:

15.1. INTRODUCTION:

Analysis of Variance (ANOVA) is a statistical technique used to determine whether there are significant differences among the means of three or more groups. It works by comparing the variation between group means to the variation within the groups, helping identify whether observed differences are due to actual effects or random chance. ANOVA is widely applied in scientific experiments, agriculture, biology, psychology, and other research fields where multiple treatments or conditions are compared simultaneously. Correlation is a statistical method used to measure the strength and direction of the relationship between two quantitative variables. It helps determine whether an increase or decrease in one variable is associated with a corresponding change in the other. The relationship is typically expressed through a correlation coefficient, such as Pearson's *r*, which ranges from –1 to +1, indicating perfect negative to perfect positive association. Linear regression is a statistical technique used to model and analyse the relationship between a dependent variable and one independent variable by fitting a straight line to the observed data. Linear regression helps quantify how strongly the variables are related, determine the direction of the relationship, and make predictions for future or unknown values.

The main topics connected with data collection are Theory of Sample Surveys and Experimental Designs. In experimentation, the researcher controls or manipulates the environment of the subjects that constitute the population. The experiments allow a researcher to study the factors of his interest and show that these factors actually cause

certain effects. Hence, whenever the objective is to study the effects of variables rather than simply to describe a population, we prefer the data collection through experimentation. R.A. Fisher developed modern concepts of experimental design in the planning of agricultural field experiments.

15.2. ANALYSIS OF VARIANCE (ANOVA):

The ANOVA is a powerful statistical tool for tests of significance. The test of significance based on t-distribution is an adequate procedure only for testing the significance of the difference between two sample means. In a situation when we have two or more samples to consider at a time, an alternative procedure is needed for testing the hypothesis that all the samples have been drawn from the same population. The term ANOVA was introduced by Prof. R.A. Fisher in 1920's to deal with problem in the analysis of agronomical data. Variation is inherent in nature the total variation in any set of numerical data is due to a number of causes which may be classified as A) Assignable causes: The variation due to assignable causes can be detected and measured B) Chance causes: The variation due to chance causes is beyond the control of humans and cannot be traced separately.

ANOVA Definition:

The ANOVA is a simple arithmetical process of sorting out the components of variation in a given data.

Types of ANOVA:

There are two types i) One-way classification and ii) Two-way classification

I) One-Way Analysis of Variance (ANOVA): It is a statistical test used to determine if there are any statistically significant differences between the means of three or more independent (unrelated) groups. The term "one-way" refers to the fact that the analysis involves only one categorical independent variable (or factor) that has at least three distinct levels (groups). It is an extension of the independent samples t-test, which is limited to comparing only two groups.

1) The Core Purpose: The ANOVA technique is based on comparing two sources of variance to calculate the **F-statistic**.

a) **Null Hypothesis (H0):** The primary assumption is that the means of all groups are equal ($\mu1=\mu2=\mu3=...=\mu k$).

b) **Alternative Hypothesis (HA):** At least one group mean is significantly different from the others (Not all μ's are equal).

The F-Statistic Ratio:

$$F = \frac{\text{Variance Between Groups (Mean Square Between/MSB)}}{\text{Variance Within Groups (Mean Square Error/MSE)}}$$

a) **Between-Group Variance (MSB):** Measures the differences **among** the group means. This variation is attributed to the treatment effect (the factor being studied).

b) **Within-Group Variance (MSE):** Measures the random variation **within** each group (error/unexplained variation). This variation is attributed to random chance or individual differences.

If the calculated F-statistic is large (meaning MSB is much greater than MSE), it suggests that the differences between the group means are larger than what would be expected by chance alone, leading to the rejection of the null hypothesis.

2) **Key Assumptions of One-way ANOVA:** For the results of a One-Way ANOVA to be reliable, the following assumptions about the data must be met:

a) **Continuous Dependent Variable:** The outcome variable (what you measure) must be continuous (interval or ratio scale, e.g., height in cm, weight in grams).

b) **Independence of Observations:** The data points within and across groups must be independent (the measurement from one plant doesn't influence another).

c) **Normality:** The dependent variable should be approximately normally distributed within each of the factor's groups (the residuals should be normally distributed).

d) **Homogeneity of Variance:** The variances (spreads) of the dependent variable across all groups must be approximately equal (tested by Levene's test or Bartlett's test).

3) **Procedure and Post-Hoc Tests**

a) **Calculate the F-statistic:** This involves calculating the Sum of Squares (Total, Between, and Within), Degrees of Freedom (df), Mean Squares (MS), and finally the F-ratio.

b) **Determine the p-value:** The p-value is found using the calculated F-statistic and the corresponding degrees of freedom.

c) **Make a Decision:**

i) If $p \leq \alpha$ (usually $\alpha=0.05$), Reject H0. Conclude that at least two group means are significantly different.

ii) If $p > \alpha$, Fail to Reject H0. Conclude that there is no significant difference between the group means.

1) **Post-Hoc Analysis (If H0 is Rejected):** ANOVA is an omnibus test, it only tells you that a difference exists somewhere, not where it is. If the F-test is significant, a post-hoc test (e.g., Tukey's HSD, Scheffé's test) is necessary to perform pairwise comparisons and identify exactly which specific group means are significantly different from one another.

Example:

The following data represent quantities of tea leaf pluckings (tender shoots from tea plants) from sixteen different plots of tea bushes intended for experimental use in Ceylon, a type of tea from Sri Lanka. The tea bushes are randomly divided into four different treatment groups. Each treatment group contains four tea bushes. The number of tea leaf pluckings for each tea bush is given below.

Treatment 1 Pluckings	**Treatment 2 Pluckings**	**Treatment 3 Pluckings**	**Treatment 4 Pluckings**
88	102	91	88
94	110	109	118
109	105	115	94
88	102	91	96

Experimenters wish to determine if the mean number of pluckings differs among the four treatments. Test this question at the 5% significance level. Assume that these samples are drawn from normal populations with equal variance

Solution:

There are $K = 4$ treatment levels. Each sample contains n = 4 bushes. The alternate hypothesis is that there is a difference in the means among the four treatments, so the null hypothesis is that the mean pluckings are the same.

$H_0 = \mu_1 = \mu_2 = \mu_3 = \mu_4$

H_a: There is difference in the means

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**P-Value**
Treatment	3	213.5	71.1667	0.650667	0.597576
Residual	12	1312.5	109.375		

The F-test statistics of 0.650667 is not significant at the 5% level and the p-value is approximately 0.60, so we fail to reject the null hypothesis. There is not enough evidence to conclude that the tea tree pluckings differ. The data do not support the claim that at least one of treatments has a mean number of pluckings that differs from the other treatments' means.

Biological Applications of One-Way ANOVA:

One-way ANOVA is used when comparing the mean of one dependent variable across three or more groups based on a single factor (independent variable).

1) Comparing growth under different fertilizer treatments
2) Comparing stress-responsive gene expression levels under different salinity levels
3) Comparing photosynthetic rates among different light intensities
4) Comparing root or shoot lengths under different hormone concentrations
5) Comparing yield among multiple plant varieties under same field conditions
6) Comparing Biochemical Constituents plant species exposed to different pollution levels.

II) Two-Way ANOVA:

Two-way Analysis of Variance (Two-way ANOVA) is a statistical technique used to evaluate how two independent factors simultaneously influence a dependent variable. It also determines whether the two factors interact with each other to produce combined effects that differ from their individual effects. Two-way ANOVA is widely used in agricultural, plant physiology, plant breeding, and ecology experiments because plant responses often depend on multiple factors such as genotype, fertilizer type, irrigation level, light intensity, salinity, soil type, or plant density.

When is to use Two-Way ANOVA: Two-way ANOVA can be used during the following conditions.

1) There are **two categorical independent variables (factors)**

   Example: Factor A = Genotype (G1, G2, G3); Factor B = Fertilizer level (Low, Medium, High)

2) The dependent variable is continuous

   Example: Grain yield (kg/plot), plant height (cm), chlorophyll content, root length.

3) You want to measure:
   a) **Main effect of Factor A**
   b) **Main effect of Factor B**
   c) **Interaction effect (A × B)** – whether the effect of one factor depends on the level of the other.

Assumptions for Two-Way ANOVA: **Like other parametric tests, the Two-Way ANOVA requires the following assumptions to be met for valid results:**

1) **Continuous Dependent Variable:** The outcome must be measured on an interval or ratio scale (e.g., yield, height, biomass).

2) **Two Categorical Independent Variables:** Each factor must have two or more levels.
3) **Independence of Observations:** The experimental units (plants/plots) must be independent of each other.
4) **Normality:** The dependent variable should be approximately normally distributed within each cell (treatment combination).
5) **Homogeneity of Variance:** The variances of the dependent variable must be approximately equal across all treatment groups (cells).

Interaction is extremely important in plant studies because genotypes often respond differently to environments or treatments.

Two-Way ANOVA Table Structure:

Source of Variation	Sum of Squares (SS)	Degrees of Freedom (df)	Mean Square (MS)	F-value
Factor A	SSA	$a - 1$	$MSA = SSA/(a - 1)$	MSA/MSE
Factor B	SSB	$b - 1$	$MSB = SSB/(b - 1)$	MSB/MSE
Interaction A × B	SSAB	$(a - 1)(b - 1)$	$MSAB = SSAB/[(a - 1)(b - 1)]$	MSAB/MSE
Error	SSE	$ab(r - 1)$	$MSE = SSE/[ab(r - 1)]$	—
Total	SST	$abr - 1$	—	—

Decision Rule: Reject H0H_0H0 if Fcal > Ftable at $\alpha = 0.05$ or 0.01.

Example:

A botanist wants to know whether or not plant growth is influenced by sunlight exposure and watering frequency. He plants 40 seeds and lets them grow for two months under different conditions for sunlight exposure and watering frequency. After two months, he records the height of each plant. The results are shown below:

	Sunlight Exposure			
Watering Frequency	None	Low	Medium	High
Daily	4.8	5	6.4	6.3
	4.4	5.2	6.2	6.4
	3.2	5.6	4.7	5.6
	3.9	4.3	5.5	4.8
	4.4	4.8	5.8	5.8
Weekly	4.4	4.9	5.8	6
	4.2	5.3	6.2	4.9
	3.8	5.7	6.3	4.6
	3.7	5.4	6.5	5.6
	3.9	4.8	5.5	5.5

In the table above, we see that there were five plants grown under each combination of conditions. For example, there were five plants grown with daily watering and no sunlight and their heights after two months were 4.8 inches, 4.4 inches, 3.2 inches, 3.9 inches, and 4.4 inches:

	Sunlight Exposure			
Watering Frequency	None	Low	Medium	High
Daily	4.8	5	6.4	6.3
	4.4	5.2	6.2	6.4
	3.2	5.6	4.7	5.6
	3.9	4.3	5.5	4.8
	4.4	4.8	5.8	5.8
Weekly	4.4	4.9	5.8	6
	4.2	5.3	6.2	4.9
	3.8	5.7	6.3	4.6
	3.7	5.4	6.5	5.6
	3.9	4.8	5.5	5.5

He Performs a two-Way ANOVA in Excel and ends up with the following output:

G	H	I	J	K	L	M
SUMMARY	None	Low	Medium	High	Total	
Daily						
Count	5	5	5	5	20	
Sum	20.7	24.9	28.6	28.9	103.1	
Average	4.14	4.98	5.72	5.78	5.155	
Variance	0.378	0.232	0.447	0.412	0.775237	
Weekly						
Count	5	5	5	5	20	
Sum	20	26.1	30.3	26.6	103	
Average	4	5.22	6.06	5.32	5.15	
Variance	0.085	0.137	0.163	0.317	0.722632	
Total						
Count	10	10	10	10		
Sum	40.7	51	58.9	55.5		
Average	4.07	5.1	5.89	5.55		
Variance	0.211222	0.18	0.303222	0.382778		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample (Watering)	0.00025	1	0.00025	0.000921	0.975975	4.149097
Columns (Sunlight)	18.76475	3	6.254917	23.04898	3.9E-08	2.90112
Interaction	1.01075	3	0.336917	1.241517	0.310898	2.90112
Within	8.684	32	0.271375			
Total	28.45975	39				

The above table shows the result of the two-way ANOVA. We can observe the following:

a) The p-value for the interaction between watering frequency and sunlight exposure was **0.310898**. This is not statistically significant at alpha level 0.05.

b) The p-value for watering frequency was **0.975975**. This is not statistically significant at alpha level 0.05.

c) The p-value for sunlight exposure was **3.9E-8 (0.000000039)**. This is statistically significant at alpha level 0.05.

These results indicate that sunlight exposure is the only factor that has a statistically significant effect on plant height.

Biological Applications of Two-Way ANOVA:

1) **Ecology and Environmental Biology:** Two-Way ANOVA is crucial for studying how multiple environmental stressors affect organisms or communities.

1) **Application:** Investigating the effect of Pollutant Type and Temperature on the growth rate of algae.
2) **Application:** Studying the impact of Habitat Fragmentation and Predator Presence on the abundance of a specific bird species.

2) Physiology and Molecular Biology: It is used to test the simultaneous effects of drugs, genetic modifications, and environmental conditions on physiological markers.

i) **Application:** Testing the effect of Drug Dosage and Patient Gender on the level of a specific hormone.

ii) **Application:** Analysing how Gene Knockout and Substrate Concentration influence the activity of an enzyme.

3) Agriculture and Crop Science: As seen in previous examples, this is essential for optimizing complex farming strategies.

i) **Application:** Determining the best combination of Cultivar (Variety) and Watering Regime to maximize plant biomass.

4) Behavioural Biology: It helps dissect the roles of innate factors and external stimuli in animal behaviour.

i) **Application:** Assessing the impact of Age and Training Schedule on the learning speed of a laboratory animal.

15.3. CORRELATION:

Correlation is a statistical measure that describes the extent and direction to which two variables are linearly related, meaning they tend to change together at a constant rate. It quantifies the degree of association between the variables using a correlation coefficient, often denoted as r, which ranges from −1 to +1. A value of +1 indicates a perfect positive correlation (both variables increase together), a value of −1 indicates a perfect negative correlation (as one variable increases, the other decreases), and a value of 0 indicates no linear correlation or relationship. It's crucial to remember that a key limitation is that correlation does not imply causation, it only measures how two variables are associated.

Definition:

If the change in one variable affects a change in the other variable, the two variables are said to be correlated and the degree of association ship (or extent of the relationship) is known as correlation. The coefficient of correlation between the two variables *x, y* is generally denoted by *r* or *r xy* or *p(x, y)* or *p.*

Types of Correlation:

Correlation is primarily classified based on three criteria 1. Direction of the relationship, 2. Constancy (form) of the relationship, and 3. Number of variables studied. These are of three types a) Positive correlation b) Negative correlation c) No or Zero correlation (Figure 15.1).

a) Positive Correlation:

If the two variables deviate in the same direction, i.e., if the increase (or decrease) in one variable results in a corresponding increase (or decrease) in the other variable, correlation is said to be direct or positive.

Ex: (i) Heights and weights, (ii) Household income and expenditure, (iii) Amount of rainfall and yield of crops, (iv) Prices and supply of commodities, (v) Feed and milk yield of an animal, (vi) Soluble nitrogen and total chlorophyll in the leaves of paddy.

b) Negative Correlation:

If the two variables constantly deviate in the opposite direction i.e., if increase (or decrease) in one variable results in corresponding decrease (or increase) in the other variable, correlation is said to be inverse or negative.

Ex: (i) Price and demand of a goods, (ii) Volume and pressure of perfect gas, (iii) Sales of woollen garments and the day temperature, (iv) Yield of crop and plant infestation

c) No or Zero Correlation: If there is no relationship between the two variables such that the value of one variable change and the other variable remain constant is called no or zero correlation.

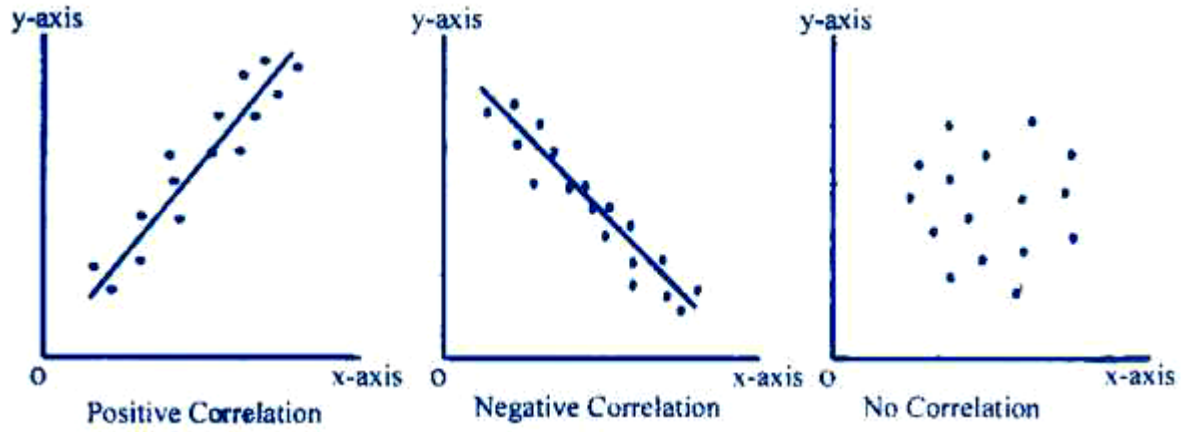


Figure-15.1: Types of Correlations

Methods of Studying Correlation: Correlation can be studied in different methods which includes 1. Scatter Diagram 2. Karl Pearson's Coefficient of Correlation 3. Spearman‟s Rank Correlation 4. Regression Lines.

1) **Scatter Diagram**: It is the simplest way of the diagrammatic representation of bivariate data. Thus for the bivariate distribution (xi,yi); i = j = 1,2,…n, If the values of the variables X and Y be plotted along the X-axis and Y-axis respectively in the xy-plane, the diagram of dots so obtained is known as scatter diagram. From the scatter diagram, if the points are very close to each other, we should expect a fairly good amount of correlation between the variables and if the points are widely scattered, a poor correlation is expected. This method, however, is not suitable if the number of observations is fairly large. If the plotted points show an upward trend of a straight line then we say that both the variables are positively correlated (Figure 15.2).

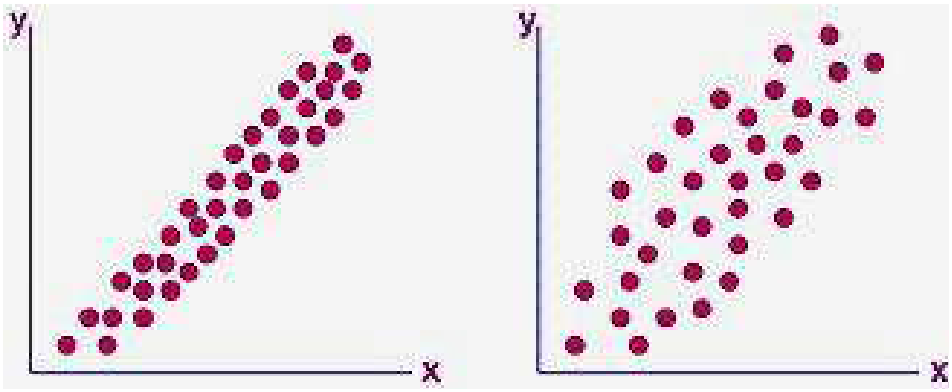


Figure-15.2: Positive Correlation

When the plotted points show a downward trend of a straight line then we say that both the variables are negatively correlated (Figure 15.3).

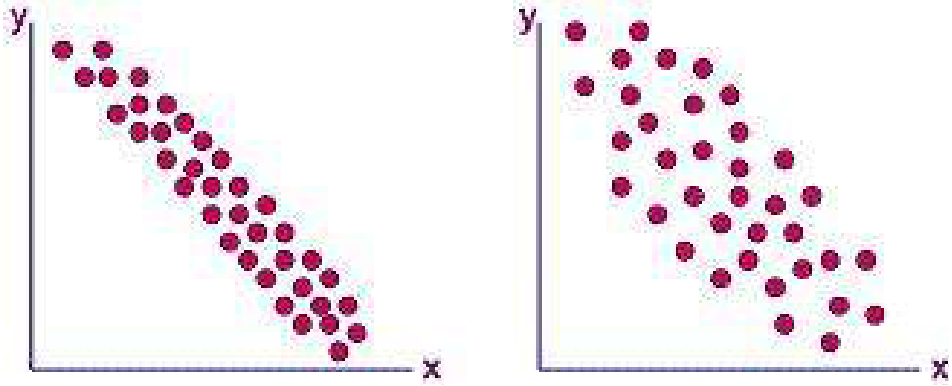


Figure-15.3: Negative Correlation

If the plotted points spread on whole of the graph sheet, then we say that both the variables are not correlated (Figure 15.4).

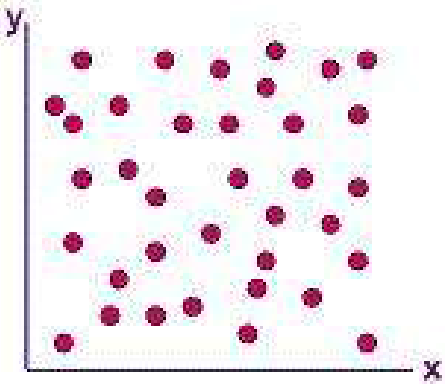


Figure-15.4: No Correlation

Karl Pearson's Coefficient of Correlation:

Prof. Karl Pearson, a British Biometrician suggested a measure of correlation between two variables. It is known as Karl Pearson's coefficient of correlation. It is useful for measuring the degree of linear relationship between the two variables X and Y. It is usually denoted by 'r'.

1. Definition and Mathematical Formulation:

A. Core Concept: Pearson's r measures the degree to which two variables, X and Y, are linearly associated. The sign of r indicates the direction of the relationship:

Positive r: As X increases, Y tends to increase (e.g., height and weight).

Negative r: As X increases, Y tends to decrease (e.g., training time and race finish time).

Strength: The magnitude (absolute value) of r indicates how closely the data points cluster around a straight line.

B. Defining Formula:

Pearson's r is defined as the ratio of the covariance of the two variables to the product of their individual standard deviations.

$$r = \frac{\text{Covariance}(X,Y)}{\text{SD}(X) \cdot \text{SD}(Y)} = \frac{\sum_{i=1}^{n}(X_i - \bar{X})(Y_i - \bar{Y})}{\sqrt{\sum_{i=1}^{n}(X_i - \bar{X})^2 \sum_{i=1}^{n}(Y_i - \bar{Y})^2}}$$

Covariance Numerator: Measures the joint variability of X and Y. If X and Y move in the same direction, the products $((Xi - \bar{X})(Yi - \bar{Y}))$ are positive, resulting in positive covariance.

Standard Deviation Denominator: Standardizes the covariance, ensuring that r is a unitless measure, allowing for comparison across different datasets.

C. Simplified (Computational) Formula: For practical calculation, the formula using raw scores is often more convenient:

$$r = \frac{n\sum XY - (\sum X)(\sum Y)}{\sqrt{[n\sum X^2 - (\sum X)^2][n\sum Y^2 - (\sum Y)^2]}}$$

2. Interpretation of the Coefficient (r):

A. Range of r: The value of r always ranges from **-1 to +1**.

Value of r	Interpretation
+1.0	Perfect positive linear correlation.
0.8 to 1.0	Very strong positive correlation.
0.5 to 0.8	Strong positive correlation.
0.3 to 0.5	Moderate positive correlation.
0.0 to 0.3	Weak or negligible correlation.
0.0	No linear correlation.
-0.3 to -0.5	Moderate negative correlation.
-1.0	Perfect negative linear correlation.

B. Coefficient of Determination (r2): reporting r^2 is crucial as it has a direct practical interpretation. r^2 = (Pearson's r)^2 and r^2 represents the **proportion of the variance** in the dependent variable (Y) that is statistically predictable from the independent variable (X).

Eg: If r = 0.70, then r^2 = 0.49. This means 49% of the variance in Y is accounted for by the linear relationship with X.

Problem: Relationship between Nitrogen level and Plant Height

Nitrogen (kg/ha)	Plant height (cm)
50	120
60	130
70	150
80	160
90	175

Compute Pearson’s Correlation.

Step 1: Prepare table

X	Y	X^2	Y^2	XY
50	120	2500	14400	6000
60	130	3600	16900	7800
70	150	4900	22500	10500
80	160	6400	25600	12800
90	175	8100	30625	15750

Step 2: Summations

$\Sigma X = 350$

$\Sigma Y = 735$

$\Sigma X^2 = 25500$

$\Sigma Y^2 = 110,025$

$\Sigma XY = 52,850$

$n = 5$

Step 3: Apply Formula

$$r = \frac{n\sum xy - (\sum x)(\sum y)}{\sqrt{[n\sum x^2 - (\sum x)^2]\,[n\sum y^2 - (\sum y)^2]}}$$

Compute Numerator:

$$5(52850) - (350)(735)$$

$$= 264250 - 257250 = 7000$$

Compute Denominator:

$$\sqrt{[5(25500) - 350^2][5(110025) - 735^2]}$$
$$= \sqrt{(127500 - 122500)(550125 - 540225)}$$
$$= \sqrt{5000 \times 9900}$$
$$= \sqrt{49,500,000} = 7035.0$$

Thus:

$$r = \frac{7000}{7035} = 0.995$$

Interpretation:

r = **0.995** → extremely strong positive correlation

Thus, plant height increases strongly with nitrogen level.

Testing Significance of r: To test whether the correlation is significantly different from zero, use:

$$t = r\sqrt{\frac{n-2}{1-r^2}}$$

df = n – 2

Example Applied:

$$t = 0.995\sqrt{\frac{3}{1-0.990025}} \approx 17.2$$

This is highly significant at **P < 0.01**.

Uses and Applications of Pearson's r:

Karl Pearson's Coefficient of Correlation (r) is widely used in statistics and research across numerous fields, particularly where the relationship between two continuous variables needs to be quantified.

1) **Quantifying Linear Association:** The most fundamental use is to determine the strength and direction of the linear relationship between two numerical variables.
2) **Validation and Reliability Assessment:** In psychometrics and measurement science, Pearson's r is crucial for evaluating the quality of tests and instruments.
3) **Data Reduction and Diagnostics:** Pearson's r helps inform decisions about data structure and modelling.

4) **Screening and Hypothesis Generation:** In exploratory data analysis (EDA), calculating r across many variables helps researchers prioritize which relationships to investigate further.

5) **Basis for Advanced Statistical Techniques:** Pearson's r is a foundational concept that forms the basis for several more complex statistical methods i.e. Simple Linear Regression, Factor Analysis and Structural Equation Modelling (SEM), Time Series Analysis.

15.4. LINEAR REGRESSION:

Regression analysis is a powerful set of statistical techniques used for modelling and analysing the relationship between a dependent variable and one or more independent variables (the predictors, or regressors, X). The term regression was first used by Sir Francis Galton in study of heredity. The term `regression‟ literally means “stepping back towards the average. The relationship between the independent and dependent variables is termed as regression. In regression analysis independent variable is also known as regressor or predictor or explanatory variable while dependent variable is also known as regressed or explained variable. When only two variables are involved the functional relationship is known as simple regression. If the relationship between two variables is a straight line, it is known as simple linear regression; otherwise it is called as simple non-linear regression.

Types of Regression:

1) Simple Linear Regression (one independent variable)
2) Multiple Linear Regression (two or more independent variables)
3) Nonlinear Regression
4) Polynomial Regression

1) Simple Linear Regression (SLR):

Simple linear regression studies the relationship between one dependent variable (Y) and one independent variable (X) with the assumption that the relationship is linear. The SLR s useful to know the dose response studies, growth prediction and trait environment relationships.

$$\mathbf{Y=a+bX+\varepsilon}$$

a = intercept

b = slope (change in Y for one unit change in X)

ε = error term

2) Multiple Linear Regression (MLR):

Multiple regression uses One dependent variable (Y) and Two or more independent variables (X_1, X_2, …, X_n). Biological traits are typically influenced by multiple factors. The MLR is used to analyse the multinutrient optimization, growth/biomass modelling and trait prediction in breeding programs.

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \cdots + \beta_p x_p + \varepsilon$$

Where:

y = dependent (response) variable

x1, x2,…,xp = independent (predictor) variables

β0 = intercept

β1, β2,…,βp = regression coefficients for each predictor

p = number of predictors

ε = random error term

3) Nonlinear Regression:

Used when the relationship between X and Y is not linear. Model takes nonlinear mathematical forms, e.g., exponential, logistic, power models. The NLR is used for plant growth modelling, to analyse the enzyme activity in plant tissues, dose–response curves and population dynamics of pests/pathogens.

$$y = f(x_1, x_2, \ldots, x_p; \beta_0, \beta_1, \ldots, \beta_m) + \varepsilon$$

Where:

y = dependent (response) variable

x1,x2,…,xp = independent (predictor) variables

β0,β1,…,βm = model parameters to be estimated

f = a non-linear function of parameters and predictors (e.g., exponential, logarithmic, power, polynomial of degree >1, logistic, etc.)

ε = random error term

4) Polynomial Regression:

When the relationship is curved but can be modeled by a polynomial. This model is used for curvilinear trends that simple linear regression cannot capture. This regression useful to analyse the growth curves, environmental gradients and germination/physiology responses.

$$y = \beta_0 + \beta_1 x + \beta_2 x^2 + \beta_3 x^3 + \cdots + \beta_n x^n + \varepsilon$$

Where:

y = dependent (response) variable

x = independent (predictor) variable

$\beta 0, \beta 1, \ldots, \beta n$ = regression coefficients (parameters)

n = degree of the polynomial

ε = random error term

Biological Applications of Linear Regression:

Linear regression is widely used in biology to analyse how one biological variable depends on another. It helps quantify relationships, make predictions, and understand biological responses under controlled or natural conditions.

1) Predicting yield from nutrient levels.
2) Relationship between plant height and time, or biomass accumulation over time.
3) Photosynthetic rate against light intensity (irradiance).
4) Relationship between species diversity and environmental gradients (e.g., rainfall, soil pH).
5) Predicting population size from resource availability.
6) Predicting gene expression levels using concentration of transcription factors.
7) Response of cells or tissues to increasing concentrations of a drug or hormone.
8) Relation between reaction rate and substrate concentration at low levels.

15.6. EXPERIMENTAL DESIGNS:

In agricultural experiments, most of the times we divide the whole experimental unit (field) into relatively homogeneous sub-groups or strata. These strata, which are more uniform amongst themselves than the field as a whole, are known as blocks.

Treatments: the objects of comparison in an experiment are defined as treatments.

For Example:

a) If different fertilizers are tried in an experiment to test the responses of a crop to the fertilizer doses, the different doses will be treatments and each dose will be a treatment.

b) A doctor treats a patient with a skin condition with different creams to see which is most effective.

Experimental Unit:

Experimental unit is the object to which treatment is applied to record the observations.

For Example:

a) In laboratory insects may be kept in groups of five or six. To each group, different insecticides will be applied to know the efficacy of the insecticides. In this study different groups of insects will be the experimental unit.

b) If treatments are different varieties, then the objects to which treatments are applied to make observations will be different plot of land. The plots will be called experimental units.

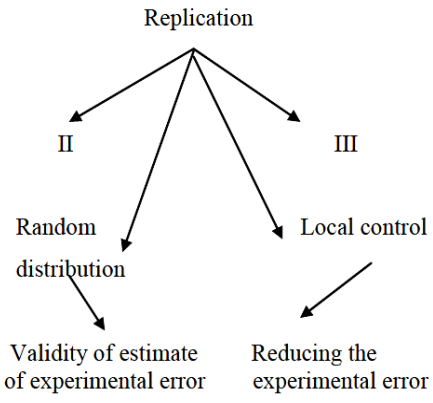
Basic Principles of Experimental Designs:

The purpose of designing an experiment is to increase the precision of the experiment. The basic principles of the experimental designs are replication, randomization and local control.

1) **Replication:** Repetition of treatment to different experimental units is known as Replication. A replication is used (i) to secure more accurate estimate of the experimental error, a term which represents the differences that would be observed if the same treatments were applied several times to the same experimental units (ii) to reduce the experimental error and thereby to increase precision, which is a measure of the variability of the experimental error.

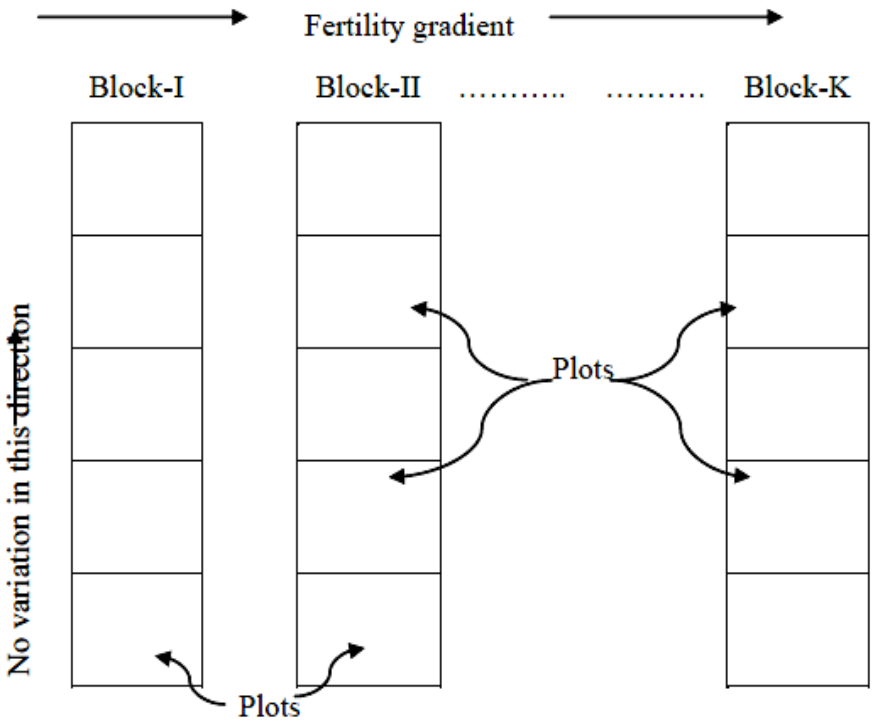
2) **Randomization:** When all the treatments have equal chances of being allocated to different experimental units it is known as randomization. The purpose of randomization is to remove bias and other sources of extraneous variation which are not controllable. Another advantage of randomization (accompanied by replication) is that it forms the basis of any valid statistical test. Hence the treatments must be assigned at random to the experimental units.

3) **Local control:** It has been observed that all extraneous sources of variation are not removed by randomization and replication. This necessitates a refinement in the experimental technique. The main purpose of the principle of local control is to increase the efficiency of an experimental design by decreasing the experimental error.



Shape of Blocks and Plots:

The shape and size of the blocks will usually depend up on the shape and size of the plots. In order to control the experimental error, it is desirable to divide the whole experimental area into different subgroups (blocks) such that within each block there is as much homogeneity as possible but between blocks there is maximum variation. Further each block is to be divided into as many plots as the number of treatments. For maximum precision the plots should be rectangular in shape with their long sides parallel to the direction of the fertility gradient and the blocks should be arranged one after the other along the fertility gradient.



A Randomized Block Design is an experimental design in which the experimental units are arranged into blocks based on a known source of variability, and within each block, the treatments are assigned randomly. Each block contains all treatments exactly once. Blocks represent homogeneous groups. Treatment effects are evaluated after removing block-to-block variation.

Randomized Block Design (RBD):

In agricultural field experiments, usually the experimental materials are not homogeneous. In such situations the principle of local control is adopted and the experimental material is grouped into homogeneous sub groups. The subgroup is commonly termed as block. Since each block consist the entire set of treatments a block is equivalent to a replication. The blocks are formed with units having common characteristics which may influence the response under study. In agricultural field experiments the soil fertility is an important character that influences the crop responses. The uniformity trial is used to identify

the soil fertility of a field. If the fertility gradient is found to run in one direction (say from north to south) then the blocks are formed in the opposite direction (from east to west). If the number of experimental units within each group is same as the number of treatments and if every treatment appears precisely once in each group, then such an arrangement is called a randomized block design.

RBD is Most Suitable When

1) Experimental units show variability due to factors like soil type, temperature, light intensity, or moisture.
2) The source of variation is known and controllable only by grouping (not by equalizing).
3) The number of treatments is moderate (commonly between 3–10).
4) Blocking factor has a strong influence on the response.

Key Terms of RBD:

a) **Factor (Treatment):** The independent variable whose effect you want to test (e.g., Fertilizer type, Drug dosage).

b) **Block:** A group of experimental units that are homogeneous (similar) with respect to some characteristic that is likely to affect the outcome (e.g., Soil type, Sunlight exposure, Age).

c) **Randomization:** Each treatment is assigned randomly to the experimental units within each block.

d) **Replication:** Each treatment appears exactly once in every block. The blocks themselves serve as the replicates for the experiment.

Layout and Structure: **The design is fundamentally a Two-Way ANOVA without replication within the main cells, as the block itself is not the factor of interest, but rather a source of variation to be accounted for.**

i) The number of Blocks (r) equals the number of replications for each treatment.

ii) The number of Treatments (t) equals the number of experimental units in each block.

iii) Total Experimental Units = r×t.

Block 1 (High Soil Quality)	**Treatment A**	**Treatment B**	**Treatment C**
Block 2 (Medium Soil Quality)	Treatment C	Treatment A	Treatment B
Block 3 (Low Soil Quality)	Treatment B	Treatment C	Treatment A

Statistical Analysis (ANOVA Model):

The data from an RBD is analyzed using a Two-Factor ANOVA (without interaction). The total variation (Sum of Squares Total, SSTotal) is partitioned into three main components:

Source of Variation $\rightarrow SS_{Total} = SS_{Treatments} + SS_{Blocks} + SS_{Error}$

Source of Variation	Degrees of Freedom (df)	Mean Square (MS)	F-ratio
Treatments	t - 1	$MS_{Treatments} = SS_{Treatments} / df_{Treatments}$	$F = MS_{Treatments} / MS_{Error}$
Blocks	r - 1	$MS_{Blocks} = SS_{Blocks} / df_{Blocks}$	(Tested if blocks are effective)
Error	(t - 1)(r - 1)	$MS_{Error} = SS_{Error} / df_{Error}$	
Total	rt - 1		

The primary test is the F-ratio for Treatments.

i) **Hypothesis:** H0: There is no significant difference between the treatment means.

ii) **Decision:** If $F_{Treatments}$ is large and the resulting p-value $\leq \alpha$ (0.05), we reject H0 and conclude that the treatments have a significant effect.

Advantages and disadvantages of RBD:

1) The principle advantage of RBD is that it increases the precision of the experiment. This is due to the reduction of experimental error by adoption of local control.

2) The amount of information obtained in RBD is more as compared to CRD. Hence, RBD is more efficient than CRD.

3) Flexibility is another advantage of RBD. Any number of replications can be included in RBD. If large number of homogeneous units is available, large number of treatments can be included in this design.

4) Since the layout of RBD involves equal replication of treatments, statistical analysis is simple. Even when some observations are missing of certain treatments, the data can be analysed by the use of missing plot technique.

5) When the number of treatments is increased, the block size will increase. If the block size is large it may be difficult to maintain homogeneity within blocks. Consequently, the experimental error will be increased. Hence, RBD may not be suitable for large number of treatments. But for this disadvantage, the RBD is a versatile design. It is the most frequently used design in agricultural experiments.

6) The optimum blocks size in field experiments is 21 plots. i.e. we cannot compare treatments which are > 21 in RBD to preserve homogeneity of plots, within a block.

Completely Randomized Design (CRD):

The CRD is the simplest of all the designs. In this design, treatments are allocated at random to the experimental units over the entire experimental material. In case of field experiments, the whole field is divided into a required number of plots equal sizes and then the treatments are randomized in these plots. Thus the randomization gives every experimental unit an equal probability of receiving the treatment. In field experiments there is generally large variation among experimental plots due to soil heterogeneity. Hence, CRD is not preferred in field experiments. In laboratory experiments and green house studies, it is easy to achieve homogeneity of experimental materials. Therefore, CRD is most useful in such experiments.

Key Features of CRD:

1) **Homogeneity:** The CRD assumes that all experimental units are uniform and that any systematic variation (like a temperature gradient or soil difference) is negligible or non-existent.
2) **Flexibility in Replication:** The number of replications (experimental units) in each treatment group does not have to be equal (though equal replication is statistically preferred for simplicity and power).
3) **Simplicity:** Both the planning of the experiment and the statistical analysis are the easiest among all basic designs.

When to Use CRD: CRD is appropriate under the following situations.

a) Experimental material is highly uniform (e.g., identical pots, uniform seedlings, controlled laboratory conditions).

b) The number of treatments is small or moderate.

c) The experiment is conducted on individual plants**,** petri dishes**,** test tubes, or pots under homogeneous conditions.

d) The main purpose is to test treatment effects, not interactions.

CRD is not suitable for field experiments where heterogeneity (soil variation, light variation) is high.

Layout of CRD:

The placement of the treatments on the experimental units along with the arrangement of experimental units is known as the layout of an experiment.

For Example:

Suppose that there are 5 treatments A, B, C, D and E each with 4 replications. We need 20 experimental units. Here, since the number of units is 20, a two-digit random number of table will be consulted and a series of 20 random numbers will be taken excluding those which are greater than 20. Suppose, the random numbers are 4, 18, 2, 14, 3, 7, 13, 1, 6, 10, 17, 20, 8, 15, 11, 5, 9, 12, 16, 19. After this the plots will be serially numbered and the treatment A will be allotted to the plots bearing the serial numbers 4, 18, 2, 14 and so on.

Statistical Analysis (One-Way ANOVA):

The data from a CRD is analyzed using a **One-Way Analysis of Variance (ANOVA)**. The total variation (SS_{Total}) in the experiment is partitioned into two major components.

Source of Variation → $SS_{Total} = SS_{Treatments} + SS_{Error}$

i) **$SS_{Treatments}$ (Between-Groups Variation):** This measures the variation in the data that is **attributable to the treatments** (the factor of interest).

ii) **SS_{Error} (Within-Groups Variation):** This measures the variation within the groups that cannot be explained by the treatments. It includes all random errors, measurement errors, and any inherent differences among the experimental units.

Source of Variation	Degrees of Freedom (df)	Mean Square (MS)	F-ratio
Treatments	t - 1	$MS_{Treatments} = SS_{Treatment} / df_{Treatments}$	$F = MS_{Treatments} / MS_{Error}$
Error	N - t	$MS_{Error} = SS_{Error} / df_{Error}$	
Total	N - 1		

Where N is the total number of experimental units and t is the number of treatments. The resulting F-ratio tests the null hypothesis (H0) that all treatment means are equal.

Advantages and Disadvantages of CRD:

1) This design is most commonly used in laboratory experiments such as in agriculture, chemistry, plant pathology, and animal experiments where the experimental material is expected to be homogeneous.

2) This design is useful in pot cultural experiments where the same type of soil is usually used. However, in greenhouse experiments care has to be taken with regard to sunshade, accessibility of air along and across the bench before conducting the experiment.

3) Any number of replications and treatments can be used. The number of replications may vary from treatment to treatment.

4) The analysis remains simple even if information on some units are missing.

5) This design provides maximum number of degrees of freedom for the estimation of error than the other designs.

6) The only drawback with this design is that when the experimental material is heterogeneous, the experimental error would be inflated and consequently the treatments are less precisely compared. The only way to keep the experimental error under control is to increase the number of replications thereby increasing the degrees of freedom for error.

Applications:

1) CRD is most useful in laboratory technique and methodological studies.

   Ex: in physics, chemistry, in chemical and biological experiments, in some greenhouse studies etc.

2) CRD is also recommended in situations where an appreciable fraction of units is likely to be destroyed or fail to respond.

15.6. SUMMARY:

Analysis of Variance (ANOVA) is a statistical method in biological sciences, commonly applied to evaluate the effect of treatments such as fertilizers, genotypes, environmental conditions, or doses on plant or animal responses. Correlation is a statistical measure that describes the strength and direction of the linear relationship between two quantitative variables. The correlation coefficient (r), ranging from –1 to +1, quantifies how closely changes in one variable are associated with changes in another. In biology, correlation analysis is widely used to study relationships such as plant height vs. biomass, nutrient level vs. yield, disease severity vs. environmental conditions, or gene expression vs. phenotype. A strong positive or negative correlation indicates a meaningful biological association, while a weak correlation suggests little to no linear relationship. Linear regression is a statistical technique used to model and predict the relationship between a dependent variable and one (simple regression) or multiple (multiple regression) independent variables. It estimates how much the dependent variable changes in response to variations in the predictors, providing an equation of the best-fit line based on least squares. In biological research, linear regression is applied to quantify relationships such as fertilizer doses vs. crop yield, environmental factors vs. growth rate, gene expression vs. trait performance, or enzyme concentration vs. reaction velocity.

Randomized Block Design (RBD) is an experimental design used to control the effects of variability among experimental units by grouping them into blocks that are relatively homogeneous. Each block contains all the treatments, and treatments are randomly assigned within each block. By comparing treatment means after accounting for block differences, RBD provides more precise estimates of treatment effects than Completely Randomized Design (CRD). A Complete Randomized Design (CRD) is the simplest and

most fundamental experimental design used in biological and agricultural research. In this design, treatments are assigned entirely at random to all experimental units, ensuring that each unit has an equal chance of receiving any treatment. CRD is most appropriate when the experimental units (such as pots, plants, seedlings, or soil samples) are homogeneous, meaning they exhibit minimal natural variation. CRD is commonly applied in laboratory experiments, growth chamber studies, and greenhouse trials where environmental variability is minimal. However, CRD is less efficient in field studies where natural variability is high, as it does not control for external sources of variation.

15.7. TECHNICAL TERMS:

Degrees of freedom (df), One-way ANOVA, Two-way ANOVA, Correlation coefficient (r), Pearson's correlation, Covariance, Positive and negative correlation, RBD, CRD.

15.8. SELF ASSESSMENT QUESTIONS:

1) What is the difference between correlation and regression?
2) Explain the principle of one-way ANOVA with an example from plant science.
3) Describe the steps involved in performing two-way ANOVA and interpret interaction effects.
4) Explain different types of correlation with suitable biological examples.
5) Explain the structure, assumptions, and statistical model of Randomized Block Design.
6) Provide a plant science example.
7) Explain the principle, layout, and analysis of Completely Randomized Design.

15.9. SUGGESTED READINGS:

1) Rosner, B. (2015). Fundamentals of Biostatistics (8^{th} ed.). Cengage Learning.
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