MICROBIOLOGY, MYCOLOGY AND PLANT DISEASES

(DBOT22)

(MSC BOTONY)

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Unit-I

Lesson 1

BACTERIA

1.0 **OBJECTIVE:**

The morphology, ultra structure of the bacterial cell and outlines of Bergey's classification of bacteria are discussed in this lesson.

1.1 INTRODUCTION
1.2 ULTRASTRUCTURE OF THE BACTERIAL CELL
1.2.1 Cell Wall
1.2.2 Components external to the cell wall
1.2.3 The Plasma membrane
1.2.4 The cytoplasmic matrix
1.2.5 The Nucleoid
1.2.6 Endospores
1.3 OUTLINES OF BERGEY'S CLASSIFICATION OF BACTERIA
1.4 SUMMARY
1.5 MODEL QUESTION PAPERS
1.6 REFERENCE BOOKS

1.1 INTRODUCTION

Bacteria are small and simple in structure when compared with eucaryotes, yet they often have characteristic shapes and sizes. Most commonly encountered bacteria have one of two shapes. **Cocci** are roughly spherical cells. They can exist as individual cells or associate in characteristic arrangements that are frequently useful in bacterial identification. When cocci divide and remain together to form pairs, they are called *Diplococci (Neisseria)*.

In *Streptococci*, **cocci** are arranged in long chains (*Streptococcus, Enterococcus* and *Lactococcus*). *Staphylococci* divide in random planes to generate irregular grape like clumps (*Staphylococcus*). Divisions in two or three planes can produce symmetrical clusters of cocci. *Micrococcus* often divides in two planes to form square groups of four cells called tetrads. In the genus *Sarcina*, cocci divide in three planes producing cubical packets of eight cells.

The other common bacterial shape is that of a rod, often called a *Bacillus*. *Bacillus megaterium* is a typical example of a bacterium with a rod shape. The shape of the rod's end often varies between species and may be flat, rounded, cigar-shaped or bifurcated. Although many rods do occur singly, they may remain together after division to form pairs or chains. Bacilli differ considerably in their length-to-width ratio. The coccobacilli are so short and wide that they

resemble cocci. A few rod-shaped bacteria, the *vibrios*, are curved to form distinctive commas (Fig. 1.1).

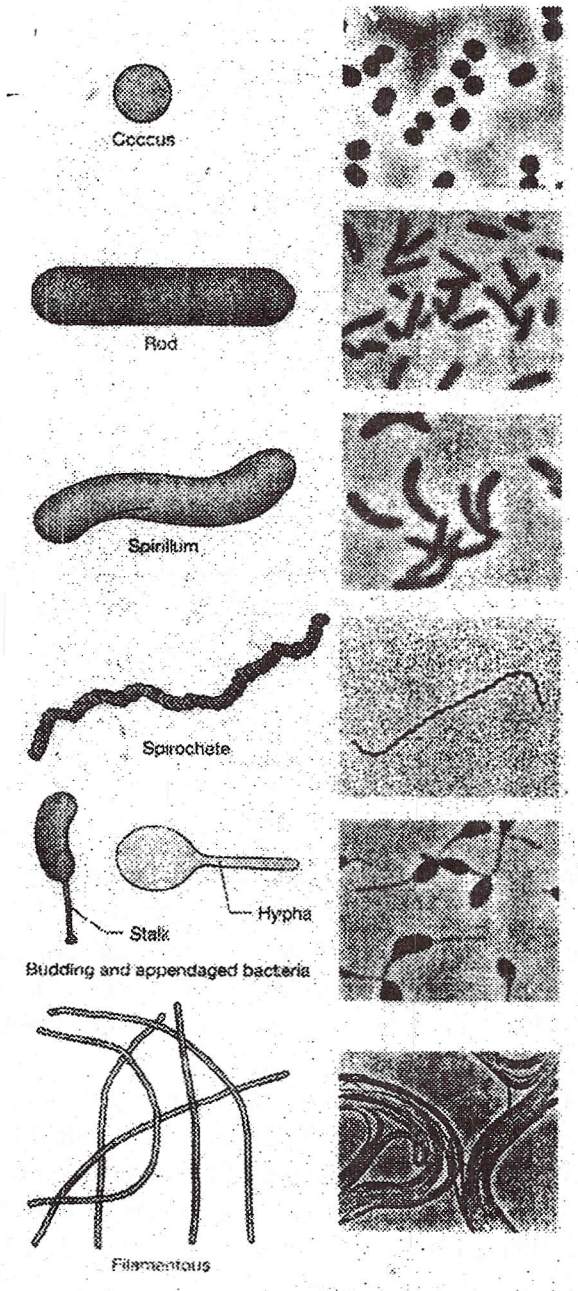

Fig. 1.1 Representative cell shapes (morphology) in prokary

Fig. 1.1 Representative cell shapes (morphology) in prokaryotes

Bacteria can assume a great variety of shapes, although they often are simple spheres or rods. Actinomycetes characteristically form long multinucleate filaments or hyphae that may branch to produce a network called a *mycelium*. Many bacteria are shaped like long rods twisted into spirals or helices; they are called *spirilla* if rigid and *spirochetes* when flexible.

Appendaged bacteria possess extensions of their cells as long tubes or stalks. The oval-to-pear-shaped *Hyphomicrobium* produces a bud at the end of a long hypha. Other bacteria such as *Gallionella* produce stalks. Finally, some bacteria are variable in shape and these are called pleomorphic.

Bacteria vary in size from cells as small as 0.1 – 0.2 μm in diameter. A few very large procaryotes, such as the surgeon fish symbiont *Epulopiscium fischelsoni* are μp to 50 μm in diameter and can be more than 0.5 millimeters in length. In 1997, Heidi Schulz discovered an even larger procaryote in the ocean sediment off the coast of Namibia. *Thiomargarita namibiensis* is a spherical bacterium, between 100 and 750 μm in diamter, that often forms chains of cells. It is over 100 times larger in volume than *E. fishelsoni*.

The smallest bacteria (some members of the genus *Mycoplasma*) are about 0.3 μm in diameter, approximately the size of the largest virus (the poxviruses). Recently there have been reports of even smaller cells. Nannobacteria or ultramicrobacteria appear to range from around 0.2 μm to less than 0.05 μm in diameter. However, the dimensions of an average rod-shaped bacterium, *Escherichia coli* are about 1 x 3 μm.

1.2 Ultrastructure of bacterial cell

All bacteria are procaryotes and much simpler structurally than eucaryotes. Procaryotic cells almost always are bounded by a chemically complex cell wall. Inside this wall, and separated from it by a periplasmic space, lies the plasma membrane. This membrane can be invaginated to form simple internal membranous structures. Since the procaryotic cell does not contain internal membrane – bound organalles, its interior appears morphologically simple. The genetic material is localized in a discrete region, the nucleoid. It is not separated from the surrounding cytoplasm by membranes. Ribosomes and inclusion bodies are scattered in the cytoplasmic matrix. Some bacteria can use flagella for locomotion. In addition, many cells are surrounded by a capsule or slime layer external to the cell wall (Fig. 1.2).

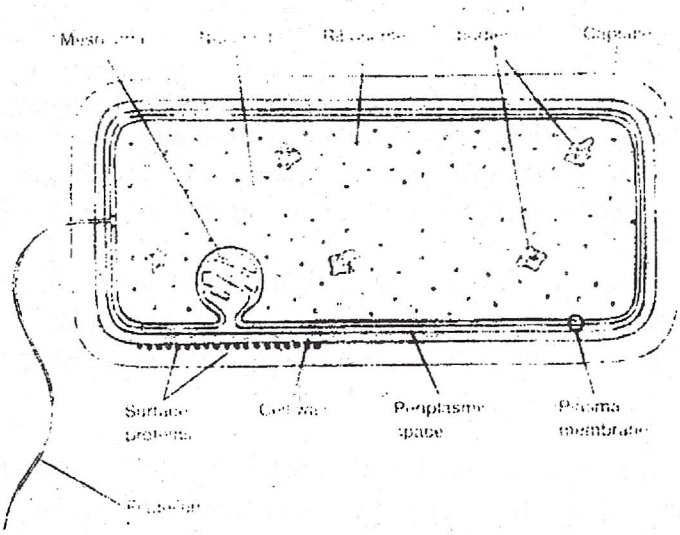

Fig. 1.2 Structure of a procaryotic cell

1.2.1 Cell wall

The cell wall is one of the most important parts of a procaryotic cell. Except for the mycoplasmas and some archaeobacteria, most bacteria have strong walls that give them shape and protect them from osmotic lysis. The cell walls of many pathogens have components that contribute to their pathogenicity. The wall can protect a cell from toxic substances and is the site of action of several antibiotics.

Bacteria can be divided into two major groups, called **gram-positive** and **gram-negative** based on their response to the Gram – stain procedure. After Gram staining, gram – positive bacteria appear purple and gram-negative bacteria appear red. This difference in reaction to the gram stain arises because of differences in the cell wall structure of gram-positive and gram-negative cells (Fig. 1.3).

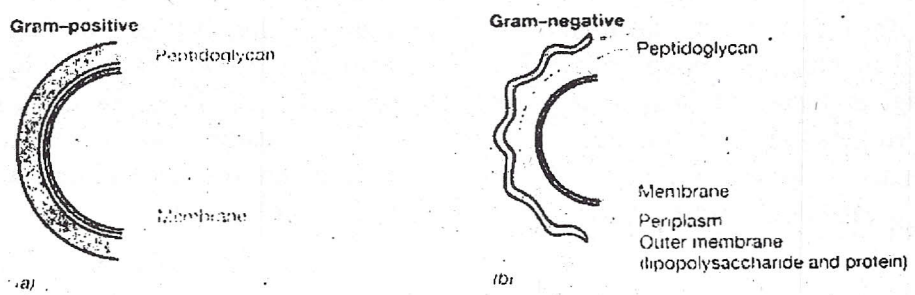

Fig. 1.3 Schematic diagram of (a) gram-positive and (b) gram-negative cell walls

The gram-positive cell wall consists of a single 20 to 80 nm thick homogeneous **peptidoglycan** or **murein** layers lying outside the plasma membrane. In contrast, the gram-negative cell wall is quite complex. It has a 2 to 7 nm peptidoglycan layer surrounded by a 7 to 8 nm thick outer membrane (envelope). Peptidoglycan is an enormous polymer composed of two sugar derivatives, N-acetylglucosamine and N-acetylmuramic acid (the lactyl ether of N-acetyl-glucosamine) and a small group of amino acids consisting of L-alanine, D-alanine, D-glutamic acid and either lysine or diaminopimelic acid. The peptidoglycan subunit present in most bacteria is shown in Fig. 1.4.

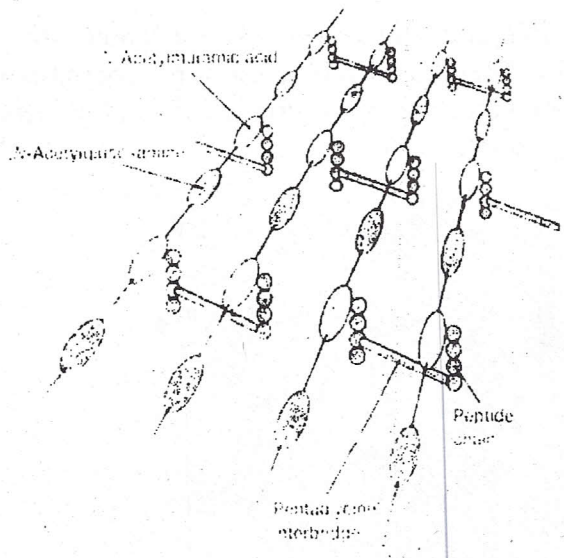

Fig. 1.4 Schematic diagram of Peptidoglycan structure

The backbone of peptidoglycan is composed of alternating N-acetylglucosamine and N-acetylmuramic acid residues. A peptide chain of four alternating D- and L- amino acids is connected to the carboxyl group of N-acetyl muramic acid. Chains of linked peptidoglycon subunits are joined by cross-links between the peptides.

In gram-positive bacteria, as much as 90% of the cell wall consists of peptidoglycan, although another kind of constituent, teichoic acid is usually present in small amounts. In gram-negative bacteria only about 10% of the wall is peptidoglycan. The outer membrane made of lipopolysaccharides lies outside the thin peptidoglycan layer.

1.2.2 Components external to the cell wall

Bacteria have a variety of structures outside the cell wall that function in protection, attachment to objects or cell movement. Several of these are discussed.

Capsules, slime layers and S-layers:

Some bacteria have a layer of material lying outside the cell wall. When the layer is well organized and not easily washed off, it is called a **capsule**. A **slime layer** is a zone of diffuse, unorganized material that is removed easily. A glycocalyx is a network of polysaccharides extending from the surface of bacteria and other cells (in this sense it could encompass both capsules and slime layers). Capsules and slime layers are composed of glycoproteins and different polysaccharides including polyalcohols and amino sugars.

Capsules are clearly visible in the light microscope when negative stains or special capsule strains are employed (Fig. 1.5). They help bacteria resist phagocytosis by host phagocytic cells. The glycocalyx also aids bacterial attachment to surfaces of solid objects in aquatic environments or to tissue surfaces in plant and animal hosts.

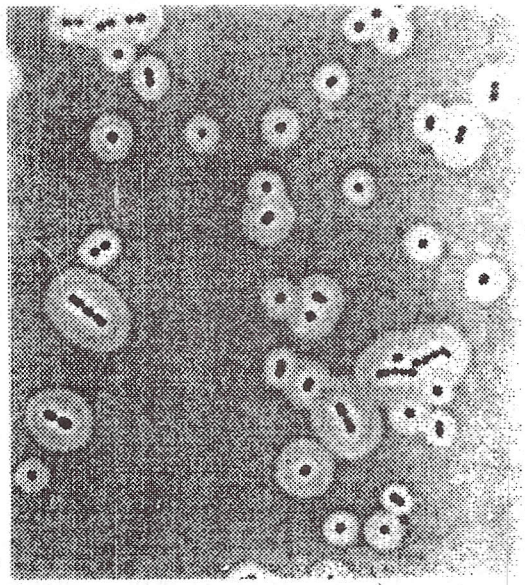

Fig. 1.56 Bacterial capsules

Many gram-positive and gram-negative bacteria have a regularly structured layer called an S-layer on their surface. It is composed of protein or glycoprotein. It may protect the cell against ion and pH fluctuations, osmotic stress, enzymes,or the predacious bacterium *Bdellovibrio*. It can promote cell adhesion to surfaces.

Fimbriae and Pili

Many gram-negative bacteria have short, fine, hair like appendages that are thinner than flagella and not involved in motility. These are usually called *Fimbriae*. They are visible only in

an electron microscope due to their small size. A cell may be covered with upto 1,000 fimbriae. Some types of fimbriae attach bacteria to solid surfaces such as rocks in streams and host tissues.

Pili are structurally similar to fimbriae but are generally longer, and only one or a few pili are present on the surface. Six pili are involved in the process of conjugation.

Flagella

Most motile bacteria move by use of flagella, thread like locomotor appendages extending outward from the plasma membrane and cell wall. Flagella are arranged differently in different bacteria (Fig. 1.6). Monotrichous bacteria have one flagellum; if it is located at an end, it is said to be a polar flagellum. *Amphitrichous* bacteria have a single flagellum at each pole. *Lophotrichous* forms have a cluster of flagella at one end. Flagella are spread fairly evenly over the whole surface of *Peritrichous* bacteria. Flagellation patterns are very useful in identifying bacteria.

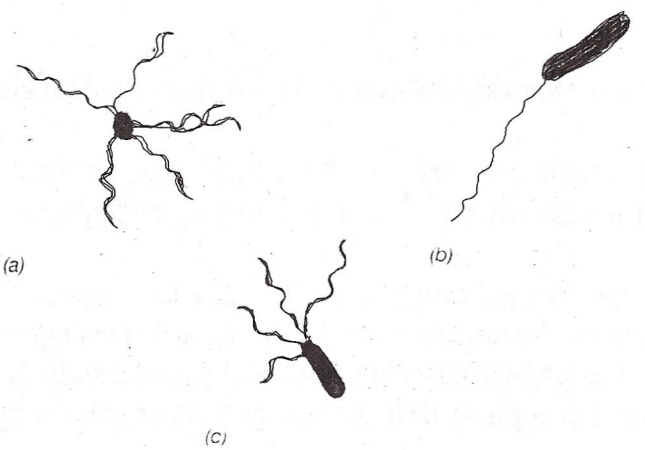

Fig. 1.6 Flagellar distribution a) Peritrichous b) monotrichous c) Lophotrichous

The bacterial flagellum is composed of three parts: (1) The longest and most obvious part is the **filament** which extends from the cell surface to the tip. (2) A **basal body** is embedded in the cell and (3) a short, curved segment, the **hook**, links the filament to the basal body and acts as a flexible coupling. The filament is a hollow, rigid cylinder constructed of a single protein called **flagellin**, which ranges in molecular weight from 30,000 to 60,000. The flagellar filament rotates like a propeller to push the bacterium through the water.

The hook is slightly wider than the filament and is made of different protein subunits. The basal body is the most complex part of a flagellum. In most gram-negative bacteria, the body has four rings connected to a central rod. The outer L and P rings associate with the lipopolysaccharide and peptidoglycan layers, respectively. The inner M ring contacts the plasma membrane (Fig. 1.7).

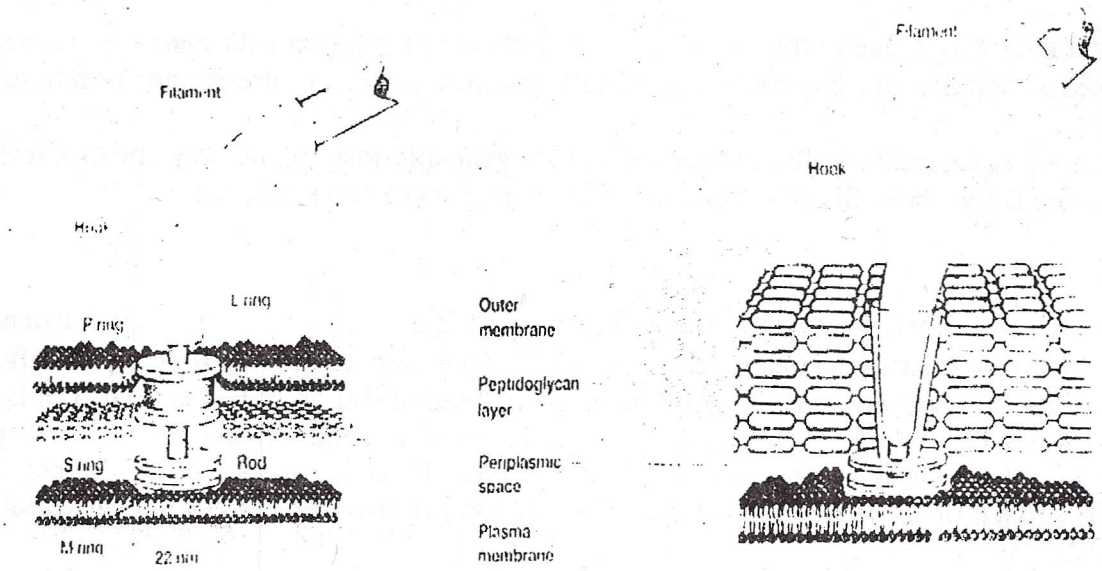

Fig. 1.7 Flagellar basal bodies and hooks in (a) gram-negative and (b) gram-positive bacteria

Gram-positive bacteria have onl 'wo basal body rings, an inner ring connected to the plasma membrane and an outer one prc ttached to the peptidoglycan.

Bacteria can move by mechanisms other than flagellar rotation. Spirochetes are helical bacteria that travel through viscous substances by flexing and spinning movements caused by a special axial filament. Certain bacterial cells can move along solid surfaces by gliding and certain aquatic microorganisms can regulate their position in a water column by gas-filled structures called gas vesicles.

1.2.3 The plasma membrane

The plasma membrane is a thin structure that completely surrounds the cell. This vital structure which is about 8 nm thick, is the critical barrier separating the inside of the cell (the cytoplasm) from its environment. If the membrane is broken, the integrity of the cell is destroyed, the internal contents leak into the environment, and the cell dies. It is also a highly selective barrier, enabling a cell to concentrate specific metabolites and excrete waste materials.

It is a phospholipid bilayer with hydrophilic exteriors and a hydrophobic interior. Other molecules such as hopanoids may strengthen the membrane. The most widely accepted current model for membrane structure is the **fluid mosaic model** of Singer and Nicholson.

Mesosomes are invaginations of the plasma membrane in the shape of vesicles, tubules or

in cell wall foramtion during division or play a role in chromosome replication and distribution to daughter cells. They may also be involved in secretory processes.

1.2.4 The Cytoplasmic matrix

The cytoplasmic matrix is the substance lying between the plasma membrane and the nucleoid. The cytoplasm of bacteria is granular in appearance and rich in ribosomes which are the site of protein synthesis. Procaryotic ribosomes are smaller than eucaryotic ribosomes. They occur either freely in the cytoplasm or may be attached to the cytoplasmic membrane. They are commonly called 70 S ribosomes, have dimensions of about 14 to 15 nm by 20 nm and are constructed of a 50 S and a 30 S subunit. The S stands for Svedberg unit. This is the unit of the sedimentation coefficient, a measure of the sedimentation velocity in a centrifuge. The sedimentation coefficient is a function of a particle's molecular weight, volume and shape.

A variety of **inclusion bodies**, granules of organic or inorganic material, is present in the cytoplasmic matrix. Organic inclusion bodies usually contain either glycogen or poly-β-hydroxybutyrate (PHB). Glycogen is a polymer of glucose units composed of long chains formed by α (1→ 4) glycosidic bonds and branching chains connected to them by α (1→ 6) glycosidic bonds. PHB contains β-hydroxybutyrate molecules joined by ester bonds between the carboxyl and hydroxyl groups of adjacent molecules.

Cyanobacteria have two distinctive organic inclusion bodies : cyanophycin granules and carboxysomes. **Cyanophycin granules** are composed of large polypeptides conta...g approximately equal amounts of the amino acids arginine and aspartic acid. **Carboxysomes** are present in many cyanobacteria, nitrifying bacteria and thiobacilli. They contain the enzyme ribulose-1,5-bisphosphate carboxylase in a paracrystalline arrangement.

Two major types of inorganic inclusion bodies are seen. Many bacteria store phosphate as **polyphosphate granules** or **volutin granules**. They function as storage reservoirs for phosphate. Some bacteria store sulfur temporarily as **sulfur granules**, a second type of inorganic inclusion body.

Magnetosomes are intracellular crystal particles of the iron mineral magnetite, Fe_3O_4. Bacteria that produce magnetosomes exhibit magnetotaxis, the process of orienting and migrating along geomagnetic field lines. Many cyanobacteria, purple and green photosynthetic bacteria and a few other aquatic forms such as *Halobacterium* and *Thiothrix* produce **gas vesicles**, which confer buoyancy on the cells. These are small gas-filled structures made of protein. Bacteria with gas vesicles can regulate their buoyancy to float at the depth necessary for proper light intensity, oxygen concentration and nutrient levels. They descend by simply collapsing vesicles and float upward when new ones are constructed.

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1.2.5 The Nucleoid

The most striking difference between procaryotes and eucaryotes is the way in which their genetic material is packaged. Eucaryotic cells have two or more chromosomes contained within a membrane-delimited organelle, the nucleus. In contrast, procaryotes lack a membrane-delimited nucleus. The procaryotic chromosome is a single circle of double-stranded **deoxyribonucleic acid** (DNA). It is located in an irregularly shaped region called the *nucleoid*. In *Escherichia coli*, the closed DNA circle measures approximately 1,400 μm. The DNA is looped and coiled extensively, probably with the aid of nucleoid proteins.

Many bacteria possess **plasmids** in addition to their chromosome. Plasmids are circular, double-stranded DNA molecules that can exist and replicate independently of the chromosome or may be integrated with it. Plasmid genes can render bacteria dry-resistant, give them new metabolic abilities, make them pathogenic or endow them with a number of other properties.

1.2.6 Endospores

Some species of bacteria produce special structures called endospores within their cells. Endospores are very resistant to heat and cannot be destroyed easily, even by harsh chemicals. The genera *Bacillus* and *Clostridium* are the best studied of endospore-forming bacteria. Endospores can be seen under the light microscope as strongly refractile bodies. Special spore-staining procedures must be used to stain spores specifically. Spores may be centrally located close to one end (subterminal), or definitely terminal (Fig. 1.8).

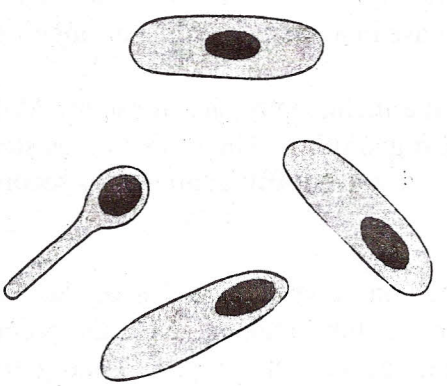

Fig. 1.8. Endospore location and size

The structure of the spore is more complex than that of the vegetative cell in that it has many layers (Fig. 1.9). The outermost layer is *exosporium*, a thin, delicate covering made or protein. Below the exosporium, **spore coats** composed of layers of spore-specific proteins are

found. **Cortex** which consists of loosely cross-linked peptidoglycan is found inside the spore coat. Inside the cortex is the **core** or **spore protoplast** which contains the usual cell wall, cytoplasmic membrane, cytoplasm, nucleoid and so on.

Dipicolinic acid is characteristic of endospores and located in the core. Spores are also high in calcium ions, most of which are combined with dipicolonic acid. Besides having an abundant calcium dipicoloniate, the core is in a partially dehydrated state. The pH of the core cytoplasm is about one unit lower than that of the vegetative cell and contains high levels of core-specific proteins called small acid-soluble spore proteins. Spores can remain dormant indefinitely but germinate quickly when the appropriate trigger is applied.

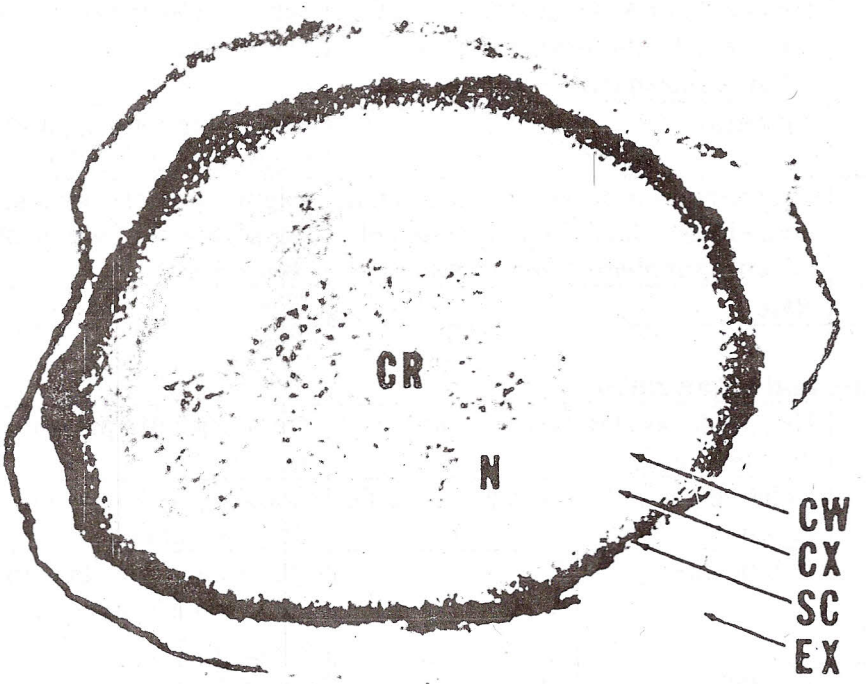

Figure 1.9 **Endospore Structure.** *Bacillus anthracis* endospore (×151,000). Note the following structures: exosporium, EX; spore coat, SC; cortex, CX; core wall, CW; and the protoplast or core with its nucleoid, N, and ribosomes, CR.

Table 1.1 compares the procaryotic and eucaryotic cell in several ways and emphasizes the great structural differences between procaryotes and eucaryotes.

Table 1.1 Comparison of the procaryotic and eucaryotic cell

Properties	Prokaryote	Eukaryote
Phylogentic groups	Bacteria, Archaea	Eukarya: Algae, fungi, protozoa, plants, animals
Nuclear structure and function:		
Nuclear membrane	Absent	Present
Nucleolus	Absent	Present
DNA	Single molecule generally covalently closed and circular, not complexed with histones (other DNA in plasmids)	Linear, present in several chromosomes, usually complexed with histones
Division	No mitosis	Mitosis; mitotic apparatus with microtubular spindle
Sexual reproduction	Fragmentary process, unidirectional; no meiosis; usually only portions of genetic complement reassorted	Regular process; meiosis; reassortment of whole chromosome complement
Introns in genes	Rare	Common
Cytoplasmic structure and organization:		
Cytoplasmic membrane	Usually lacks sterols; hopanoids may be present	Sterols usually present; hopanoids absent
Internal membranes	Relatively simple; limited to specific groups	Complex; endoplasmic reticulum; Golgi apparatus
Ribosomes	70S in size	80S, except for ribosomes of mitochondria and chloroplasts, which are 70S
Membranous organelles	Absent	Several present
Respiratory system	Part of cytoplasmic membrane; mitochondria absent	In mitochondria
Photosynthetic pigments	In internal membranes or chlorosomes; chloroplasts absent	In chloroplasts
Cell walls	Present (in most), composed of peptidoglycan (Bacteria), other polysaccharides, protein, glycoprotein (Archaea)	Present in plants, algae, fungi, usually polysaccharide; absent in animals, most protozoa
Endospores	Present (in some), very heat-resistant	Absent
Gas vesicles	Present (in some)	Absent

Forms of motility:		
Flagellar movement	*Flagella composed of a single type of protein arranged in a fiber and anchored into the cell wall and membrane; flagella rotate*	*Flagella or cilia; composed of microtubules; do not rotate*
Nonflagellar movement	Gliding motility; gas vesicle-mediated	Cytoplasmic streaming and ameboid movement; gliding motility
Cytoskeleton containing microtubules	Absent	Present, microtubules are present in flagella, cilia, basal bodies, mitotic spindle apparatus, centrioles
Size	Generally small, usually <2 μm in diameter	Usually large, 2 to >100 μm in diameter

1.3 OUTLINES OF BERGEY'S CLASSIFICATION OF BACTERIA

Bergey's Manual of Systematic Bacteriology is a major taxonomic compilation of bacteria. The second edition is divided into 30 sections that are organized in five volumes. Comparisons of nucleic acid sequences, particularly 16 S rRNA sequences, are the foundation of this new classification. The general organization of the five volumes is summarized in Table 1.2 and briefly outlined below:

Table 1.2 Organization of Bergey's Manual of Systematic Bacteriology

Taxonomic Rank and Section	**Representative Genera**
Volume 1. The *Archaea*, Cyanobacteria, Phototrophs, and Deeply Branching Genera	
The Archaea Kingdom *Crenarchaeota*	
Section I – Thermoprotei, Sulfolobi, and Barophiles Kingdom *Euryarchaeota*	*Thermoproteus, Sulfolobus*
Section II – The Methanogens	*Methanobacterium*
Section III – The Halobacteria	*Halobacterium,Halococcus*
Section IV – The thermoplasms	*Thermoplasma, Picrophilus*
Section V – The Thermococci	*Archaeoglobus, Thermococcus*
Deeply Branching Genera The Bacteria (Eubacteria)	
Section VI – *Aquifex* and Relatives	*Aquifex, Hydrogenobacter*

Section VII – Thermotogas and Geotogas	*Thermotoga, Geotoga, Thermodesulfobacterium*
Section VIII – The Deinos	*Deinococcus*
Section IX – Thermi	*Thermus, Magnetobacterium*
Section X – *Chrysiogenes*	*Chrysiogenes*
Section XI – The Chloroflexi and Herpetosiphons	*Chloroflexus, Herpetosiphon*
Section XII – Thermomicrobia	*Thermomicrobium*
Section XIII – *Prochloron* and Cyanobacteria	*Prochloron, Synechococcus, Pleurocapsa, Oscillatoria, Anabaena, Nostoc, Stigonema*
Section XIV – *Chlorobia*	*Chlorobium, Pelodictyon*
Volume 2. The Proteobacteria The Bacteria Kingdom *Proteobacteria*	
Section XV – The α-Proteobacteria	*Rhodospirillum, Rickettsia, Caulobacter, Rhizobium, Brucella, Nitrobacter, Methylobacterium, Beijerinckia, Hyphomicrobium*
Section XVI – The β-Proteobacteria	*Neisseria, Burkholderia, Alcaligenes, Comamonas, Nitrosomonas, Methylophilus, Thiobacillus*
Section XVII – The γ-Proteobacteria	*Chromatium, Leucothrix, Legionella, Pseudomonas, Azotobacter, Vibrio, Escherichia, Klebsiella, Proteus, Salmonella, Shigella, Yersinia, Haemophilus*
Section XVIII – The δ-Proteobacteria	*Desulfovibrio, Bdellovibrio, Myxococcus, Polyangium*
Section XIX – The ε-Proteobacteria	*Campylobacter, Helicobacter*
Volume 3. The Low G + C Gram Positives	
Section XX – the Clostridia and Relatives	*Clostridium, Peptostreptococcus, Eubacterium, Epulopiscium, Desulfotomaculum, Veillonella, Haloanaerobium*
Section XXI – The Mollicutes	*Mycoplasma, Ureaplasma, Spiroplasma, Acholeplasma*
Section XXII – The Bacilli and Lactobacilli	*Bacillus, Caryophanon, Paenibacillus. Thermoactinomyces, Lactobacillus, Streptococcus, Enterococcus, Listeria, Staphylacoccus*
Volume 4. The High G + C Gram Positives	

Section XXIII – Class *Actinobacteria*	*Actinomyces, Micrococcus, Arthrobacter, Corynebacterium, Mycobacterium, Norcardia, Actinoplanes, Propionibacterium, Streptomyces., Thermomonospora, Frankia, Actinomadura, Bifidobacterium*
Volume 5. The Planctomycetes, Spirochaetes, Fibrobacters, Bacteroides and Fusobacteria	
Section XXIV – The Planctomycetes, Chlamydia and Relatives	*Planctomyces, Chlamydia*
Section XXV – The Spirochetes	*Spirochaeta, Borrelia, Treponema, Scrpulina, Leptospira*
Section XXVI – The Fibrobacters	*Fibrobacter*
Section XXVII – The Bacteriodes	*Bacteroides, Porphyromonas, Prevotella*
Section XXVIII – The Flavobacteria	*Flavobacterium*
Section XXIX – The Sphingobacteria, Flexibacteria and *Cytophaga*	*Sphingobacterium, Flexibacter, Cytophaga*
Section XXX – The Fusobacteria	*Fusobacterium*

(1) **Volume 1:** The Archaea, cyanobacteria, phototrophs and deeply branching genera are placed in this volume.

(2) **Volume 2:** All of the proteobacteria are placed in this volume and are divided into five major groups based on rRNA sequences and other characateristics: α-proteobacteria, β-Proteobacteria, γ-proteobacteria, δ-proteobacteria and ε-proteobacteria.

(3) **Volume 3:** This volume contains gram-positive bacteria with G + C content below about 50%. Some of the major groups are the clostridia, bacilli, streptococci and staphylococci. Mycoplasmas are also placed here.

(4) **Volume 4:** Gram-positive bacteria with G + C content above around 50 to 55% are in this volume. Groups such as *Corynebacterium, Mycobacterium, Nocardia* and the actinomycetes are placed in this volume.

(5) **Volume 5:** It has a variety of different gram-negative eubacterial groups. It includes the planctomycetes, spirochaetes, fibrobacters, Bacterioides and Fusobacteria.

1.4 SUMMARY

All bacteria are procaryotes and much simpler structurally than eucaryotes. Bacteria may be spherical (cocci), rod-shaped (bacilli), spiral or filamentous or even have no characteristic

shape at all (pleomorphic). Most bacteria have a cell wall outside the plasma membrane to give them shape and protect them from osmotic lysis. Bacterial walls are chemically complex and usually contain peptidoglycan or murein.

Bacteria are often classified as either gram-positive or gram-negative based on differences in cell wall structure and their response to gram staining. Gram-positive walls have thick, homogeneous layers of peptidoglycan and teichoic acids. Gram-negative bacteria have a thin peptidoglycan layer surrounded by a complex outer membrane containing lipopolysaccharides and other components. Structures such as capsules, fimbriae and pili are found outside the cell wall. Many bacteria are motile by means of flagella. Bacterial species differ in the number and distribution of their flagella.

The plasma membrane is a phospholipid bilayer. It may invaginate to form mesosomes. The cytoplasmic matrix contains inclusion bodies and ribosomes. The genetic material is located in an area called the nucleoid and is not enclosed by a membrane. Some bacteria survive adverse environmental conditions by forming endospores, dormant structures resistant to heat, desiccation and many chemicals.

Bergey's Manual of Systematic Bacteriology gives the accepted system of bacterial taxonomy. Comparisons of nucleic acid sequences, particularly 16 S rRNA sequences are the foundation for the classification given in the second edition of Bergey's Manual, which is organized in five volumes.

1.5 MODEL QUESTIONS

1. Describe the structure of a procaryotic cell using a labelled diagram.
2. Explain the structure of cell wall in gram-positive and gram-negative bacteria.
3. Write short notes on:
   a) Bacterial flagella
   b) Endospores
   c) Outlines of Bergey's classification of bacteria
   d) Inclusion bodies
   e) Nucleoid

1.6 REFERENCE BOOKS

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■ **Dr. M. VIJAYALAKSHMI**

Unit-I

Lesson 2

NUTRITIONAL TYPES OF BACTERIA

2.0 **OBJECTIVE:**

Phototrophic, chemoautotrophic and hetrotrophic bacteria are described in this lesson.

2.1 INTRODUCTION
2.2 NUTRITIONAL TYPES OF BACTERIA
2.3 PHOTOSYNTHETIC BACTERIA
2.4 CHEMOLITHTROPHIC BACTERIA
2.5 CHEMOHETEROTROPHIC BACTERIA
2.6 SUMMARY
2.7 MODEL QUESTIONS
2.8 REFERENCE BOOKS

2.1 INTRODUCTION

Microorganisms require nutrients for biosynthesis and energy production. Analysis of microbial cell composition reveals that over 95% of cell dry weight is made up of a few major elements: carbon, oxygen, hydrogen, nitrogen, sulfur, phosphorus, potassium, calcium, magnesium and iron. These are called macroelements or macronutrients as they are required by microbes in relatively large amounts. The first six (C, O, H, N, S and P) are components of carbohydrates, lipids, proteins and nucleic acids.

The remaining four macroelements exist in the cell as cations and play a variety of roles. For example, potassium (K^+) is required for activity by a number of enzymes. Calcium (Ca^{2+}), among other functions, contributes to the heat resistance of bacterial endospores. Magnesium (Mg^{2+}) serves as a cofactor for many enzymes, complexes with ATP and stabilizes ribosomes and cell membranes. Iron (Fe^{2+} and Fe^{3+}) is a part of cytochromes and a cofactor for enzymes and electron carrying proteins.

All microorganisms require several **trace** elements (also called microelements or micronutrients) besides macroelements. The trace elements - manganese, zinc, cobalt, molybdenum, nickel and copper are needed by most cells. Trace elements are normally a part of enzymes and cofactors. They aid in the catalysis of reactions and maintenance of protein structure.

Manganese (Mn^{2+}) aids many enzymes catalyzing the transfer of phosphate groups. Zinc (Zn^{2+}) is present at the active site of some enzymes. It is also involved in the association of

regulatory and catalytic subunits in *Escherichia coli* aspartate carbamoyltransferase. Molybdenum (Mo^{2+}) is required for nitrogen fixation and cobalt (Co^{2+}) is a component of vitamin B^{12}.

Besides the common macroelements and trace elements, microorganisms may have particular requirements that reflect the special nature of their morphology or environment. For example, most bacteria do not require large amounts of sodium. But bacteria growing in saline lakes and oceans depend on the presence of high concentrations of sodium ions.

Growth factors are organic compounds that are required in very small amounts and only by some cells. Growth factors include vitamins, amino acids, purines and pyrimidines. Species which have the ability to synthesize the growth factor requirements are called **prototrophs**. A prototrophic microorganism may mutate so that it cannot synthesize the growth factors required for its growth. The mutated microorganisms that lack the ability to synthesize growth factors and therefore must obtain it from the surroundings are called **auxotrophs**. The requirement for a specific amino acid is a common form of auxotrophy.

2.2 NUTRITIONAL TYPES OF BACTERIA

All bacteria require sources of carbon, energy and hydrogen/electrons for growth to take place (Table 2.1). Bacteria can be grouped into nutritional classes based on how they satisfy these requirements. Basing on the carbon source utilized, bacteria can be grouped into Autotrophs and Heterotrophs. **Autotrophs** use CO_2 as their primary or sole carbon source, while **heterotrophs** employ organic compounds.

Table 2.1 Sources of carbon, Energy and Hydrogen/Electrons

Carbon Sources	
Autotrophs	CO_2 sole or principal biosynthetic carbon source
Heterotrophs	Reduced, preformed, organic molecules from other organisms
Energy Sources	
Phototrophs	Light
Chemotrophs	Oxidation of organic or inorganic compounds
Hydrogen or Electron Sources	
Lithotrophs	Reduced inorganic molecules
Organotrophs	Organic molecules

Microorganisms can be classified based on their energy and electron sources. **Phototrophs** use light as their energy source; **chemotrophs** obtain energy from the oxidation of chemical compounds (either organic or inorganic). Microorganisms also have only two sources for hydrogen

atoms or electrons. **Lithotrophs** use reduced inorganic substances as their electron source, whereas **organotrophs** extract electrons or hydrogen from organic compounds (Table 2.2).

Table 2.2 Major nutritional types of bacteria

Major Nutritional Types	Sources of Energy, Hydrogen/ Electrons and Carbon	Representative Microorganisms
Photolithotrophic autotrophy (Photolithoautotrophy)	Light energy Inorganic hydrogen/electron (H/e^-) donor CO_2 carbon source	Algae Purple and green sulfur bacteria Blue-green bacteria (cyanobacteria) Purple nonsulfur bacteria Green nonsulfur bacteria
Photoorganotrophic heterotrophy (Photoorganoheterotrophy)	Light energy Organic H/e^- donor Organic carbon source (CO_2 may also be used)	Purple nonsulfur bacteria Green nonsulfur bacteria
Chemolithotrophic autotrophy (Chemolithoautotrophy)	Chemical energy source (inorganic) Inorganic H/e^- donor CO_2 carbon source	Sulfur-oxidizing bacteria Hydrogen bacteria Nitrifying bacteria Iron bacteria
Chemoorganotrophic heterotrophy (Chemoorganoheterotrophy)	Chemical energy source (organic) Organic H/e^- donor Organic carbon source	Protozoa Fungi Most nonphotosynthetic bacteria (including most pathogens)

The microorganisms may be placed in one of four nutritional classes based on their primary sources of energy, hydrogen and/or electrons and carbon (Table 2.2). The majority of microorganisms thus far studied are either photolithotrophic autotrophs or chemoorganotrophic heterotrophs.

Photolithotrophic autotrophs (often called **Photoautotrophs** or **photolithoautotrophs**) use light energy and CO_2 as a carbon source. **Chemoorganotrophic heterotrophs** (often called **chemoheterotrophs, chemoorganoheterotrophs** or even **heterotrophs**) use organic compounds as sources of energy, hydrogen, electrons and carbon for biosynthesis. Frequently the same organic nutrient will satisfy all these requirements.

The other two classes have fewer microorganisms but often are very important ecologically. **Photoorganotrophic heterotrophs** (**photoorganoheterotrophs**) are common inhabitants of

polluted lakes and streams. These include some purple and green bacteria which are phototrophic and use organic matter as their electron donor and carbon source.

The fourth group, the **chemolithotrophic autotrophs (chemolithoautotrophs),** oxidizes reduced inorganic compounds such as iron, nitrogen or sulfur molecules to derive both energy and electrons for biosynthesis. Carbon dioxide is the carbon source for these organisms. Chemolithotrophs contribute greatly to the chemical transformations of elements that continually occur in the ecosystem. Although a particular species usually belongs to one of the four nutritional classes, some show great metabolic flexibility and alter their metabolic patterns in response to environmental changes.

2.3 Photosynthetic bacteria

There are three groups of photosynthetic procaryotes: the purple bacteria, the green bacteria and the cyanobacteria (Table 2.3). The cyanobacteria differ most fundamentally from the green and purple photosynthetic bacteria in being able to carry out **oxygenic photosynthesis**. They use water as an electron donor and generate oxygen during photosynthesis.

Table 2.3 Characteristics of the major groups of photosynthetic bacteria

Characteristic	**Anoxygenic Photosynthetic Bacteria**				**Oxygenic Photosynthetic Bacteria**
	Green Sulfur	**Green Nonsulfur[a]**	**Purple Sulfur**	**Purple Nonsulfur**	**Cyanobacteria**
Major photosynthetic pigments	Bacteriochlor o-phylls *a* plus *c*, *d*, or *e* (the major pigment)	Bacteriochlor o-phylls *a* and *c*	Bacteriochlor o-phyll *a* or *b*	Bacteriochlor o-phyll *a* or *b*	Chlorophyll *a* plus phycobiliproteins
Morphology of photosynthetic membranes	Photosyntheti c system partly in chlorosomes that are independent of the plasma membrane	Chlorosomes present when grown anaerobically	Photosynthetic system contained in spherical or lamellar membrane complexes that are continuous with the plasma membrane	Photosynthetic system contained in spherical or lamellar membrane complexes that are continuous with the plasma membrane	Membranes lined with phycobilisomes
Characteristic	**Anoxygenic Photosynthetic Bacteria**				**Oxygenic Photosynthetic Bacteria**

	Green Sulfur	Green Nonsulfur[a]	Purple Sulfar	Purple Nonsulfur	Cyanobacteria
Photosynthetic electron donors	H_2, H_2S, S	Photoheterotrophic donors—a variety of sugars, amino acids, and organic acids; photoautotrophic donors—H_2S, H_2	H_2, H_2S, S	Usually organic molecules; sometimes reduced sulfur compounds or H_2	H_2O
Sulfur deposition Nature of photosynthesis	Outside of the cell Anoxygenic	Anoxygenic	Inside the cell[b] Anoxygenic	Outside of the cell Anoxygenic	Oxygenic (sometimes facultatively anoxygenic)
General metabolic type	Obligately anaerobic photolithoautotrophs	Usually photoheterotrophic; sometimes photoautotrophic or chemoheterotrophic (when aerobic and in the dark)	Obligately anaerobic photolithoautotrophs	Usually anaerobic photoorganoheterotrophs; some facultative photolithoautotrophs (in dark, chemoorganoheterotrophs)	Aerobic photolithoautotrophs
Motility	Nonmotile; some have gas vesicles	Gliding	Motile with polar flagella; some are peritrichously flagellated	Motile with polar flagella or nonmotile; some have gas vesicles	Nonmotile or with gliding motility; some have gas vesicles
Percent G + C	48-58	53-55	45-70	61-72	35-71

[a]Characteristics of *Chloroflexus*.
[b]With the exception of *Ectothiorhodospira*.

In photosynthesis, cyanobacteria trap light energy with chlorophyll and accessory pigments and move electrons through photosystems I and II to make ATP and NADPH (the light reaction). Cyclic photophosphorylation involves the activity of photosystem I alone. In noncyclic photophosphorylation, photosystems I and II operate together to more electrons from water to $NADP^+$ producing ATP, NADPH and O_2.

Purple and green bacteria carry out **anoxygenic photosynthesis**. These bacteria are unable to use water as an electron source. They employ reduced molecules such as hydrogen sulfide, sulfur, hydrogen and organic matter as their electron source for the generation of NADH and NADPH. Consequently, purple and green bacteria do not produce oxygen but many form sulfur granules.

Purple bacteria can be categorized into purple sulfur bacteria and purple non-sulfur bacteria. Purple sulfur bacteria are photolithotrophs while purple non-sulfur bacteria are photoorgano-heterotrophs. They trap light energy and use organic molecules as electron source. Green bacteria are of two types: green sulfur bacteria and green non-sulfur bacteria. Green sulfur bacteria are photolithoautotrophs, while green non-sulfur bacteria are photoorganoheterotrophs.

Purple sulfur bacteria accumulate sulfur granules within their cells, whereas green sulfur bacteria deposit the sulfur granules outside their cells.

Green and purple bacteria possess slightly different photosynthetic pigments, **bacteriochlorophylls**, many with absorption maxima at longer wave lengths (Table 2.4). Bacteriochlorophylls *a* and *b* have maxima in ether at 775 and 790 nm respectively. *In vivo* maxima are about 830 to 890 nm (bacteriochlorophyll *a*) and 1,020 to 1,040 nm (bacteriochlorophyll *b*). This shift of absorption maxima to the infrared region better adapts these bacteria to their ecological niches.

Many differences found in green and purple bacteria are due to their lack of photosystem II; they cannot use water as an electron donor in noncyclic electron transport. Without photosystem II they cannot produce O_2 from H_2O photosynthetically and are restricted to cyclic photo-phosphorylation. Almost all purple and green sulfur bacteria are strict anaerobes.

Table 2.4 Procaryotic bacteriochlorophyll and chlorophyll absorption maxima

Pigment	Long Wavelength Maxima (nm)	
	In Ether or Acetone	Approximate Range of Values in Cells
Chlorophyll a	665	680-685
Bacteriochlorophyll *a*	775	850-910 (purple bacteria)[a]
Bacteriochlorophyll *b*	790	1,020-1,035
Bacteriochlorophyll *c*	660	745-760
Bacteriochlorophyll *d*	650	725-745
Bacteriochlorophyll *e*	647	715-725

[a]The spectrum of bacrteriochlorophyll *a* in green bacteria has a different maximum, 805-810 nm.

The fixation of CO_2 by most phototrophic organisms occurs via the calvin cycle, in which the enzyme ribulose bisphosphate carboxylase (RubisCo) plays a key role. The calvin cycle is an

energy-demanding process in which CO_2 is converted into sugar. The reverse citric acid cycle and the hydroxypropionate cycle are pathways of CO_2 fixation found in green sulfur and green nonsulfur bacteria, respectively.

2.4 CHEMOLITHOTROPHIC BACTERIA

Chemolithotrophs obtain energy from the oxidation of inorganic compounds. Most chemolithotrophic bacteria are also able to obtain all their carbon from CO_2, so they are also autotrophs. Two components are needed for growth on CO_2 as sole carbon source: energy in the form of ATP and reducing power.

In chemolithotrophs, ATP generation is in principle similar to that in chemoorganotrophs, except that the electron donor is inorganic rather than organic. Thus, ATP synthesis is coupled to oxidation of the electron donor. Reducing power in these bacteria is obtained from the inorganic compound. These chemolithotrophs fix CO_2 by the calvin cycle.

Depending on the nature of the inorganic compound oxidized, aerobic chemolithotrophs are categorized into six groups. They include hydrogen oxidizing bacteria, sulfur oxidizers, Fe^{2+} oxidizers, ammonia oxidizers, nitrite oxidizers and carbon monoxide oxidizing bacteria. Representative chemolithotrophs and their energy sources are presented in Table 2.5.

Table 2.5 Representative Chemolithotrophs and their energy sources

Bacteria	**Electron Donor**	**Electron Acceptor**	**Products**
Alcaligenes and *Pseudomonas* spp.	H_2	O_2	H_2O
Nitrobacter	NO_2^-	O_2	NO_3^-, H_2O
Nitrosomonas	NH_4^+	O_2	NO_2^-, H_2O
Thiobacillus denitrificans	S^0, H_2S	NO_3^-	SO_4^{2-}, N_2
Thiobacillus ferrooxidans	Fe^{2+}, S^0, H_2S	O_2	Fe^{3+}, H_2O,H_2SO_4

Hydrogen Oxidizing Bacteria

These bacteria use molecular hydrogen as energy source. They are highly diverse in their physiological and morphological features. The hydrogen oxidizing bacteria include *Pseudomonas saccharophila, P. facilis, Alcaligenes eutrophus* and *Nocardia autotrophica.* Generation of ATP during H_2 oxidation comes from the oxidation of H_2 by O_2 leading to the formation of a proton motive force.

Carbon Monoxide Oxidizing Bacteria

Some hydrogen bacteria can grow on carbon monoxide as energy source, with electrons from the oxidation of CO to CO_2 entering the electron transport chain to drive ATP synthesis. All CO-oxidizing bacteria are H_2 oxidizers (not vice versa). They include *Pseudomonas carboxydovorans*, *Alcaligenes carboxydus* and the thermophilic *Bacillus schlegelli.*

Sulfur Oxidizers

The concept of chemolithotrophy emerged from studies of the sulfur bacteria, as the great Russian Microbiologist Winorgradsky first proposed the idea of chemolithotrophy from studies of these organisms. Many reduced sulfur compounds can be used as electron donors by a variety of sulfur bacteria. The ability to grow chemolithotropically on reduced sulfur compounds is a property of a diverse group of proteobacteria such as *Thiobacillus thioparus, T. denitrificans, T. novellus, Beggiatoa, Thiothrix, Thiomicrospira, Thiosphaera* and *Thermothrix.*

The most common sulfur compounds used as electron donors are hydrogen sulfide, elemental sulfur and thiosulfate. The final product of sulfur oxidation inmost cases is sulfate and the total number of electrons involved between H_2S (oxidation state, -2) and sulfate (oxidation state, +6) is eight.

Iron Oxidizers

Iron is one of the most abundant elements in Earth's crust. On the surface of the Earth, iron exists naturally in two oxidation states, ferrous (Fe^{2+}) and ferric (Fe^{3+}). Fe^{o} is a major product of human activities in the smelting of ferrous or ferric iron ores to form cast iron. In nature, iron cycles primarily between the ferrous and ferric forms, the reduction of Fe^{3+} occurring both chemically and as a form of anaerobic respiration, and the oxidation of Fe^{2+} occurring both chemically and as a form of chemolithotrophic metabolism.

The aerobic oxidation of iron from the ferrous (Fe^{2+}) to the ferric (Fe^{3+}) state is an energy-yielding reaction for a few bacteria. Only a small amount of energy is available from this oxidation. Hence iron bacteria must oxidize large amounts of iron in order to grow. The ferric iron produced forms insoluble ferric hydroxide {$Fe(OH_3)$} precipitates in water.

The best-known iron-oxidizing bacteria (*Thiobacillus ferrooxidans* and *Leptospirillum ferrooxidans*) are able to grow autotrophically using ferrous iron as electron donor. These bacteria are very common in acid-polluted environment such as coal-mining dumps. *Gallionella* is active under neutral pH conditions and *Sulfolobus* functions under acidic, thermophilic conditions.

Ammonia Oxidizers and Nitrite Oxidizers

Bacteria able to grow chemolithotrophically at the expense of reduced inorganic nitrogen compounds (NH_3 and NO_2^-) are called **nitrifying bacteria**. Nitrification in nature results from the sequential action of two separate groups of organisms, the **ammonia oxidizing bacteria** (Nitrosofyers) and the **nitrite oxidizing bacteria** (the true nitrifying bacteria). Nitrosofyers typically have genus names beginning in Nitroso (*Nitrosomonas, Nitrosococcus, Nitrosospira, Nitrosolobus, Nitrosovibrio*) while true nitrifyers usually begin with Nitro (*Nitrobacter, Nitrospina, Nitrococcus* and *Nitrospira*). *Nitrosomonas* and *Nitrobacter* are major genera of nitrifying bacteria. The nitrifying bacteria were the first organisms to be shown to grow chemolithotrophically.

2.5 CHEMOHETEROTROPHIC BACTERIA

These bacteria use organic compounds as sources of energy and carbon. The survival of heterotrophs is dependent either directly or indirectly on the activities of autotrophs. Major groups of bacteria are heterotrophic in their mode of nutrition. They may lead either saprophytic or parasitic mode of life.

Organisms which feed on dead or decaying organic matter are called **saprophytes**. They secrete enzymes on to their food where it is digested. The soluble end-products of this extracellular digestion are then absorbed and assimilated by the saprophyte. They feed on the dead organic remains of plants and animals and contribute to the removal of such remains by decomposing it.

Organisms which obtain nutrients from another organism (host) are called **parasites**. Parasitism is a close association between two living organisms of different species which is beneficial to one (the parasite) and harmful to the other (the host). When a parasite is growing and multiplying within or on a host, the host is said to have an infection. The nature of infection can vary widely with respect to severity, location and number of organisms involved. An infection may or may not result in disease. Any parasitic organism that causes a disease is called pathogen. The ability to cause disease is influenced by complex host-parasite interactions.

2.6 SUMMARY

Basing on the primary sources of energy, hydrogen and/or electrons and carbon, microorganisms can be grouped into four nutritional classes – photolithotrophic autotrophs, photoorganotrophic heterotrophs, chemolithotrophic autotrophs and chemoorganotrophic heterotrophs.

Photolithotrophic autotrophs (photo autotrophs) use light energy and CO_2 as a carbon source. Photoorganotrophic heterotrophs (photoheterotrophs) use light energy and organic carbon as carbon source. Chemolithotrophic autotrophs (Chemolithotrophs) oxidize reduced inorganic compounds to obtain energy and use CO_2 as carbon source. Chemoorganotrophic heterotrophs (Heterotrophs) use organic compounds as sources of energy and carbon.

2.7 MODEL QUESTIONS

1. Explain the nutritional types of bacteria.
2. Give an account of photosynthetic bacteria.
3. Write short notes on:
   a) Chemolithotrophs
   b) Heterotrophs
   c) Green and purple bacteria

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Dr. M. VIJAYALAKSHMI

Unit-I

Lesson 3

ROLE OF BACTERIA IN CARBON, NITROGEN AND PHOSPHORUS CYCLES

3.0 **OBJECTIVE:**

The role of bacteria in the transformations of carbon, nitrogen and phosphorus is discussed in this lesson.

3.1 INTRODUCTION
3.2 CARBON CYCLE
3.3 NITROGEN CYCLE
3.3.1 Nitrogen mineralization
3.3.2 Nitrification
3.3.3 Immobilization
3.3.4 Denitrification
3.3.5 Nitrogen fixation
3.4 PHOSPHORUS CYCLE
3.5 SUMMARY
3.6 MODEL QUESTIONS
3.7 REFERENCE BOOKS

3.1 INTRODUCTION

The microbial community plays a major role in biogeochemical cycling. As this term suggests, both biological and chemical processes are involved in the cycling and transformations of nutrients important to microorganisms, plants and animals. This often involves oxidation-reduction reactions that can change the chemical and physical characteristics of the nutrients.

The microbes convert complex organic compounds in to simple inorganic compounds during the process of mineralization. Mineralization of organic carbon, nitrogen, sulphur and phosphorus by microorganisms makes these elements available for reuse. These elements are again absorbed by plants and microorganisms and are reincorporated in to protoplasm. The reutilization of elements in nature thus makes it a closed, self-sustaining system.

3.2 CARBON CYCLE

Carbon is the most important element in the biological realm. It serves as the corner stone of cell structure and energy system. About 50 per cent of the dry weight of living organisms is composed of carbon. The main source of carbon for photoautotrophs is the CO_2 present in the atmosphere.

CO_2 is the most oxidized state of carbon and it is reduced to carbohydrates by photoautotrophs during the process of photosynthesis in which the radiant energy of the sun is used. In the process, plants split water and release free oxygen into the atmosphere. The CO_2 comes back into the atmosphere by plant, animal and microbial respiration in which O_2 is taken up and CO_2 is released. However, the major part of CO_2 comes to the atmosphere from microbial degradation of organic compounds. Thus the carbon cycle revolves around the fixation and regeneration of CO_2.

The vegetation of earth's surface has been estimated to consume some 1.3×10^{14} kg CO_2 per annum. The carbon cycle would be disrupted in the absence of microbial activities. The transfer of carbon between the biotic and abiotic environment is shown in Fig. 3.1.

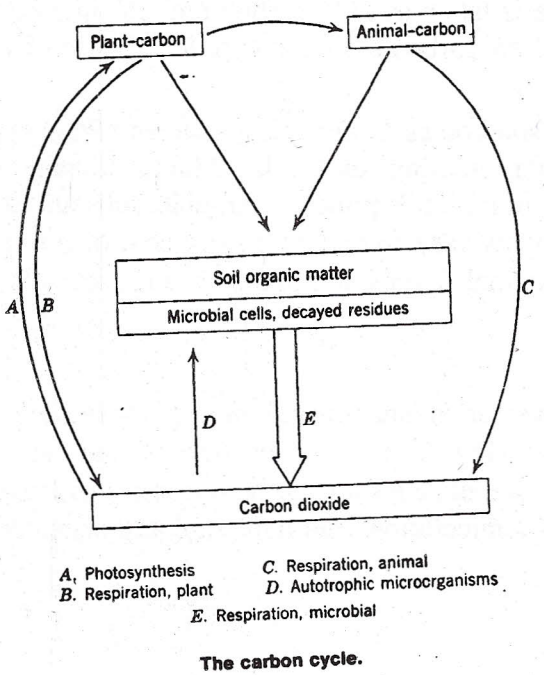


Fig. 3.1 The carbon cycle

Degradation of organic compounds : Microorganisms are regarded as nature's garbage-disposal agents. They convert the carbon in organic materials to CO_2. They obtain energy for growth by decomposing the organic materials. They also get carbon for the formation of new cell

material in this process. They convert substrate carbon to protoplasmic carbon. This process is known as **assimilation**. Under aerobic conditions, microorganisms assimilate 20-40% of substrate carbon. The remainder is released as CO_2 or accumulated as waste products.

Fungi are more efficient in the assimilation of carbon under aerobic conditions when compared to the other microbial groups. Efficient cultures convert the major quantity of substrate carbon into cell carbon and release only small quantity of organic products and CO_2. Anaerobic bacteria are not efficient in carbon assimilation as they can utilize only small quantity of substrate carbon to form cell carbon.

Along with the assimilation of carbon, the microorganisms also take up inorganic substances such as nitrogen, sulphur, phosphorus and potassium. They utilize these nutrients for the generation of new protoplasm. This process is called **immobilization**. Assimilation of inorganic nutrients has great practical significance since it is an important means of immobilization. Microorganisms reduce the quantity of plant-available nutrients in the soil by this process.

The magnitude of immobilization is related to carbon assimilation by a factor governed by the C : N, C : P, C : K or C : S ratio of the newly generated protoplasm. If the average cell composition of the microflora is taken as 50% carbon and 5% nitrogen, the nitrogen immobilized would be equal to one-tenth of the carbon assimilated or C : N ratio of 10.

The organic matter in soil comes from several sources. Plant residues contribute the largest fraction of all organic matter entering the soil. Plants contain 15-60% cellulose, 10-30% hemicellulose, 5-30% lignin and 2-15% protein. Soluble substances constitute 10% of the dry weight. The mineral constituents vary from 1 to 13 per cent of the total issue. Besides the vast quantities of plant residues, animal tissues and excretory products are also subjected to microbial attack.

Cellulose: Cellulose is the most abundant constituent of plant residues. It is composed of glucose units with β $(1 \rightarrow 4)$ linkages (Fig. 3.2). It is localized in the cell wall as submicroscopic rod shaped units called micelles. The micelles are further arranged to form microfibrils. A number of other polysaccharides such as hemicellulose and lignin are associated with the cellulose in the plant cell wall.

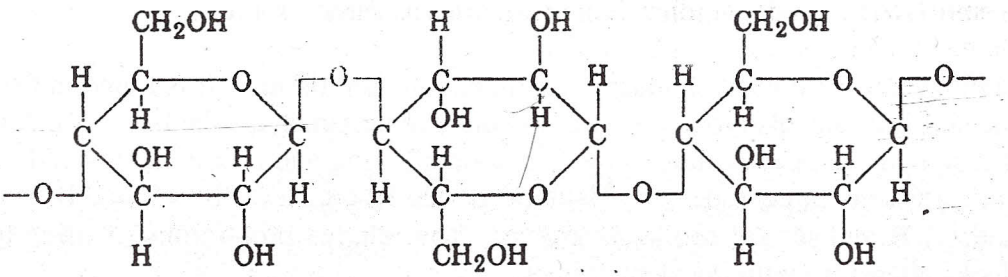


Fig. 3.2 Structure of cellulose

Aerobic and anaerobic mesophilic bacteria, thermophilic bacteria, actinomycetes and filamentous fungi utilize cellulose. These cellulolytic microbes are commonly found in field and forest soils, in manure and on decaying plant tissues. They produce an enzyme complex **cellulase** which acts in two distinct stages. In the first step, the crystalline structure of the substance is lost. Depolymerization occurs in the second step. The resultant cellobiose (disaccharide) is hydrolyzed by the enzyme **cellobiase** to glucose. Some important cellulolytic microorganisms are listed below:

Bacteria: *Bacillus, Clostridium, Chromobacterium, Cellulomonas, Cytophaga* and *Pseudomonas.*

Actinomycetes: *Streptomyces, Nocardia* and *Micromonospora.*

Fungi: *Aspergillus, Chaetomium, Fusarium, Penicillium, Thielavia, Trichoderma and Rhizoctonia.*

Cellulose decomposition is influenced by several environmental factors such as the available nitrogen level, aeration, moisture, temperature, pH and the relative proportion of lignin in the plant residues.

Hemicellulose: Hemicelluloses are the heterogeneous group of compounds. They are the polymers of hexoses, pentoses and some times uronic acids. They are classified into two categories: **Homoglycans** and **Heteroglycans.** Homoglycans contain only a single monosaccharide type. They never constitute the dominant fraction of hemicelluloses in plants e.g. xylan, mannan, galactan. Heteroglycans are abundant in plants. They contain more than one kind of monosaccharide or uronic acid e.g. Arabinoxylans, glucomannans, arabinoglucuronoxylans.

In the pure state, hemicelluloses are easily decomposed. But in nature they are frequently complexed with other substances which may make the breakdown more difficult. Several enzymes collectively known as **hemicellulases** are involved in the decomposition process. Bacteria such as *Bacillus, Cytophaga, Erwinia, Pseudomonas* and *Sporocytophaga* are able to utilize hemicelluloses

as carbon sources. Among fungi, species of *Alternaria, Aspergillus, Chaetomium, Fusarium, Penicillium* and *Trichoderma* can utilize hemicelluloses as carbon source.

Lignin: Lignin is the third most abundant constituent of plant tissues. It is found in the secondary layers of the cell wall and also to some extent in the middle lamella. The basic structure of lignin consists of the phenyl-propanoid unit. It has an aromatic ring and a three carbon side chain (Fig. 3.3). It may exist in three types in which (a) R and R^1 are H; (b) R is H and R^1 is a methoxyl ($-OCH_3$) and (c) R and R^1 are methoxyl groups. The relative proportions of these three major building blocks often vary with the plant species.

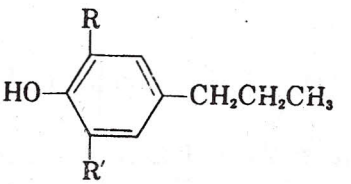


Fig. 3.3 Phenyl-propanoid unit

Lignin is resistant to enzymatic degradation. Lignin decomposition under natural environments proceeds slowly when compared with the other carbohydrates. Fungi play an important role in the decomposition of lignin. White rot fungi such as *Coriolus, Pleurotus, Polyporus* and *Trametes* degrade lignin only in the presence of some other readily degradable substrate as the primary energy source.

Brown-rot fungi such as *Poria* and *Gloeophyllum* degrade the polysaccharides associated with lignin. Actinomycetes, such as *Streptomyces* and *Nocardia* and aerobic, Gram-negative bacteria such as *Azotobacter* and *Pseudomonas* reduce the size of lignin molecule.

A number of simple aromatic compounds appear during the degradation of purified lignins by microorganisms in culture. Several intermediates frequently recovered from culture filtrates include ferulic acid, p-hydroxybenzoie acid, p-hydroxycinnamic acid, syringic acid, vanillin, dehydrodivanillin, coniferaldehyde, syringylaldehyde, guaiacylglycerol and guciacylglycerol-β-coniferyl ether. A scheme has been proposed for the microbial metabolism of lignin basing on the products identified in culture (Fig. 3.4).

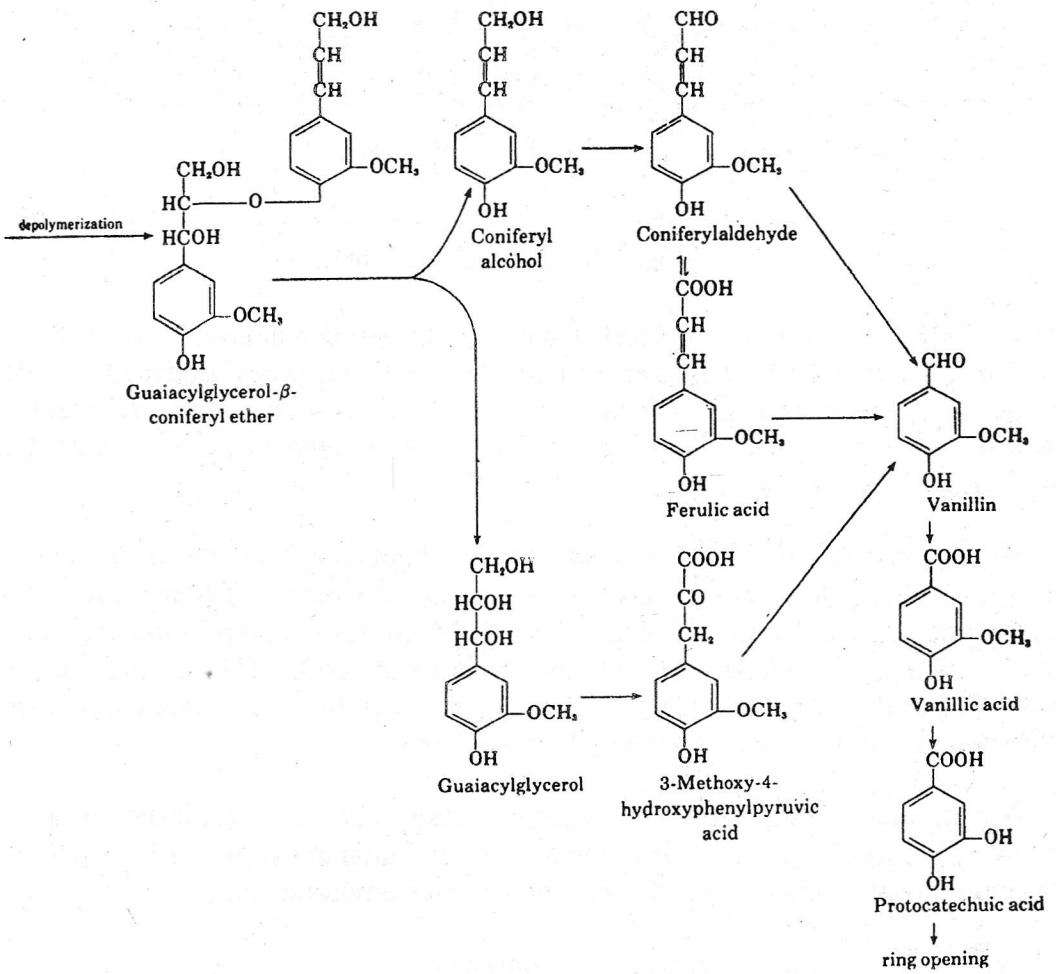


Fig. 3.4 Proposed pathway for the microbial metabolism of lignin

Starch: Starch is the major food reserve of plants. It contains two glucose polymers, amylose and amylopectin. Amylose consists of an unbranched chain of glucose units attached by α (1→ 4) glucosidic bonds (Fig. 3.5). Amylopectin has the same structure with side chains joined by α (1 → 6) bonds. Many bacteria and fungi hydrolyze starch by producing extra cellular enzymes collectively called **amylases**.

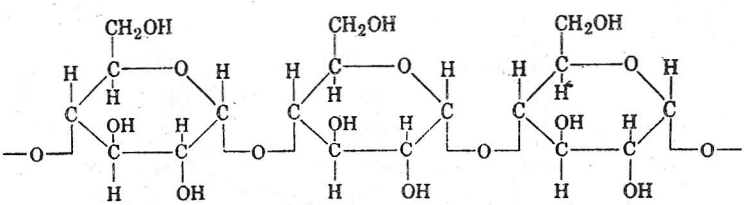


Fig. 3.5 Structure of amylose

Proteins: Proteins constitute the most abundant nitrogen-containing constituents of organisms. They are readily attacked by microorganisms. Proteolytic enzymes produced by microorganisms hydrolyze the peptide bonds. Fibrous proteins such as keratin, are resistant to microbial degradation. However some actinomycetes such as *Streptomyces* and some fungi such as *Penicillium* can degrade them.

The decomposition of native organic matter (humus) reflects the biological availability of soil carbon. The release of CO_2 following the addition of simple substrates is an estimation of the biodegradability of the test compound. The rate of humus decomposition may be enhanced or reduced following the addition of fresh substrates to the soil. This enhancement is known as **priming.** Factors such as organic matter level of soil, aeration, moisture, temperature, pH, depth and cultivation govern the rate of humus decomposition.

During the decomposition of organic matter, organic complexes of a substance are converted into inorganic state. This process is called **mineralization**. It is exactly the reverse of that of immobilization and releases the nutrients into the environment.

Organic	Mineralization →	Inorganic
Complexes	← Immobilization	Compounds

The microorganisms convert the carbon in organic materials to CO_2, thus completing the biological carbon cycle that was initiated during photosynthesis.

The main products of aerobic carbon mineralization are CO_2, water, cells and humus components. In the absence of O_2, organic carbon is incompletely metabolized, intermediary substances accumulate, and abundant quantities of CH_4 and smaller amounts of H_2 are evolved. At the same time, the energy yield during anaerobic fermentation is low, resulting in the formation of fewer microbial cells per unit of organic carbon degraded. Consequently, organic matter breakdown is consistently slower under total anaerobiosis than in environments containing adequate O_2; the rate in waterlogged soils is intermediate between the two extremes.

3.3 NITROGEN CYCLE

The cycling of nitrogen in nature has been studied more extensively than that of any other nutrient. The nitrogen cycle in soil is an integral part of the over all cycle of nitrogen in nature. The source of soil nitrogen is the atmosphere, where N_2 is the predominant gas. The cycling of other nutrients, notably phosphorus and sulfur, is closely associated with biochemical nitrogen transformations.

This element is a key building block of the protein molecule, thus becomes a major indispensable component of the protoplasm of living organisms. Nitrogenous compounds and molecular nitrogen undergo a number of transformations that are commonly mediated through microbial activities.

A small fraction of N_2 in the atmosphere is converted into organic compounds through biological nitrogen fixation. The utilization of inorganic nitrogen (NH_4^+ and NO_3^-) by plants and soil organisms constitutes **assimilation** and **immobilization**, respectively. The nitrogen present in the proteins of nucleic acids of plant tissues is consumed by animals. It is again converted to other simple and complex compounds in the animal body. Microorganisms act on the dead tissues of plants and animals and convert the organic forms of nitrogen into NH_3 and NO_3^- by a process called **mineralization**.

The conversion of organic forms of nitrogen such as proteins and nucleic acids to NH_3 is termed **ammonification**. The oxidation of NH_3 to NO_3^{--} is termed **nitrification**. The nitrate may serve as plant nutrient or it may be lost by leaching or reduced to ammonium or to gaseous nitrogen. Organic form of nitrogen is returned to the atmosphere as molecular N_2 by the process of **denitrification**, thereby completing the nitrogen cycle. The important features of N-cycle are presented in Fig. 3.6.

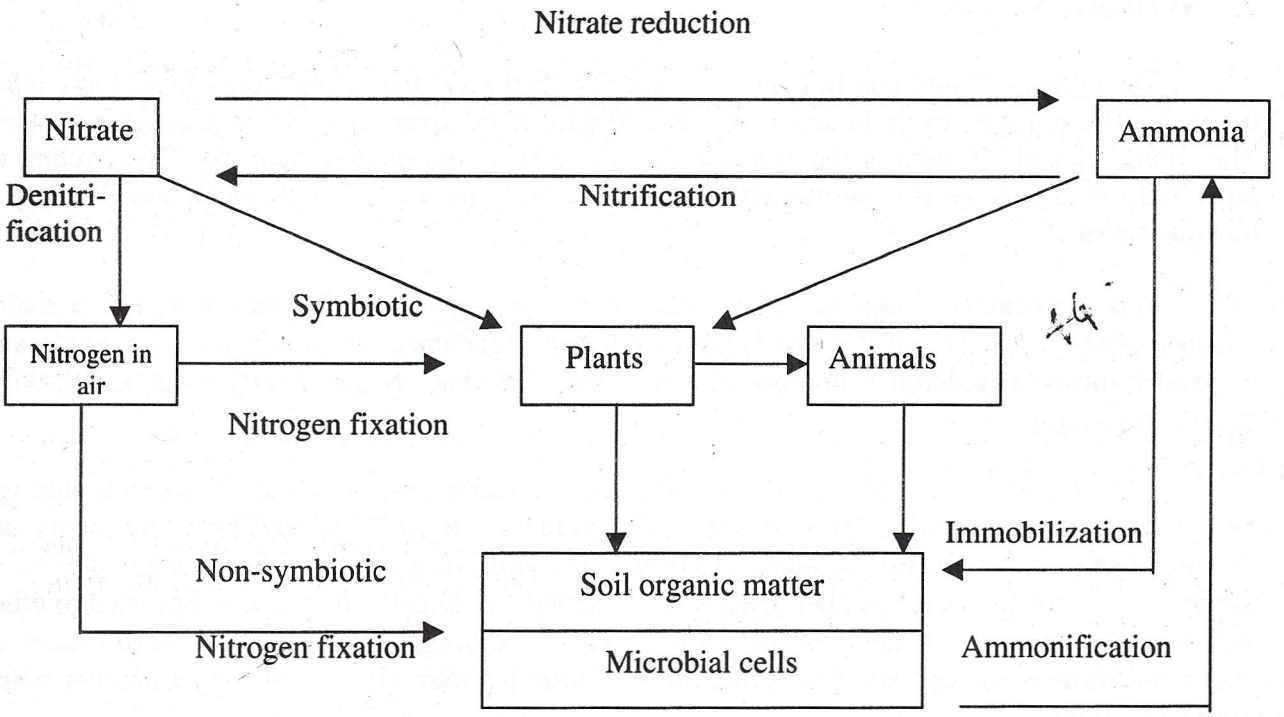


Fig. 3.6 The nitrogen cycle

Nitrogen transformations: Nitrogen transformations mediated by microorganisms are categorised into the following types: (a) Nitrogen mineralization (b) Nitrification (c) Immobilization (d) Denitrification and (e) Nitrogen fixation.

3.3.1 Nitrogen mineralization

The conversion of organic nitrogen to the more mobile, inorganic state is termed **nitrogen mineralization**. The major forms of organic nitrogen are proteins, nucleic acids, amino sugars and related compounds. The conversion of organic nitrogen to NH_3 is termed **ammonification**. It is an enzymatic process. A wide array of enzymes are involved in this process, each acting on a specific group of organic compounds.

Ammonification is carried out by heterotrophic microorganisms. Both aerobic and anaerobic microbes are involved in this process. Bacteria such as *Arthrobacter, Bacillus, Clostridium, Micrococcus* and *Pseudomonas* and fungi such as *Aspergillus, Fusarium, Mucor, Penicillium* and *Rhizopus* play active role in this process. Characteristics of the habitat such as aeration, temperature, moisture, pH and the inorganic nutrient supply govern this process.

3.3.2 Nitrification

It includes the oxidation of NH_3 to NO_2^- and NO_3^-. Winogradsky (1889) established the biological nature of nitrification. Two groups of chemoautotrophic bacteria carry out this process, these bacteria are obligate aerobes. The oxidation of NH_4^+ to NO_2^- is carried out by five genera of bacteria viz., *Nitrosomonas, Nitrosococcus, Nitrosolobus, Nitrosospira* and *Nitrosovibrio*. The oxidation of NO_2^- to NO_3^- is carried out by bacteria such as *Nitrobacter, Nitrococcus* and *Nitrospira.*

$$NH_3 \underset{-H^+}{\overset{+H^+}{\rightleftharpoons}} NH_4^+ \xrightarrow{Nitrosomonas} NO_2^- \xrightarrow{Nitrobacter} NO_3^-$$

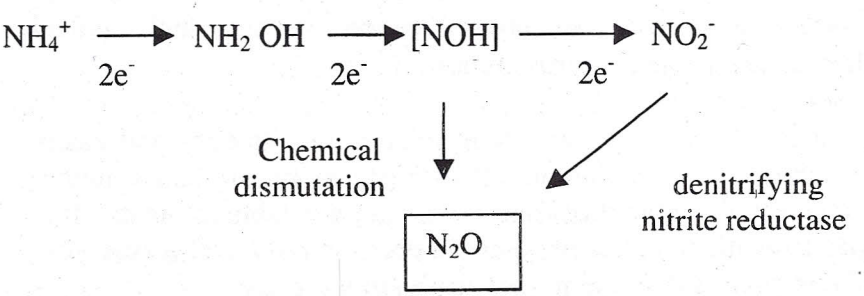
Nitrosomonas europaea and *Nitrobacter winogradskyi* are primarily responsible for the oxidation of NH_4^+ and NO_2^- in soil, respectively.

Pathway of nitrification: The oxidation of NH_4^+ (oxidation state of -3) to NO_2^- (oxidation state of +3) is accompanied by six-electron transfer. Two intermediate compounds such as hydroxylamine (NH_2OH) and nitroxyl (NOH) are formed during this process.

$$\underset{-3}{NH_4^+} \xrightarrow[-H^+]{+\,{}^1/_2\,O_2} \underset{-1}{NH_2OH} \xrightarrow[-2H^+]{} \underset{+1}{NOH} \xrightarrow[-H^+]{+\,{}^1/_2\,O_2} \underset{+3}{NO_2^-}$$

Ammonium Hydroxylamine Nitroxyl Nitrite

In this process 65 k cal/mole energy is released. It is used by the bacteria to carry out their life activities. Several studies have indicated that some of the N_2O produced in soil is generated during nitrification. It may arise by chemical dismutation of nitroxyl or through the action of nitrite reductase, as shown below:



The oxidation of NO_2^- (oxidation state of +3) to NO_3^- (oxidation state of +5) involves the transfer of two electrons. Energy released during this process (17.8 k cal/mole) is utilized by the bacteria.

$$NO_2^- + {}^1/_2\ O_2 \xrightarrow[2e^-]{} NO_3^-$$

Nitrite +3 Nitrate +5

In most soils, oxidation of nitrite by *Nitrobacter* proceeds at a rapid rate when compared to the oxidation of NH_4^+ by *Nitrosomonas*. Consequently, nitrite seldom persists in detectable amounts. Several factors such as acidity, aeration, moisture, temperature and organic matter affect the nitrification process in soil.

Heterotrophic nitrification: In addition to the autotrophic bacteria, several heterotrophic bacteria, actinomycetes and fungi are able to produce NO_2^- or NO_3^- when grown in culture media containing NH_4^+ (Table 3.1).

Table 3.1 Heterotrophic nitrifying microorganism

Bacteria	Actinomycates	Fungi
Aerobacter *Arthrobacter* *Azotobacter* *Bacillus* *Proteus* *Pseudomonas*	*Streptomyces* *Nocardia*	*Aspergillus* *Fusarium* *Neurospora* *Penicillium*

The production of nitrate by heterotrophic microorganisms in agricultural soils is not significant when compared to that brought about by chemoautotrophs. The ecological importance of heterotrophic nitrification has yet to be established with certainity.

Biological nitrification is used as an index of soil fertility. However, nitrification can also lead to some undesirable consequences such as eutrophication, infant and animal methemoglobinemia and the formation of carcinogenic nitrosamines.

Ammonium is a cation that gets adsorbed to soil. It is relatively stationary and easily available to plants. Nitrate, on the other hand, is an anion. It is freely mobile in soil solution. Under certain conditions, it will leach away from the root zone, hence not available to plants. It is also susceptible to losses through denitrification. In recent years in order to reduce N-losses from soils, efforts have been made to find compounds that inhibit the nitrification process.

3.3.3 Immobilization

Microbial assimilation of inorganic nitrogen is called **nitrogen immobilization.** Microorganisms take up inorganic forms of nitrogen such as ammonium and nitrate and convert

them into organic forms such as proteins, nucleic acids and other organic complexes. Thus immobilization is the converse of mineralization.

Organic	Mineralization →	Inorganic
Complexes	← Immobilization	Compounds

Since both mineralization and immobilization occur simultaneously, the amount of mineral nitrogen (NH_4^+ and NO_3^-) found at any time represents the differences in the magnitude of the two opposing processes. A decrease in mineral nitrogen level with time indicates net immobilization. An increase in mineral nitrogen level indicates net mineralization.

The removal of available ions by microorganisms has agronomic importance. Microorganism reduce the quantity of plant available nutrients by this immobilization process.

3.3.4 Denitrification

Microbial reduction of nitrate to nitrate and then to gaseous nitrous oxide and nitrogen is known as **denitrification**. Mostly heterotrophic microorganisms carry out this process. Genera of bacteria such as *Alcaligenes, Agrobacterium, Bacillus, Flavobacterium, Propionibacterium* a *Pseudomonas* reduce nitrate under anaerobic condi Several chemoautotrophs such as *Thiobacillus* are able to utilize nitrate with the production of gaseous nitrogen. However, they are of little importance in most soils. The probable sequence of bacterial denitrification is as follows:

$$\underset{+5}{NO_3^-} \longrightarrow \underset{+3}{NO_2^-} \longrightarrow \underset{+2}{NO} \longrightarrow \underset{+1}{N_2O} \longrightarrow \underset{0}{N_2}$$

The reduction of nitrate in bacteria serves two different physiological purposes.

a) **Assimilatory nitrate reduction**: In this type, NO_3^- is reduced to NH_3 and is utilized for cell synthesis.

b) **Dissimilatory nitrate reduction**: In this process, NO_3^- is used as a terminal electron acceptor during respiration. It is also referred to as denitrification.

The initial step in denitrification (reduction of NO_3^- to NO_2^-) is catalyzed by the enzyme **nitrate reductase**. It contains molybdenum, iron and labile sulfide groups. The second step (reduction of NO_2^- to NO) is catalyzed by **nitrite reductase**. **Nitric oxide reductase** and **nitrous oxide reductase** are responsible for the reduction of nitric oxide and nitrous oxide, respectively. These enzymes are not yet adequately characterized.

Several factors such as nitrate levels, available organic matter, aeration, pH, temperature and moisture status of the soil affect this process.

Chemodenitrification: High levels of nitrite may be sometimes found in soil when NH_3 or NH_4^+ type fertilizers are applied to it at high rates. The high NO_2^- accumulations are attributed to inhibition of the second step of nitrification process, probably due to NH_3 toxicity to *Nitrobacter*. Nitrite is relatively unstable. It undergoes a series of reactions leading to the formation of gases. Under aerobic conditions, nitrite in soil is subject to chemical reactions that lead to production of gaseous nitrogen. This process is called chemodenitrification which takes place in a non-enzymatic way. The term side-tracking of nitrification has been used sometimes to designate nitrogen loss by this mechanism.

3.3.5 Nitrogen fixation

A major reservoir for nitrogen on earth is the atmosphere (386×10^{16} kg), where it exists as an inert gas. Plants and animals cannot use the gaseous form of nitrogen. However, a restricted group of microorganisms, called **diazotrophs**, can utilize the gaseous nitrogen (N_2) as a source of nitrogen.

The utilization of gaseous nitrogen as a source of nitrogen is called **nitrogen fixation**. It is carried out by certain procaryotes such as bacteria and cyanobacteria (Table 3.2). A wide range of free-living bacteria can fix nitrogen unde robic and anaerobic conditions. In addition there are some bacteria, often called symbiotic ba that fix nitrogen only when associated with the roots of specific host plants.

In the fixation process, nitrogen is reduced to ammonium. The mechanism of nitrogen fixation appears to be similar in most of the nitrogen fixing procaryotes. **Nitrogenase** enzyme catalyzes the reduction process. The enzyme consists of two proteins in a ratio of 2:1 azoferredoxin and molybdoferredoxin. Both proteins contain iron-sulfur centers. In addition, molybdoferredoxin contains molybdenum. The enzyme system is encoded by a genetic region called nif regulon.

Nitrogen fixation is a highly reductive process. Six electrons are required for the reduction of N_2 to $2NH_3$. N_2 is an extremely stable molecule. It contains a triple bond ($N \equiv N$). Its activation is a high energy demanding process. Hence, high levels of ATP are required for N_2-fixation.

Ferredoxin and flavodoxin, low potential iron-sulfur proteins, serve as electron donors. Reduced ferredoxin or flavodoxin transfers electrons to the azoferredoxin. Only the azoferredoxin component has ATP binding sites. At the expense of the energy of ATP hydrolysis, a super reduced molybdoferredoxin is formed. It binds N_2 and reduces it stepwise to ammonia. The sequence of electron transfer in nitrogen fixation is as follows.

$$\text{Electron donor} \rightarrow \text{Azoferredoxin} \rightarrow \text{Molybdoferredoxin} \rightarrow N_2 \rightarrow 2NH_3$$

Oxygen sensitivity: The enzyme nitrogenase is extremely sensitive to oxygen. It is irreversibly inactivated by oxygen. Hence N_2 fixation is regarded as a strictly anaerobic process. In strict anaerobes and phototrophs, to keep the nitrogenase system anaerobic is not a problem. However, it is a problem for aerobic bacteria and cyanobacteria. In aerobic bacteria the enzyme is protected from oxygen inactivation either by removal of oxygen by a very active branched respiratory system or by the production of oxygen retarding slime layer.

In most filamentous cyanobacteria, heterocysts are the sites of nitrogen fixation. They are larger than vegetative cells. They are devoid of photo system II. Therefore they cannot produce oxygen. They gain ATP by photophosphorylation. Vegetative cells adjacent to heterocysts provide metabolites that produce reducing power for N_2-fixation. These cells, in turn, receive ammonia from the heterocysts. Although only six electrons are sufficient to reduce N_2 to $2NH_3$, consumption of eight electrons has been recorded in this process. The two electrons are involved in the production of hydrogen.

$$N_2 + 16ATP + 8e^- + 8H^+ \xrightarrow{Mg^{2+}} 2NH_3 + H_2 + 16ADP + 16Pi$$

Steps in the reduction of N_2 to $2NH_3$ are given below:

$$N \equiv N$$
$$\downarrow \; 4H \; (H_2)$$
$$H\,N = NH$$
$$\downarrow 2\,H$$
$$H2N - NH_2$$
$$\downarrow 2H$$
$$H_3N - NH_3$$

Table 3.2 Some important nitrogen-fixing organisms

Free-living aerobes	Free-living anaerobes	Symbiotic
a) *Heterotrophs* *Azospirillum* *Azotobacter* *Beijerinckia* *Citrobacter* *Klebsiella*	a) *Heterotrophs* *Clostridium* *Desulfotomaculum* *Desulfovibrio*	a) *Legumes:* Legume plants such as beans, clover, peanut and soybean in association with *Rhizobium* or *Bradyrhizobium.*

b) Photorophs Cyanobacteria such as *Anabaena* *Gloeocapsa* *Nostoc* c) Chemotrophs *Alcaligenes* *Thiobacillus*	*b) Phototrophs* *Chromatium* *Chlorobium* *Rhodomicrobium* *Rhodopseudomonas* *Rhodospirillum* *c) Chemotrophs* *Methanococcus* *Methanosarcina*	b) *Non-legumes Alnus, Casuarina* and *Myrica* in association with *Frankia* (actinomycetes).

Symbiotic nitrogen - fixation in leguminous plants: Legumes represent a large group of plants that includes many economically important plants such as alfalfa, beans, clover, groundnut, peas and soybean. Infection of the roots of legumes with appropriate species of *Rhizobium* or *Bradyrhizobium* leads to the formation of root nodules which are the sites for nitrogen fixation. Under normal conditions, neither legume nor the bacterium alone, is able to fix nitrogen; only when the two are associated this process occurs. It is a mutualistic symbiosis in which the plant provides the organic energy source needed by the bacteria, and the bacteria provide fixed nitrogen for plant growth.

There is a marked specificity between the species of legume and the strains of *Rhizobium*. A *Rhizobium* strain is generally able to infect certain species of legumes and not others. A cross-inoculation group consists of a group of *Rhizobium* strains that are able to infect a group of related legumes. The major cross-inoculation groups are listed in Table 3.3.

Table 3.3 Major cross-inoculation groups

Cross-inoculation group	**Plant hosts**
Fast growing	
Rhizobium leguminosarum	*Pisum, Vicia,Lathyrus*
R. phaseoli	*Phaseolus*
R. trifolii	*Trifolium*
R. meliloti	*Medicago, Melilotus, Trigonella*
Slow growing	
Bradyrhizobium lupini	*Lupinus*
B. Japonicum	*Glycine,* some cowpeas
B. spp "Cowpea"	*Vigna*

Nodule formation: The roots of legumes secrete a variety of organic compounds which stimulate the growth of microflora surrounding the root zone. Rhizobia in soil are attracted to root surface and proliferate there by using the organic compounds. The bacteria attach to root hair by specific mechanisms that involve the interaction of complementary macromolecules. The initial penetration

of *Rhizobium* cells into the root hair is through the root-hair tip. Following binding, the root-hair tip tightly curls into a form resembling a shephard's crook. Bacteria enter the root hair and induce the formation of an infection thread, which spreads down the root-hair. The bacteria multiply rapidly in the host cortical cells. They loose their rod shaped structure and become pleomorphic, club shaped cells called **bacteroids**. They also develop the enzyme complex **nitrogenase** required for nitrogen fixation. Bacteroids are surrounded by portions of the plant cell membrane called peribacteriod membrane. Nitrogenase is localized within the bacteroids. It is never released into the plant cytosol. It has characteristics similar to the enzyme from free-living nitrogen-fixing bacteria. It is able to reduce acetylene as well as nitrogen. It is extremely sensitive to oxygen.

Following infection the host tissue enlarges by cell division forming root nodule. In an active nodule up to 10,000 bacteroids may be found per root cell. As the N_2-fixing enzyme nitrogenase is extremely oxygen-sensitive, the level of oxygen in nodules is controlled by the oxygen binding protein called **leghemoglobin**. It is a red, iron-containing protein found in healthy nodules. Neither plant nor *Rhizobium* alone synthesizes leghemoglobin. Its heme and globin portions are synthesized by the bacterium and plant, respectively.

The mechanism of N_2-fixation in root nodules is similar to that found in free-living nitrogen fixers. The first stable product of N_2-fixation is ammonia. Assimilation of ammonia into organic nitrogen compounds in the root nodule is carried out by the plant.

Nitrogen fixation by the legume - *Rhizobium* symbiosis has considerable agricultural importance, as it leads to a significant increases in combined nitrogen in soil.

3.4 PHOSPHORUS CYCLE

Phosphorus exists in nature in a variety of organic and inorganic forms but primarily in either insoluble or only very poorly soluble inorganic forms. The total amount of phosphorus in the earth's crust is of the order of 10^{15} metric tons. Estimates of the amounts of phosphorus lodged in certain terrestrial and oceanic reservoirs are shown in Table 3.4

Table 3.4 Major Reservoirs of phosphorus in the earth

Reservoir	Total Phosphorus ($X10^{12}$ kg)
Land	
Soil	96-160
Mineable rock	19
Biota	2.6
fresh water (dissolved)	0.090
Ocean	
Sediments	840,000
Dissolved (inorganic)	80
Detritus (particulates)	0.65
Biota	0.050 – 0.12

Microorganisms are intimately involved in the cycling of phosphorus. They bring about a number of transformations of the element (Fig. 3.7). These include:

- altering the solubility of inorganic compounds of phosphorus.
- mineralizing organic compounds with the release of inorganic phosphate.
- converting the inorganic, available anion into cell components (Immobilization process)
- bringing about an oxidation or reduction of inorganic phosphorus compounds.

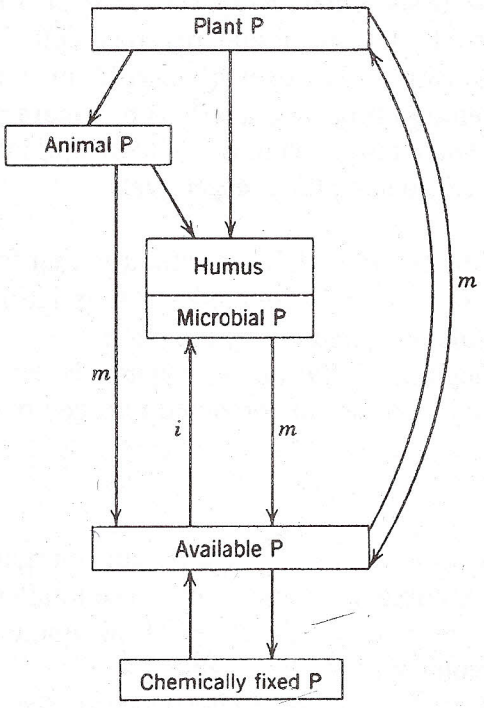


Fig. 3.7 A simplified phosphorus cycle. The letters *m* and *i* denote mineralization and immobilization of phosphorus.

Solubilization of inorganic phosphorus: Soil organisms and plant roots participate in the solubilization of soil phosphorus, mainly through their production of CO_2 and organic acids. Attempts were made to isolate the specific bacteria responsible for solubilization and to use such organisms as soil or seed inoculants. Most frequently isolated were species of *Pseudomonas* and *Bacillus*. In past years, *Bacillus megaterium* var. *phosphaticum* was widely used in a bacterial inoculant known as phosphobacterin.

Mineralization of organic phosphorus: The organically bound phosphorus usually constitutes 30-50% of the total phosphorus in most soils, although it may range from as low as 5% to as high

as 95%. Organic phosphorus occurs in soils principally as phytates, nucleic acids and their derivatives, and phospholipids.

Microorganisms play an important role in converting the organic phosphorus to inorganic forms. This process of mineralization is generally more rapid in virgin soils than in their cultivated counter parts. The enzymes that cleave phosphorus from the more frequently encountered organic substrates are collectively called **phosphatases**. The enzyme phytase liberates phosphate from phytic acid or its calcium – magnesium salt, phytin with the accumulation of inositol. Phytase activity is widespread, and some 30 to 50% of the isolates from soil synthesizing the enzyme. Species of *Aspergillus, Penicillium, Rhizopus, Cunninghamella, Arthrobacter, Streptomyces, Pseudomonas* and *Bacillus* can synthesize the enzyme.

Bacteria, actinomycetes and fungi are able to use nucleic acids and phospholipids as a phosphorus source. Mycorrhizal fungi frequently have a dramatic and important effect on plants whose roots harbor these symbionts. Growth of plants in phosphorus – poor soils is markedly enhanced if the roots develop mycorrhizae as contrasted with those not bearing the fungus. The symbiotic association frequently allows for as extensive phosphate uptake in phosphorus – deficient environments as would occur on the addition of fertilizer phosphorus.

Immobilization: Microbial growth requires the presence of available forms of phosphorus. In environments where phosphorus is limiting, its addition will therefore stimulate microbiological activities. The assimilation of phosphorus into microbial nucleic acids, phospholipids or other protoplasmic substances leads to the accumulation of non-utilizable forms of the element.

Phosphorus, like nitrogen, is therefore both mineralized and immobilized. The process that predominates is governed by the percentage of phosphorus in the plant residues undergoing decay and the nutrient requirements of the responsible populations.

Oxidation - reduction reactions: Phosphorus, like nitrogen, may exist in a number of oxidation states ranging from the –3 of the phosphine, pH_3 to the oxidized state, +5 of orthophosphate. Biological oxidation of reduced phosphorus compounds is evident when phosphite is added to soil. The phosphite disappears with a corresponding increase in the concentration of phosphate.

$$H PO_3^{=} \rightarrow H PO_4^{=}$$

A number of heterotrophic bacteria, actinomycetes and fungi utilize phosphite as sole phosphorus source in culture media and oxidize phosphite within the cell to organic phosphate compounds.

The possibility of the reverse process, a reductive pathway, has also received some attention. When certain soil samples are incubated anaerobically in a mannitol – $NH_4H_2PO_4$ medium, the phosphate disappears relatively rapidly. Phosphate apparently is reduced to phosphite and hypophosphite.

3.5 SUMMARY

Microorganisms play an active role in the transformations of carbon, nitrogen and phosphorus. The carbon cycle revolves about CO_2 and its fixation and regeneration. Chlorophyll containing plants utilize the gas as their sole carbon source and the carbonaceous matter thus synthesized serves to supply the animal world with preformed organic carbon. Upon the death of the plant or animal, microbial metabolism assumes the dominant role in the cyclic sequence. The cycle is completed and carbon made available with the final decomposition and production of CO_2 from humans and the rotting substances.

Nitrogen undergoes a number of transformations that involve organic, inorganic and volatile compounds. The portions of the nitrogen cycle governed by microbial metabolism are composed of by several individual transformations such as nitrogen mineralization, nitrogen immobilization, denitrification and nitrogen fixation.

Micoorganisms are also intimately involved in the cycling of phosphorus. They bring about a number of transformations of the element including – altering the solubility of inorganic phosphorus compounds, mineralizing organic compounds with release of inorganic phosphorus, converting the inorganic forms into cell components and bringing about the oxidation or reduction of inorganic phosphorus compounds.

3.6 MODEL QUESTIONS

1. Discuss the role of microorganisms in the transformation of nitrogenous compounds.
2. Bring out the salient features of carbon cycle.
3. Write in detail about the role played by microorganisms in phosphorus transformations.
4. Write short notes on:
   a) Organic matter decomposition
   b) Nitrogen fixation
   c) Nitrification

3.6 REFERENCE BOOKS

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Dr. M. VIJAYALAKSHMI

Unit-I

Lesson 4

PLANT VIRUSES

4.0 **OBJECTIVE:**

In this lesson, general account of Plant Viruses, their classification, transmission and control are discussed.

4.1 INTRODUCTION

4.2 GENERAL ACCOUNT OF VIRUSES

4.3 CLASSIFICATION OF VIRUSES

4.4 TRANSMISSION OF VIRUSES

4.4.1 Transmission of viral diseases not mediated by vectors

4.4.2 Vector mediated transmission

4.5 CONTROL OF VIRAL DISEASES

4.6 SUMMARY

4.7 MODEL QUESTIONS

4.8 REFERENCE BOOKS

4.1 INTRODUCTION

Plant viruses are submicroscopic, infectious, nucleoprotein particles that multiply only intracellularly and are potentially pathogenic. In 1882, Mayer described a disease of the tobacco plant which he called **Mosaikkrankheit**, and this term is now widely used to describe the mottling type of virus disease. Mayer showed that this mosaic disease of tobacco could be transmitted to a healthy tobacco plant by inoculation with the sap of the infected plant. Iwanowski (1892) showed that the cause of the mosaic disease of tobacco passed through a bacteria-proof filter. A few years later, Beijerinck confirmed Iwanowski's results and concluding that the pathogen causing tobacco mosaic was different from a bacterium. He called it *Contagium vivum fluidum* or contagious living fluid.

Mulvania (1926) found that the infections agents of tobacco mosaic could be precipitated by protein precipitants, such as $(NH_4)_2\ SO_4$ without the loss of infectivity. Vinson (1929) and Petre

(1931) obtained an infectious, colorless preparation by this method and concluded that it contained nitrogen. Stanley (1935) crystallized tobacco mosaic virus (TMV) and concluded that it was a protein. For his work, he was awarded the Nobel Prize. Bawden and Pirie (1936) reported TMV as a nucleoprotein.

Stream birefringence (double refraction) by Takahashi and X-ray analyses by Franklin of TMV preparations indicated that it was rod-like in shape. Since the work on TMV, many viruses have been isolated from plants and found to vary in their morphology, for example, rods, flexuous rods, polyhedral bodies etc. All plant viruses were earlier reported to contain only RNA. Later, it was found that cauliflower mosaic virus and perhaps other similar viruses contain DNA.

4.2 GENERAL ACCOUNT OF VIRUSES

A virus particle is made up of a single type of nucleic acid (either RNA or DNA) which is enclosed in a protein coat. The nucleic acid is called a core and the particle is referred to as a virion. The protein coat of virus particle is called capsid and along with the nucleic acid, it is called a nucleocapsid. In some viruses, the virion is enclosed in a lipid or lipoprotein envelope. Viruses do not possess any metabolism of their own and use the host metabolic and genetic apparatus for their multiplication. This process is called replication. The virus particles are extremely small, ranging from about 20 nm to 400 nm.

Shape and size of viruses: Shapewise, most viruses would fall in any one of the following categories: a) spheroidal, also known as cuboidal, b) elongated or rod-like, and c) mixed or combination particles with shapes which are an admixture of the first two types (Fig. 4.1) Shapes of virus species falling into a particular category may show variation amongst themselves. For example, rod shaped particles could be simple elongate (tobacco rattle virus), coir shaped (tobacco mosaic virus) or flexuous (potato virus x). Similar variation within spheroidal types are also quite common. Shapes and sizes of some plant viruses are shown in Table 4.1.

Table 4.1 Shapes and sizes of some groups of plant viruses

Virus	Shape	Size dimension (nm)	Special feature
Alfalfa Mosaic	Pleomorphic	18x18; 18x36	Aggregation of three bullet shaped and one spheroidal particle
Beet Yellow	Plexuous rods	10x1250	
Brome mosaic	Spheroidal	25	
Carnation latent	Rods	15x650	Slight bents
Cauliflower mosaic	Spheroidal	50	
Virus	**Shape**	**Size dimension (nm)**	**Special feature**
Clover wound tumour	Spheroidal	70	

Cowpea mosaic	Spheroidal	28-30	
Cucumber mosaic	Spheroidal	30	
Dahlia mosaic	Spheroidal	50	
Lettuce necrotic yellow	Bullet shaped	66x227	Enveloped
Pea enation mosaic	Spheroidal	28	
Potato virus X	Rods	13x480-580	Flexuous
Potato virus Y	Rods	15x730-790	Flexuous
Potato yellow dwarf	Rod shaped	50-100x200-300	With envelope
Southern bean mosaic	Spheroidal	30	Filamentous
Tobacco mosaic	Rod shaped	18x300	Cylindrical rod
Toabcco necrosis	Spheroidal	28	
Tobacco ringspot	Spheroidal	30	
Tomato bushy stunt	Spheroidal	36	
Tomato spotted wilt	Spheroidal	70-80	Enveloped
Turnip yellow mosaic	Spheroidal	30	

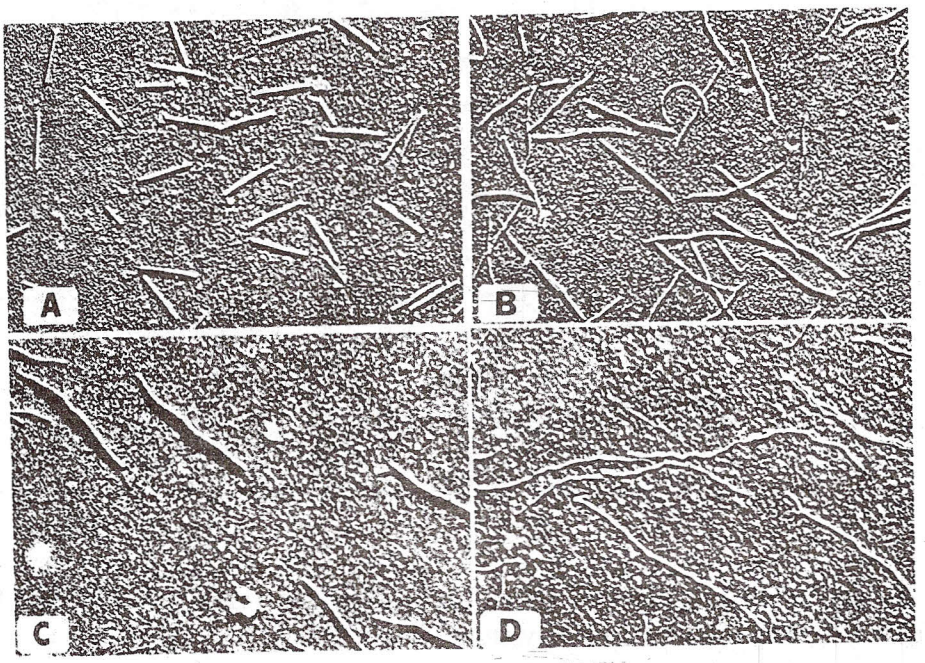

Fig. 4.1 Some rod-shaped plant viruses; (A) tobacco mosaic virus; (B) potato virus X; (C) common bean mosaic virus; (D) beet yellows virus.

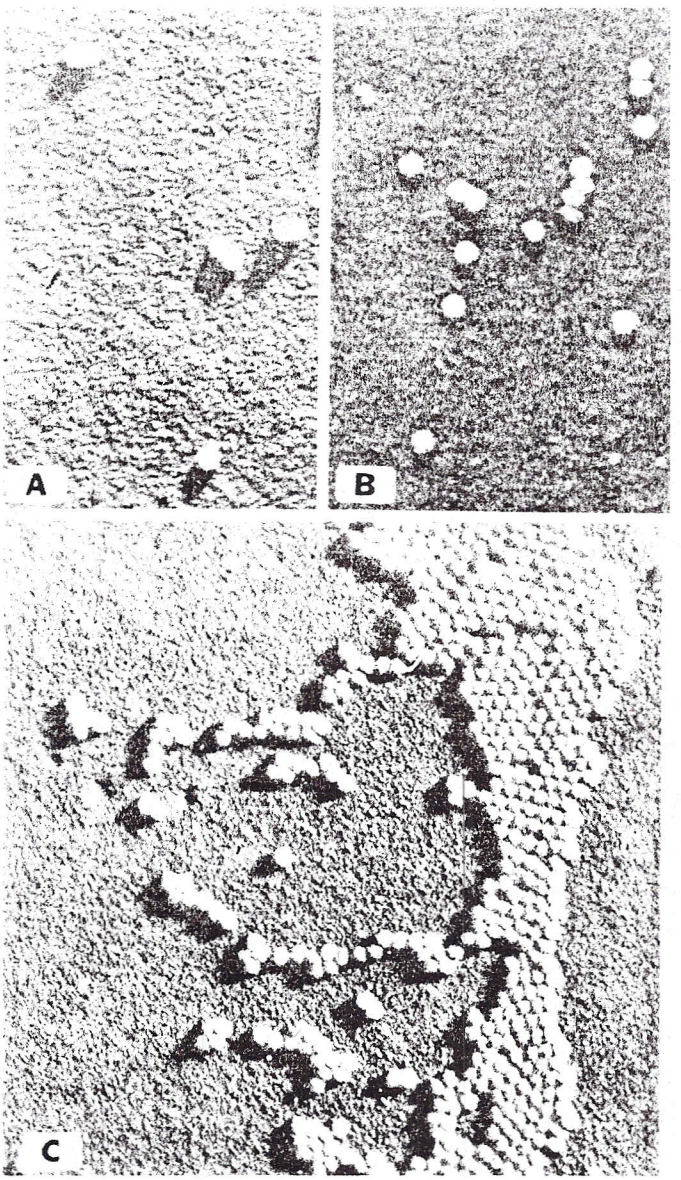

Fig. 4.1a Some polyhedral viruses; (A) tobacco ring spot virus x 170,000; (B) turnip yellow mosaic virus x 170,000; (C) tomato bushy stunt virus x 130,000.

The chemical composition of some plant viruses is presented in Table 4.2

Table 4.2 Chemical composition of some plant viruses
(As percentages)

Virus	RNA	DNA	Proteins	Lipids	Carbo-hydrates
Alfalfa mosaic	19	--	81	--	--
Broad bean mottle	22	--	78	--	--
Carnation latent	6	--	94	--	--
Cauliflower mosaic	--	16	84	--	--
Cucumber mosaic	18	--	82	--	--
Cyanophage LPP I	--	40	60	--	--
Golden yellow mosaic	--	23	77	--	--
Pea enation mosaic	29	--	71	--	--
Potato spindle tuber (Viriod)	100	--	--	--	--
Potato X	6	--	94	--	--
Tobacco necrosis	19	--	81	--	--
Tobacco mosaic	5	--	95	--	--
Tomato bushy stunt	17	--	83	--	--
Tomato spotted wilt	5	--	71	19	5
Turnip yellow mosaic	34	--	67	--	--
Wound tumour	23	--	77	--	--

Symmetry of Viruses: The manner in which the coat proteins and the nucleic acid core are arranged determines the symmetry of virus particles. Two main types of symmetry are recognized in viruses which correspond to the two primary shapes, rod and spherical.

Helical symmetry: The nucleic acid core is arranged in a coiled spring-like manner and is enclosed in a protein coat in which the subunits of protein are also arranged in a helical manner. Such virions appear as rods or filaments and are called rod-shaped, tubular or filamentous viruses, and their symmetry is said to be helical symmetry.

Tobacco mosaic virus is a rigid helical rod, 300 nm long and 18 nm in diameter (Fig. 4.2). It contains approximately 95% protein and 5% RNA. X-ray diffraction analyses gave a detailed picture about the arrangements of the protein subunits and the RNA in the rod. The particle contains approximately 2130 subunits that are closely packed in a helical array. The coat protein comprises of 158 amino acids with a molecular weight of 17-18 kDa.

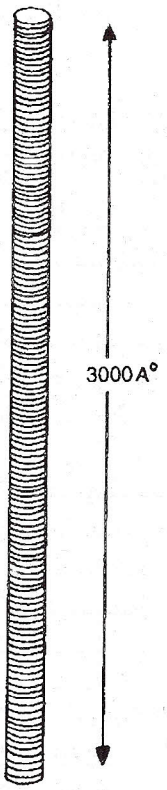

Fig. 4.2 Tobacco mosaic virus

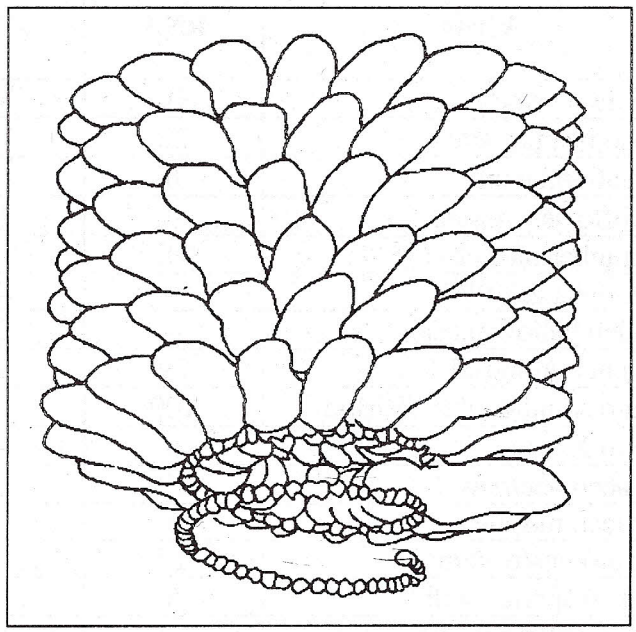

Fig. 4.2a Arrangement of nucleic acid and capsid in TMV

Icosahedral symmetry: An icosahedron is a symmetric structure roughly spherical in shape that has 20 triangular faces and 12 vertices (Fig. 4.3). Icosahedral symmetry is the most efficient arrangement for subunits in a closed shell because it uses the smallest number of units to build a shell. Ex: turrip yellow mosaic virus.

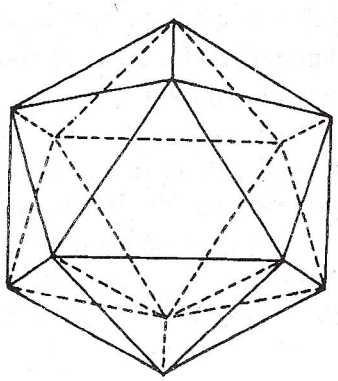

Fig. 4.3 Icosahedron

Enveloped viruses: Many viruses have a complex membranous structures surrounding the nucleocapsid. These are called Enveloped viruses. These viruses are common in the animal world, but enveloped bacterial and plant viruses are also known. The virus envelope consists of a lipid bilayer with proteins, usually glycoproteins, embedded in it.

Complex viruses: Some virions are more complex, being composed of several separate parts, each with separate shapes and symmetries. The most complicated viruses in terms of structure are some of the bacterial viruses, which possess not only icosahedral heads but also helical tails.

The greatest single advancement in elucidating the structure of viruses was the invention of the electron microscope by Knoll and Ruska in 1932. Because of their small size, virus particles cannot be seen through a light microscope. Instead of using a beam of light, the electron microscope emits a beam of electrons with a wavelength of about 0.05 A°. This beam is aligned and adjusted by magnetic rather glass lenses.

Three tec' . iques used to enhance the visibility of viruses are **shadow casting, positive staining** and **negative staining**. In shadow casting, the virus particles are subjected to bombardment at an oblique angle with atoms of a heavy metal such as platinum or uranium. The metal sprayed at an angle forms a coat over the particle, which in turn casts a 'shadow' where the metal does not fall.

In positive staining, an electron-dense material such as a heavy metal compound actually combines with the specimen; and in negative staining, the electron-dense material surrounds or infiltrates the pores of the specimen. These staining techniques can also be used to detect whole virus particles in plant cells. The use of each of these techniques is illustrated on specimens of barley stripe mosaic virus in Fig. 4.4. The shadow-casted specimen shows the shape of the virus and the stained specimens show the central hole of the virus.

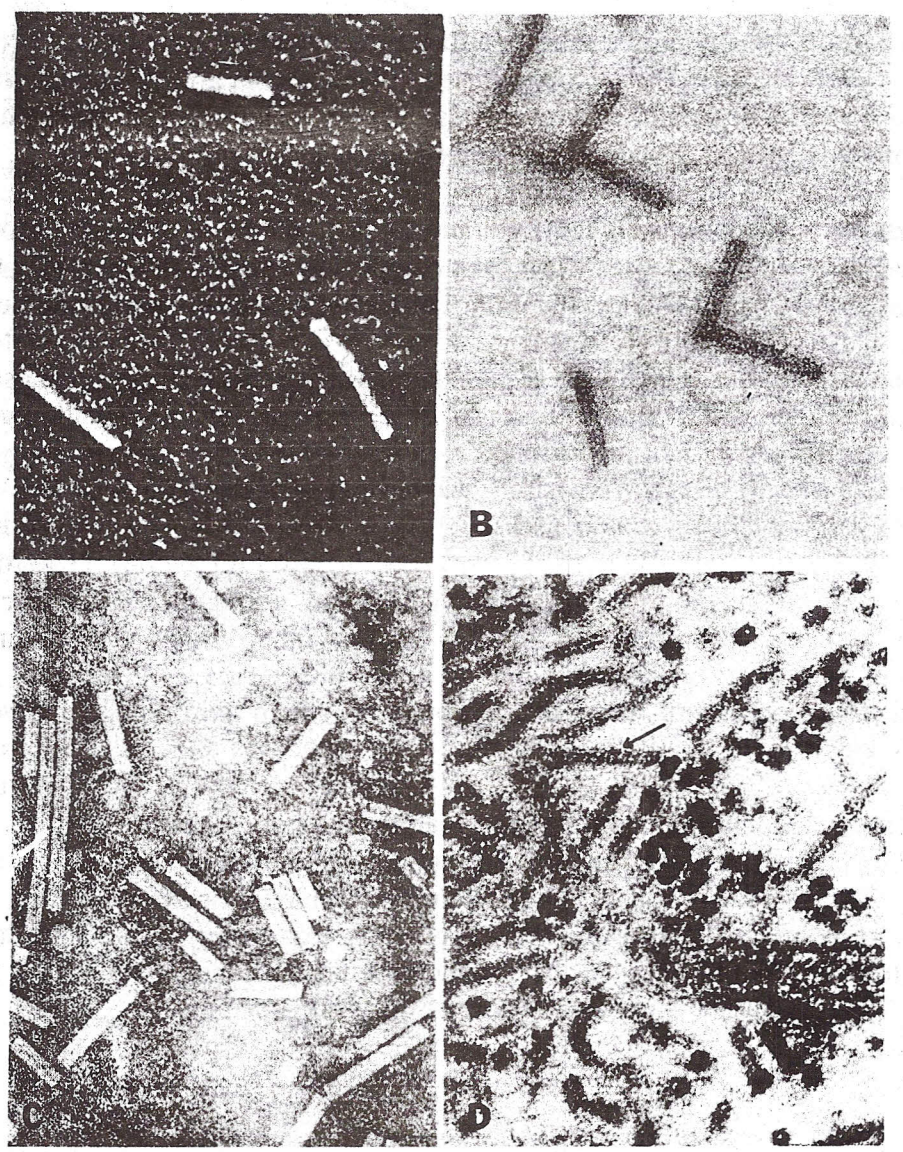

Fig. 4.4 Electron microscopy of barley stripe mosaic virus. The virus particles in the photo (A) have been shadowed with palladium. The particles in the photos in (B) and (C) have been positively and negatively stained; respectively, with uranyl acetate. The photo in (D) shows the presence of intact virus particles in the cytoplasm of the host cell.

Viruses can be isolated from plant tissue extracts in a suitable environment of pH, temperature and ionic strength by treatment with organic solvents, filtration and centrifugation. Various techniques, including gel filtration, electrophoresis and density gradient centrifugation can be employed for further purification of virus.

Viruses are commonly characterised according to their size and shape and relative per cent of nucleic acid and protein. Other chemical and physical measurements of a virus may reveal its particle weight, kinds and arrangement of amino acids in the coat protein, relative arrangement of

nucleic acid and protein and serological reactivity. Identity of a virus is based on such features as its host range, thermal inactivation point, longevity *in vitro*, dilution end point, transmission characteristics, cross protection reactions, serological reactivity, plus any chemical or physical features of it that are known.

Replication of plant viruses: Replication of plant viruses is different from the process observed in the case of bacteriophages. The initial process of entry of the virus particle into the host cell has been found to involve entry of virus particles *in toto*. There is no prior separation of the protein coat from the nucleic acid core. The process of initial entry thus is analogous to the process of swallowing or pinocytosis. However, often injury on the surface of the host cell facilitates infection. A major number of plant viruses are vector transmitted. The mouth parts of the vectors play an important role in injecting the virus particles into the host cells.

Plant viruses do not generally replicate in the cells into which they are initially injected. They are transported through plasmodesmata from cell to cell till they reach site for multiplication. Sometimes specific sites of infection are needed for successful multiplication. For example, barley yellow dwarf virus is phloem limited and would multiply only in phloem sieve element.

The information concerning multiplication of viruses in plants are based mostly upon investigations carried with tobacco mosaic virus. Although direct evidences illustrating the sequential occurrence of the process of replication in TMV are not as yet fully available, a reasonably clear picture does emerge out of the various studies mentioned earlier. These could be summarised as follows:

(1) The virus particles make entry into the host tissue directly, either through the stomatal openings or through microscopic injuries on the surface.

(2) The particles enter the cell *in toto*, possibly by means of a mechanism analogous to pino or *phagocytosis*.

(3) The particles remain intact and may pass on from one cell to the next via the plasmodesmata.

(4) Some of the particles invade the nucleus through the nuclear membrane.

(5) Inside the nucleus, the nucleolus becomes the main centre of activity.

(6) The metabolic functions of the host cell nucleus comes under the control of the viral nucleic acid.

(7) RNA dependent RNA synthesis occurs in the nucleus.

(8) Viral capsid proteins are also synthesised within the nucleus.

(9) Assembly of protein subunits and the nucleic acid starts within the nucleus. However, incomplete particles may also come out of the nucleus into the cytoplasm and assembly completed there.

The above scheme seems to be operative in the case of other plant viruses as well. In tomato spotted wilt virus (TSWV) the process has been found to be similar except that the process of assembly probably occurs in association with the endoplasmic reticulum. In sugar beet yellow virus (SBYV), vescicles are reported to develop around newly synthesised nucleic acid molecules. These vescicles remain within the nucleus and particles assembly occurs within them.

4.3 CLASSIFICATION OF VIRUSES

Viruses were earlier classified on the basis of their natural hosts as plant viruses, invertebrate viruses, vertebrate viruses and dual host viruses. In recent years, several systems of classification have been proposed. All of them proved to be controversial and failed to find general acceptance. To resolve the problem, a separate agency, the International Committee on Nomenclature of Viruses (ICNV) was set up in 1966. The ICNV set up different sub-committees particularly the International Committee for Taxonomy of viruses (ICTV) to look into various broad groups of viruses and devise syste of classification for them on the basis of a uniform set of principles.

ICTV specifies the suffixes for the various taxa and rules for written descriptions of viruses. Names for genera, subfamilies, families and orders must be ending with suffixes – virus, - virinae, -viridae and – virales respectively. Several criteria such as virion properties, genome organization and replication, antigenic properties and biological properties are taken into consideration for the classification of viruses. The details are as follows:

1) Virion properties:

a) Morphological properties of virions
   - i) Shape
   - ii) Size
   - iii) Presence or absence of an envelope
   - iv) Capsomeric symmetry and structure

b) Physical properties of virions
   - i) Molecular mass
   - ii) Buoyant density
   - iii) Sedimentation coefficient
   - iv) pH stability
   - v) Thermal stability
   - vi) Cation (Mg^{2+}, Mn^{2+}, Ca^{2+}) stability
   - vii) Solvent stability

viii) Detergent stability
ix) Radiation stability

c) Properties of the genome
   i) Type of nucleic acid, DNA/RNA
   ii) Strandedness: Single-stranded / double-stranded
   iii) Linear or circular
   iv) Sense: Positive / negative / ambisense
   v) Size of genome or genome segments

d) Properties of proteins
   i) Number
   ii) Size
   iii) Functional activities
   iv) Amino acid sequence comparisons

e) Lipids
   i) Presence or absence
   ii) Nature

f) Carbohydrates
   i) Presence or absence
   ii) Nature

2. Genome organization and replication
   a) Genome organization
   b) Strategy of replication of nucleic acid
   c) Characteristics of transcription
   d) Characteristics of translation and post-translational processing
   e) Sites of accumulation of virion proteins, site of assembly, site of maturation and release.
   f) Cytopathology, inclusion body formation

3. Antigenic properties
   a) Serological relationships
   b) Mapping epitopes

4. Biological properties
   a) Host range, natural and experimental
   b) Pathogenecity, association with disease
   c) Pathology, Histopathology
   d) Mode of transmission
   e) Vector relationships
   f) Geographic distribution

5)

Basing on these characters, ICTV has identified a number of families, subfamilies, genera and species in six major groups: 1. Double stranded DNA viruses, 2. Single stranded DNA viruses, 3. DNA and RNA reverse transcribing viruses, 4. Double stranded RNA viruses, 5. Negative sense single stranded RNA viruses and 6. Positive sense single stranded RNA viruses.

Some examples of plant viruses belonging to the major groups are given below:

Single-stranded DNA viruses: Maize streak virus, beet curly top virus, tomato golden mosaic virus belong to Geminiviridae.

DNA and RNA reverse transcribing viruses: Cauliflower mosaic virus, commelina yellow mottle virus.

Double-stranded RNA viruses: Wound tumor virus, Fiji disease virus, rice ragged stunt virus, while clover cryptic virus.

Negative-sense, single-stranded RNA viruses: Lettuce necrotic yellows virus, potato yellow dwarf virus, tomato spotted wilt virus.

Positive-sense, single-stranded RNA viruses: Parsnip yellow fleck virus, rice tungro virus, cowpea mosaic virus, tobacco ring spot virus, potato virus 4, barley yellow mosaic virus, southern bean mosaic virus, tomato bushy stunt virus, tobacco necrosis virus, tobacco mosaic virus, tobacco rattle virus, barley stripe mosaic virus, cucumber mosaic virus, alfalfa mosaic virus, turnip yellow mosaic virus.

Subviral agents: Viroids: Diener (1971) discovered the plant viroids in the potato crop which causes spindle tuber disease. They are small circular molecules and a few hundred nucleotides long. They are classified into two families, the Pospiviroidae and the Avsunviroidae. Potato spindle tuber viroid and coconut cadang cadang viroid belong to pospiviroidae. Avocado sunblotch viroid belongs to Avsunviroidae. The disease symptoms caused by viroids include stunting, mottling, leaf distortion and necrosis. They are readily transmitted by mechanical means through the contaminated tools and implements.

Most viroids have a relatively high G + C content. They replicate in the nucleus of the host cells. Diagnostic procedures for viroids include biological tests, gel electrophoresis, nucleic acid hybridization and reverse transcription polymerase chain reaction.

4.4 TRANSMISSION OF VIRUSES

The unit of inoculum for plant viruses is the virus particle itself. Although, it is known that the nucleic acid portion of the virus particle is the infectious entity, in nature the entire virus particle is undoubtedly the unit that is transmitted from one host to another. The modes of spread of

viruses and consequently the diseases spread of them, are many. To some extent, these means are dependent upon the nature of the host organism. In general, two principal categories of means of transmission are noted to be operative. These are: (a) those transmitted without the intervention of any vectors, and (b) those mediated through an intermediary organism, called the vector.

4.4.1 Transmission of viral diseases not mediated by vectors

Transmission through contact, direct or indirect, is the main method of transmission with many plant viruses. This is particularly true of disease of crop plants. For example, potato virus-X spreads in the field by contact between both roots and crown of infected and healthy individuals. Contact between aerial parts is most common in strong breeze.

Transmission of viral diseases of plants through the agency of various propagating agents is of common occurrence. Transmission through seeds is known to occur in lettuce mosaic virus caused diseases. Transmission of viral diseases through the means of vegetative propagation is of most common occurrence. All plant viruses which are systemic in their hosts are carried through such propagating organs as tubers, bulbs, rhizomes, cuttings and buds. Rattoon stunt disease of sugarcane is transmitted in this manner from one generation to another.

Plant viruses causing soil-borne diseases are generally carried through the agency of soil microorganisms. Direct mechanical contact between roots and virus is sufficient for the spread of a disease as with tobacco necrosis. Wound or abrasion on the root surface facilitates such transmission.

Artificial transmission of viruses has been practised from very early days of virus pathology. Two procedures of artificial transmission are generally prevalent, namely: (1) mechanical inoculation, and (2) grafting.

Mechanical inoculation of hosts is achieved simply. The sap or extract of the infected host is used as the inoculum. Sometimes, chemical treatment of the extracted sap is necessary to prevent its destruction by oxidation. For example, sap of tomato spotted wilt has been found remain viable only when treated with sodium sulphite.

The leaves of plants to be inoculated are dusted lightly with some abrasive material such as carborandum powder. Then a piece of muslin cloth is dipped into the inoculum and the leaves are rubbed gently. The excess inoculum is washed off the leaf surface. Almost all the important plant virus diseases, like tobacco mosaic, tobacco ring spot, tomato spotted wilt and mosaic of bean have been sub-transmitted artificially.

Viruses that are systemic in their hosts can be transmitted by **grafting** between susceptible or compatible plants. In case of herbaceous plants, the leafy upper portions (scion) of one individual are suitably trimmed and inserted into the cut stem of another (stock). Usually, the scion

is virus infected rather than the stock. Grafting has been found a successful means for transmission of such viruses as alfalfa mosaic virus, potato virus Y, tomato spotted wilt virus etc.

4.4.2 Vector mediated transmission

Many viruses are dependent upon other organisms for their transmission from one host to other potential hosts. The agents responsible for such transport are called **vectors**. Most, though not all, organisms acting as vectors are insects. A few nematodes and even fungi are known to act as vectors for virus diseases.

Insects: A large number of economically important plant viruses are transmitted by insects, including aphids, leaf hoppers, thrips, white flies, beetles and mealy bugs. The ephids and leaf hoppers are probably the most important. The mites, while not true insects are also known to carry several important viruses (Table 4.3).

Table 4.3 Plant viruses and their insect vectors

Virus	Vectors
Bean mosaic	*Myzus persicae* (Aphid)
Citrus quick decline	*Myzus persicae* (Apid)
Maize streak	*Balclutha nibila* (leaf hooper)
Papaya mosaic	*Aphis gossypi* (Aphid)
Potato leaf roll	*Myzus solanifolia*
Rice Tungro	*Nephotettix apicalis*
Tobacco leaf curl	*Bemisia tobacci* (white fly)
Tomato spotted wilt	*Thysanoptera* (Thrips)
Wheat striate mosaic	*Delphacodes pellucida*

The relationship of the virus to its vector may range from a strictly mechanical contamination of mouth and body parts to one of intimate biological association of the virus with the insect. The case where a virus has no intimate biological relationship with an insect vector is illustrated by TMV and grasshoppers. These insects can pick up the virus on their mouth parts or on their feet and transmit it to healthy plants. Thus the virus has only a mechanical relationship to the vector.

Most viruses have a relationship with the insect more intimate than a mechanical contamination of body parts. These relationships may be classified into three main categories: stylet-borne, circulative and propagative viruses. The **Stylet-borne viruses** are carried by the insect on the tip of the stylet. Most stylet-borne viruses are associated with aphids but the specificity of association varies.

The next category of virus-vector relationship is the **circulative viruses**. This derives from the fact that these viruses are acquired by the insect through its mouth parts and are accumulated internally and passed through the tissue of the insect to be introduced later back into the plant via the mouth parts. Viruses of this type are transmitted by aphids and leaf hoppers. With most viruses of this type there is a "latent" period of from a few hours to several days between the time when the insect acquires the virus and the time the insect is capable of transmitting the virus.

The **Propagative Viruses** are similar in most respects to the circulative viruses but have the additional property of multiplying within the insect vector. The latent period with these viruses is probably due to both the time for the virus to circulate within the insects body and the time required for the virus to multiply within the insect to a point where the virus titer is high enough for transmission and infection to take place. Table 4.4 summarises the differences between the three categories of virus – insect vector relationships.

Table 4.4 A comparison of the relationships between stylet-borne, circulative, and propagative viruses and their vectors.

	Stylet-Borne	Circulative	Propagative
1.Most common vector	1. Aphids	1. Leafhoppers and Aphids	1. Leafhoppers and Aphids
2. Retention period within vector	2. Short	2. Long	2. Long
3. Time of feeding period necessary to acquire virus	3. Short	3. Long	3. Long
4. Latent period	4. Little or none	4. Hours to days	4. Hours to days
5. Efficiency of trans-mission increased by fasting	5. Yes	5. No	5. No
6. Vector specificity	6. Low	6. High	6. High
7. Multiplication within vector	7. No	7. No	7. No

Fungi: Several viruses have been shown to be transmitted by fungi. The virus causing big-vein disease of lettuce is transmitted by the zoospores of *Olpidium* and survives within the resting spores of this fungus. *Olpidium* also transmits other viruses including tobacco necrosis virus. Various other viruses that are termed "soil-borne" are also known to be transmitted by various chytridiomycetes.

Nematodes: Some nematodes have been shown to be disseminators of viruses. For example, grapevine fan leaf virus is transmitted by *Xiphinema index.*

Man: Man serves to disseminate pathogens in two ways – on his person, or in the things that he transports from one area to another. Tobacco mosaic virus is known to survive in dry tobacco leaves. Hence, workers who handle tobacco plants in the field are advised not to smoke while they are working and to thoroughly wash their hands before entering a field. Also, workers who tend tomatoes in glass houses are advised to keep a separate set of cover alls for each house to prevent

spread of TMV by their clothing. The long-distance dissemination of plant pathogens by man is usually accomplished by the transport of infected plant parts such as seeds or nursery stock.

4.5 CONTROL OF VIRAL DISEASES

There are various methods of approach to the problem of the control of plant virus diseases. These methods can be classified under the following six heads.

a) Elimination of the source of infection
b) Avoiding the vectors
c) Direct attack on the vectors
d) Breeding resistant varieties of crops
e) Cure of virus-infected plants
f) Special methods of propagation

Elimination of the sources of virus infection

Wild plants infected with viruses may act as sources of infection for cultivated crops. Cucumber mosaic, cabbage black-ring spot, lucerne mosaic and potato Y viruses are known to infect many wild hosts. Viruses which have a fairly wide host range can be brought to one crop from another cultivated crop. For example, clover is the host of several viruses which affect peas and beans. Remnants of the previous year's crop are frequently important sources of virus. 'Volunteer' potatoes and sugarbeet, which are usually infected with one or more viruses, are good examples. Another important point is careful attention to roguing out any obviously virus diseased plants and also any ground-keepers which are usually a prolific source of virus infection.

Avoiding the vectors: This can be achieved in various ways, by isolation, by breaking the cycle of vector, virus and host plant, and by artificial barriers to exclude the vector.

Direct attack on the vectors: Much progress has been made in the control of the aphid-borne viruses affecting the potato crop by means of insecticides. Both contact and systemic insecticides prevent the spread of the circulative virus of leaf-roll of potato from infected plants within the crop. Among other successful cases of control of virus vectors by insecticides are the use of demetonmethyl against strawberry viruses, and of parathion sprays against pea enation mosaic in peas.

Breeding resistant varieties of crops: One of the promising methods of control lies in the production of virus-immune or resistant varieties of plants. Several good varieties of mosaic-resistant sugarcane have been produced and the substitution of these for susceptible varieties in most of the sugarcane growing areas has reduced the incidence of disease. Several varieties of

cotton resistant to the leaf-curl disease have also been evolved and these seem to combine vigour and fruitfulness with a high degree of resistance.

Cure of virus-infected plants: Two methods of eliminating the virus from infected plants may be considered, firstly inactivation of the virus by heat and secondly by chemicals. Ratoon stunt, a serious virus disease of sugarcane is now controlled on a commercial scale by exposing the setts for 2 hours in hot water at 50°C.

The principle underlying the application of the chemical therapeutics is that multiplication of a virus can be delayed by compounds which interfere with the nucleic acid metabolism. If nucleic acids are the most important part of viruses and the bases the most important part of nucleic acids, it seems reasonable to look for virus-inhibitory agents among synthetic analogues of those natural bases. Spraying 8-azaguanine on to plants had a marked effect on the spread of virus within the plant. It was found most effective against the viruses of lucerne and cucumber mosaics in the tobacco plant. Experiments with 6-azauracil suggest that the analogue does not affect the multiplication of tobacco mosaic virus but there may be impaired transport from cell to cell.

Special method of propagation: By taking advantage of the rate, or lack, of movement of a virus in a plant, it is sometimes possible to propagate from tissues which are temporarily free of invading virus. This is a useful technique in cases of valuable plants or where it is desired to build up a virus-free clone of a particular variety. For example, virus-free plants from dahlias infected with the virus of tomato spotted wilt may be obtained by taking cuttings from the tips of shoots as they arise from the tubers. Since some viruses fail to invade the growing-point, the apical meristem may be cut off and grown in tissue culture. Potato plants obtained from meristem cultures of clones infected with viruses A and Y were found to be 85 to 95% virus-free. All carnation viruses including those of mottle, ring spot, vein-mottle, streak and the latent virus have been eliminated by meristem culture.

4.6 SUMMARY

Viruses are submicroscopic, infectious, nucleoprotein particles that multiply only intracellularly and are potentially pathogenic. The virus particle is made up of either DNA or RNA which is enclosed in a protein coat. In some cases, the virion is enclosed in a lipid or lipoprotein envelope. Shadow casting, positive staining and negative staining techniques have been used to enhance the visibility of viruses. Stanley (1935) crystallized tobacco mosaic virus and was awarded Nobel Prize.

Viruses were classified on the basis of their natural hosts in earlier times. Now the International Committee for Taxonomy of viruses (ICTV) has devised systems of classification of viruses on the basis of a uniform set of principles. Several criteria such as virion properties, genome organization and replication, antigenic properties and biological properties are taken into consideration for the classification of viruses.

The two principal categories of modes of transmission of plant viruses include those transmitted without the intervention of any vectors and those mediated through vectors. The methods of approach to control plant viruses diseases include – elimination of the source of infection, avoiding the vectors, direct attack on vectors, breeding resistant varieties of crops, cure of virus-infected plants and special methods of propagation.

4.7 MODEL QUESTIONS

1. Write in detail about the transmission of plant viruses.
2. Give an account of the control of plant virus diseases.
3. Write short notes on:
   a) General characters of viruses
   b) Symmetry of viruses
   c) Replication of plant viruses

4.8 REFERENCE BOOKS

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LESSON 5: STATUS OF FUNGI, JUSTIFICATION FOR THE KINGDOM MYCETAE AND CLASSIFICATION OF FUNGI (AINSWORTH SYSTEM)

OBJECTIVE: To explain the status of fungi in the living world and reasons for treating fungi as a separate kingdom; and also to give the details of classification of fungi proposed by Ainsworth(1966,73)

CONTENTS:

5.1. Introduction
5.2. Status of fungi
5.3. Justification for the kingdom Mycetae
5.4. Classification of fungi (Ainsworth system)
5.5. Summary
5.6. Model questions
5.7. Reference books

5.1. INTRODUCTION

The fungi are generally understood as eukaryotic, spore bearing, achlorophyllous, filamentous organisms that show absorptive type of heterotrophic nutrition and reproduce both asexually and sexually. The fungi are known to man since times immemorial, and the term fungus is derived from Greek word sphongos which means sponge. It refers to the soft sponge like texture of agaric fruit bodies. The study of fungi is referred to as Mycology meaning study of mushrooms (Mykes = mushrooms; logos = Science), since agarics or mushrooms are the earliest group of fungi known to man.

Fungi are ubiquitous and widely distributed in nature all over the world. The activities of the fungi are both beneficial and harmful to man. Hence, the study of fungi received much attention. The fungi as we now know include both macroscopic and microscopic forms and more than 1,00,000 species of fungi have been described so far. Most of these are microscopic in size. Until 16th century only macroscopic fungi are known to man, and after the invention of microscopes, the study on the microscopic structure of fungi began in 17th century. The founder of modern mycology is Pier Antonio Micheli, an Italian Botanist, who in 1729 published the first book on fungi entitled "**Nova plantarum genera**", meaning genera of new plants. These new plants are nothing but fungi and included both macroscopic and microscopic forms. The studies on fungi were firmly established on sound lines with contributions of a number of mycologists in 19th

century. The publication classic studies on fungi such as "Synopsis methodica Fungorum" by Christian Hendrick Persoon a Dutch Mycologist in 1801, "Systema Mycologicum" by Elias Magnus Fries, a Swedish mycologist in 1821, "Selecta Fungorum Carpologia" by French mycologists Tulasne brothers- Louis Tulasne and Charles Tulasne, during 1861-65 are important land marks in the studies on fungi during 19th century. Common Wealth Mycological Institute (CMI), now called International Mycological Institute (IMI), was established during early 20th century in England and it is carrying out intensive taxonomic studies on various groups of fungi.

5.2 STATUS OF FUNGI:

The fungi were traditionally treated as plants and studied by botanists. Theophraustus, the 'Father of Botany' in the first book on plants, "Historia plantarum", described fungi or agarics as rootless and leafless plants that arose from the excess moisture of soil or putrefying organic matter during rainy season. The mushrooms and other higher fungi are mostly rooted in soil or other substrata and do not move from place to place. Hence, fungi are treated as plants. It finely fits into the concept that all living organisms fall into only two categories viz. plants and animals. The superficial similarity of filamentous fungi and algae, is the reason for studying algae and fungi as two groups of Thallophyta, and regarding fungi as having evolved from algae by the loss of photosynthetic capability. Carl Van Linnaeus (1753) who is considered as father of plant taxonomy also treated both algae and fungi as two groups in his 24th class of plants – cryptogamia.

Anton Von Leeuwen hoek of Holland and Robert Hooke of England started using microscopes to study the biological specimens in 1660s and over a period of time a great deal of knowledge accumulated on microscopic forms of life. With increasing knowledge on microorganisms, the basic concept of treating all livings organisms either as plants or animals was questioned. Hogg (1860) for the first time proposed 3 kingdom system for living organisms. The three kingdoms of Hogg (1860) are 1. Plant kingdom 2. Animal kingdom and 3. Regnum primigenum which include primitive forms of life (protosticta). Ernst Haeckel (1866) a famous German zoologist proposed 4 kingdom system. A part from Metaphyta (green plants) and Metazoa (animals) he recognized two more kingdoms viz. Protozoa and Protophyta. He included fungi in Protophyta. However, proposal of 4 kingdom system is considered revolutionary at that time and,

biologists of the time began to treat microscopic forms of life as Protista, and fungi are considered as an important group of protista.

In early 20th century, the importance of nucleus was recognized and basing on the presence or absence of membrane bound nucleus, the microorganisms of protista are recognized as two groups viz. Procaryotes and Eukaryotes.

Cope Land (1956) proposed 4 kingdom system viz.

1. Monera – prokaryotic microbes, bacteria
2. Protista – Eukaryotic protists – algae, fungi and protozoa
3. Plant kingdom
4. Animal kingdom.

In addition to presence or absence of membrane bound nucleus, level of organization and mode of nutrition was also taken into consideration as a major character by Whittaker (1969) who proposed 5 kingdom system and for the first time elevated the status of fungi and treated them as a separate kingdom on par with plants and animals(fig. 5.1).

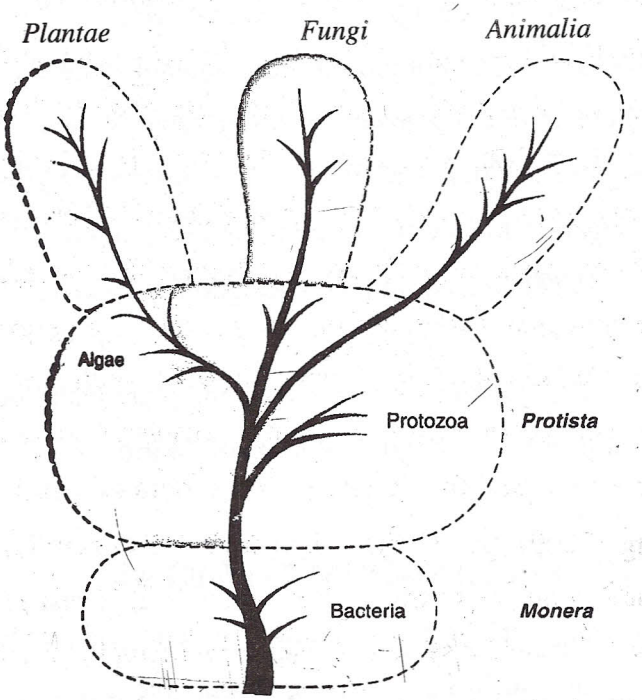

Figure 5.1 Whittaker's proposals of 5 kingdom system

The 5 kingdoms of Whittaker are as follows

1. Monera : Procaryotic blue green algae, bacteria and related forms.
2. Protista : Eucaryotic unicellular organisms mainly algae and protozoa.
3. Fungi : Eucaryotic, predominantly multinucleate organisms, with nuclei dispersed in a walled and often septate mycelium, showing absorptive type of nutrition'
4. Plantae : Multicellular plants with walled eukaryotic cells, showing photoautotrophic nutritions.
5. Animalia : Multicellular animals with wall-less eukaryotic cells showing phagotrophic or ingestive type of nutrition

Later, Cavalier-Smith proposed eight kingdom system with two empires viz. Empire **Bacteria** with two kingdoms (Eubacteria and Archaeobacteria) and Empire Eucaryota with six **kingdoms** (Arc'iezoa, Protozoa, Animalia, Fungi, Chromista and Plantae). In this 8-**kingdom system** also Fungi were treated as belonging to a separate kingdom Fungi, on par with **Animalia** and **Plantae**. Later Carl-Woese (1991) proposed three domain system viz. Archaea, **Procarya** and Eucarya. He included all eukaryotic organisms including fungi in the domain Eucarya.

Since the proposals of Whittaker to treat fungi as a separate kingdom, all mycologists are considering fungi as a separate kingdom. Ainsworth (1973) treated fungi as kingdom Fungi. Alexopoulos and Mims (1979) treated fungi as kingdom Mycetae. Von Arx(1981) treated as kingdom Mycota.

Philip H. Gregory (1981) a famous mycologist and plant pathologist expressed the opinion that "there are grounds for classifying fungi in a group of their own".

5.3 JUSTIFICATION FOR KINGDOM MYCETAE

The characters of fungi are very distinct and there is justification for treating fungi as a separate kingdom basing on nutrition, storage material, biochemistry of cell wall, pattern of growth, protoplasmic continuity, hyphal anastomoses and genetic plasticity.

5.3.1 Nutrition: Fungi are heterotrophic like animals while plants are photosynthetically autotrophic. Even though fungi are heterotrophic like animals, there is a fundamental difference in

nutrition of fungi and animals. Animals show phagotrophic or ingestive type of nutrition i.e. taking in of solid food material and digesting them inside their body. Fungi show absorptive type of nutrition i.e. they secrete extracellular enzymes into the surrounding and degrade them into smaller units which can be absorbed by the cells directly. Such absorptive type of nutrition is seen in bacteria also but they are fundamentally different from fungi in nuclear organization, bacteria being procaryotes and fungi eukaryotes.

5.3.2. Biochemistry of cell wall: Fungi are having true cell wall made of mostly fungal chitin and chitin and cellulose in oomycetes, while the cell wall of plants is mainly composed of cellulose. The animal cells are lacking in cell wall. Bacteria also posses a rigid cell wall but it is mainly made up of peptidoglycan layer but not of cellulose or chitin. Since biochemical composition of cell wall is considered as a fundamental characteristic feature plants ' fungi are considered as distinctly different.

5.3.3. Storage material: In plants the reserve food material is mainly starch (amylose 20% + amylopectin 80%) while in fungi reserve food material is mainly glycogen, (animal starch), It is typical of animal cells but not of higher plants Chemically it is similar to amylopectin but different in mol. wt. and degree of branching.

5.3.4. Growth: The filamentous fungi show primary growth by apical growth i.e. hyphae grow only at apices, their walls may thicken considerably behind this point but they do not extend. This form of growth contrast with intercalary growth which is seen in other filamentous organisms in which almost any cell of the filament can enlarge and divide. Indeed this type of apical growth is parallel only in one group of algae, in the root hairs and pollen tubes of higher plants. Therefore, apical growth of a filament is essentially specialized feature of the fungi

5.3.5. Protoplasmic continuity: The mycelium of filamentous forms of zoosporic fungi and zygomycetes is made up of aseptate, coenocytic hyphae, hence protoplasm is continuous throughout the fungal body.

The mycelia of ascomycotina, basidiomycotina and most deuteomycotina fungi consist of branched hyphae which are septate at frequent intervals. However, the septa have central perforations or pores which help in easy communication between the cells, thus maintaining the protoplasmic continuity throughout the mycelium. In basidiomycotina the septal perforations are

characteristic and described as dolipore septa, while in the members of ascomycotina and deuteromycotina septal pores are simple.

5.3.6. Hyphal anastomoses: The most remarkable feature of fungal hyphae is the process of hyphal fusion or anastomosis. The growing hyphal tip, which may be terminal or young peg like lateral, evokes an unknown stimulus, acting over a distance of about 10 µm, on the development of another growing point on an adjacent hyphae of the same mycelium or on another mycelium of same species. The two hyphal growing points then grow towards one another and make contact. The cell walls dissolve at the point of contact, and continuity of the filaments established through the newly formed bridge allowing the passage of cytoplasm and even nuclei.

The anostomoses and their consequences are the most out standing characteristic of the higher fungi. The mycelium of the higher fungi is thus a three dimensional net work.

Separate mycelia originated from germinating spores of same spores can anstomose and exploit the substratum more efficiently as a unit, instead of competing with each other.

5.3.7. Genetic plasticity: The fungal cells contain membrane bound nuclei. The cells are coenocytic. Mostly they are haploid and are of same genotype. However, because of fungal anastomoses and nuclear migration throughout, the two or more nuclei in the same cell may be of different genotype and a condition of Heterokaryosis develops. The nuclear fusion may take place between homozygous nuclei or nuclei of different genotypes and hence, fungal cells some times may contain five types of nuclei viz. A type nuclei, B type nuclei, AA nuclei, BB type nuclei and AB type nuclei. These nuclei are assorted during spore formation. This type of high genetic plasticity of possessing nuclei of different types at a time giving higher variability to the fungi, not found in any other groups of living organism.

Because of all these specialized characters shown by the fungi, there is every justification for treating the fungi as a separate Kingdom.

5.4 CLASSIFICATION OF FUNGI:

The fungi are known to man since long time, but systematic study began only since 18th century and it is still continuing. When only the macro fungi are known, man divided them into edible and poisonous ones basing on their effect on man on eating. Naturalists, during 15th and 16th centuries divided the macrofungi into three groups basing on their occurrence. The three

groups are 1) terrestrial fungi - growing free on soil, 2) Hyphogean fungi - growing in the soil and 3) Epiphytic fungi – growing on plants.

Basing on microscopic studies, Micheli, Persoon, Freies, Tulasnes etc proposed various groupings, which are of historical importance. In 1866 Anton Van deBary a famous German Mycologist proposed a classification which was widely followed for a long time. He recognized 4 classes in fungi. They are

1. Phycomycetes: Fungi with tubular coenocytic thallus that produce spores in a sporangium.
2. Hypodermi: The plant pathogenic fungi which cause rust and smut diseases.
3. Basidiomycetes: The fungi that produce basidiospores.
4. Ascomycetes: The fungi that produce ascospores.

Slime moulds were described as Mycetozoa and are treated separately.

In 1926, Gwynne-Vaughan and Barnes divided the fungi into 4 classes. They are phycomycetes, ascomycetes, Basidiomycetes and deuteromycetes. They abolished the class hypodermi of deBary and recognized deuteromycetes as a separate class for fungi which do not show sexual reproduction in their life cycle.

Later Gauman and Dodge (1928), Bessey (1950), Martin (1961), Alexopoulos (1962) and others proposed various systems of classification of fungi. In 1966 G.C. Ainsworth of Common Wealth Mycological Institute proposed a general purpose classification for fungi in Bibliography of Systematic Mycology. It was followed in "Ainsworth and Bisby's Dictionary of fungi". 6th Edition (Ainsworth, 1971). This classification system was elaborated and followed in taxonomic treatment of fungi in "Fungi-an advanced treatise" vol. 4A,B, edited by G.C. Ainsworth, F.K. Sparrow and A.S. Sussman, published in 1973. All important Mycologists of the world contributed to the taxonomic treatments on groups of they are interested. Since 1973, this system of classification of fungi is generally followed in all Common Wealth Countries. It came to be known as Ainsworth system of classification.

5.4.1. Classification of fungi (Ainsworth system)

Ainsworth (1973) treated Fungi as a separate kingdom and recognized two divisions viz. Myxomycota and Eumycota. The fundamental criterion used for this division is presence or absence of rigid cell wall around vegetative structures. Those fungi that lack a rigid defined cell

wall are placed in Myxomycota and those with a clear rigid cell wall are placed in Eumycota or true fungi.

5.4.2. DIVISION MYXOMYCOTA:

This is a relatively small group of wall less fungi, generally described as slime moulds. The vegetative thallus is a plasmodium and as it lacks a rigid cell wall, it is pleomorphic. Around the plasmodium, plasma membrane and a slime layer are usually present. They resemble protozoa in their vegetative stage and resemble true fungi in their methods of reproduction. Hence they are described as Mycetozoa by deBary (1866) and as Myxomycota by Ainsworth (1973). 4 classes are recognized in this division.

Class Acrasiomycetes: These are called cellular slime moulds. The myxamoebae aggregate to form a pseudoplasmodium, which give rise to sporulating structures. This class comprise 3 orders viz protosteliales, acrasiales and dictyosteliales each with two families. *Dictystelium* is the well studied organism of the group.

Class Hydromyxomycetes: These are aquatic fungi with a net work of slime tubes in which plasmodium crawls. Hence, these are net slime moulds. A single order Labyrinthulales, and single family Labyrinthulaceae are recognized.

Class Myxomycetes: The members of this group are called true slime moulds. Plasmodium is formed by the fusion of two compatible myxamoebae or zoospores. Three types of plasmodia viz. Protoplasmodium, Aphnoplasmodium or Phaneroplasmodium are recognized. The fruit bodies formed by slime moulds may be sporangium, aethalium or plasmodiocarp. The class is divided into 6 orders viz. Ceratiomyxales, Echinosteliales, Liceales, Trichiales, Physarales and Stemonitales. *Physarum* and *Stemonitis* are well known genera of the group.

Class Plasmodiophoromycetes: This is a small group of obligately parasitic slime moulds. *Plasmodiophora brassicae*, which causes club root disease of cruciferous hosts, is the best studied member. A single order plasmodiophorales with a single family plasmodiophoraceae are recognized.

5.4.3. DIVISION: EUMYCOTA

This is the large group of fungi with a rigid cell wall made up of chitin or cellulose or both This division comprises 5 sub divisions each with a number of classes, orders and families. Th

five subdivsions are Mastigomycotina, Zygomycotina, Ascomycotina, Basidiomycotina and Deuteromycotina.

5.4.3.1. SUB DIVISION MASTIGOMYCOTINA: The fungi that produce zoospores asexually are placed in this sub division. These are generally called zoosporic fungi. Basing on the structure and flagella arrangement and structure three classes are recognized in this subdivision.

5.4.3.1.1. Class Chytridiomycetes: The fungi that produce zoospores with a single posteriorly situated whiplash type flagellum are placed in this class. Three orders viz. Chytridiales, Blastocladiales and Monoblepharidales are recognized in this class. Sexual reproduction may be planoisogamy, planoanisogamy or heterogamy.

5.4.3.1.2. Class Hyphochytridiomycetes: The fungi that produce uniflagellate zoospores with an anteriorly situated tinsel type flagellum are placed in this class. A single order Hyphochytriales is recognized.

5.4.3.1.3. Class Oomycetes: The fungi that produce biflagellate zoospores with one whiplash type and one tinsel type are placed in this class. The flagella may be apical or lateral. The sexual reproduction is typically oogamous type. The class includes 4 orders viz. Lagenidiales, Saprolegniales, Leptomitales and Peronosporales. The last order includes very important plant pathogenic fungi like *Pythium*, *Phytophthora*, *Albugo* and downy mildew fungi.

5.4.3.2. SUB DIVISION ZYGOMYCOTINA: The fungi with coenocytic tubular mycelium, reproduce asexually by non motile sporangiospores and sexually by zygospores are placed in this group. The subdivision comprises two classes viz. Zygomycetes - very typical of the subdivision, and Trichomycetes, a group of uncertain affinity. The class zygomycetes comprise three orders viz. Mucorales - mostly saprophytic, Entomophthorales - mostly insect parasites; and Zoopagales which are parasitic or predaceous on soil fauna like protozoans and nematodes.

5.4.3.3. SUB DIVISION ASCOMYCOTINA: The fungi that produce ascospores sexually are placed in this sub division. The asci in which ascospores are produced are formed in fruit bodies called ascocarps. Depending on the over all structure, the ascocarps are recognized as four types viz.

cleistothecium - completely closed peridium enclosing prototunicate asci randomly distributed,

perithecium - generally flask shaped structures with unitunicate asci in a hymenium,

Apothecium - a cup shaped ascocarp with open hymenium of unitunicate asci and

pseudothecium - bitunicate asci formed in locules.

Basing on the ascocarp type and other characters, 6 classes are recognized in ascomycotina. They are

1. Hemiascomycetes: The fungi that do not produce ascocarps and zygote directly tranform into ascus.

2. Plectomycetes: The fungi which produce cleistothecium.

3. Pyrenomycetes: The fungi which produce perithecia.

4. Discomycetes: Fungi which produce apothecia.

5. Loculoascomycetes: Asci formed in pseudothecia.

6. Laboulbeniomycetes: Ecologically distinct group, parasitic on insects or red algae. They produce asci in perithecia.

Each class comprises a number of orders and families.

5.4.3.4. SUBDIVISION BASIDIOMYCOTINA: The fungi which produce basidiospores externally on basidium are placed in this subdivision. Basidium may be a single celled structure or divided into 4 cells. Basidiocarps may be produced or not produced. The basidiocarps, when formed may be open at maturity or completely closed throughout. Other important characters are formation of clamp connections in mycelium, and presence of dolipore septa in the mycelium.

Basidiomycotina is divided into three classes viz. Teliomycetes, Hymenomycetes and Gasteromycetes.

5.4.3.4.1. Class Teliomycetes: Plant pathogenic fungi which produce rust and smut diseases on higher plants are placed in this class. Mycelium in these fungi is limited without clamp connections and dolipore septa. They do not produce basidiocarps. Teleutospores in rust fungi and chlamydospores in smut fungi are perfect stages equivalent to probasidium in which karyogamy occurs. The 4 celled promycelium arising from these spores on germination is equivalent to metabasidium which bear 4 basidiospores. The class teliomycetes comprise two orders viz. Uredinales (rust fungi) and Ustilaginales (smut fungi).

5.4.3.4.2. Class Hymenomycetes: The fungi which produce basidiocarps with open hymenium are placed in the class Hymenomycetes. The class is divided into two subclasses basing on basidium structure. They are *holobasidiomycetidae and phragmobasidiomycetidae.*

Sub class Holobasidiomycetidae: Basidium is a holobasidium (single celled structure). This is the largest group which comprises two large orders Agaricales and Aphyllophorales and 4 small orders viz. Exobasidiales, Brachybasidiales, Dacrymycetales and Tulasnellales.

Sub class: phragmobasidiomycetidae: This is a very small group with septate metabasidium. The subclass comprise 3 orders viz. Tremellales(with vertically divided metabasidium) Auriculariales and septobasidials(with tranversely septate metabasidium).

5.4.3.4.3.Class Gasteromycetes: This is an unnatural assemblage of basidiocarp producing fungi, whose basidiocarps are completely closed. The sporiferous region of the fruit body is called gleba and hymenium may or not formed. The class comprise 9 orders of which 5 orders have hymenium at some stage of development and 4 orders do not have hymenium.

Hymenial orders: Podaxales, phallales, hymenogastrales, Lycoperdales and Gautieriales.

Nonhymenial orders: Melanogastrales, Tulostomatales, Sclerodermatales and Nidularales.

5.4.3.5. SUB DIVISION DEUTEROMYCOTINA :

The fungi in which sexual sage is lacking or not discovered so a are placed in this subdivision. They are generally called fungi imperfecti. They reproduce mainly by conidia. Some reproduce by budding while a few fungi do not produce any asexual spores even.

The class comprises three class viz. Blastomycetes, Hyphomycetes and Coelomycetes.

5.4.3.5.1. Class Blastomycetes: Yeasts that do not posses perfect stage but reproduce only by budding are placed in this class. It includes two orders viz. 1. Sporobolomycetes-in which blastospores are actively liberated and 2. Crytococcales-in which blastospores are passively released.

5.4.3.5.2. Class Hyphomycetes: The fungi which reproduce by conidia and conidiophores may be variously organized into different structures but not into pycnidia or acervulus. This class comprise some of the very common conidial fungi. Two orders viz. Moniliales and Agonomycetales. The order Moniliales include fungi which produce conidia on free conidiophores (Moniliaceae, Dematiaceae) or on synnemata (stilbellacea) or on sporodochium (Tuberculariace).

The order agonomycetales include fungi which do not produce even conidia. A single family agonomycetaceae is recognized.

5.4.3.5.3. Class Coelomycetes: These are conidial fungi in which the conidia are produced either in pycnidia or acervuli. The class comprise two orders viz. Sphaeropsidales (pycnidial fungi) and Melanconiales (acervulus producing fungi). In the order sphaeropsidales four families viz. sphaeropsidaceae, nectrioidaceae, leptostromataceae and excipulaceae are recognized. In the order melanconiales a single family melanconiaceae is recognized.

5.5 SUMMARY

The fungi are eukaryotic nonchlorophyllous, unicellular or filamentous organisms that reproduce both asexually and sexually by producing specialized spores. The fungi are traditionally treated as plants and studied along with algae because of superficial similarity between the two groups of organisms. Because of the distinctive characters of fungi, Whittaker (1969) for the first time proposed to treat Fungi as a separate kingdom on par with plants and animals. The elevation of the status of fungi as a separate kingdom is justified on a number of distinctive characters such as nutrition, storage material, biochemistry of cell wall, pattern of growth, protoplasmic continuity, hyphal anastomoses and genetic plasticity.

The fungi are classified by Ainsworth (1966, 73) into two Divisions viz. Myxomycota and Eumycota. The division myxomycota is a very small group of wall less organisms generally called slime moulds. Four classes are recognized in Myxomycota. They are 1. Acrasiomycetes, 2. Hydromyxomycetes, 3. Myxomycetes and 4. Plasmodiophoromycetes. The division Eumycota or true fungi is a very large group that comprises organisms with a rigid cell wall. The division Eumycota is divided into 5 sub divisions. They are 1. Mastigomycotina – zoosporic fungi; 2. Zygomycotina – which reproduce sexually by producing zygospores 3. Ascomycotina – ascospore producing fungi, 4. Basidiomycotina – basidiospore producing fungi and 5. Deuteromycotina – which reproduce only by asexual means.

5.6 MODEL QUESTIONS

Essay type questions

1. Discuss the status of fungi and justification for kingdom mycetae.
2. Give an account of classification of fungi according to Ainsworth system

Short answer type questions

3. Status of fungi
4. Kingdom mycetae
5. Outline classification of fungi
6. Classification of ascomycotina

5.7. REFERENCE BOOKS

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LESSON-6. GENERAL ACCOUNT OF MYXOMYCOTA AND MASTIGOMYCOTINA

OBJECTIVE: To explain the general characters, reproduction and classification of fungi belonging to the division Myxomycota and subdivision of Mastigomycotina of Division Eumycota.

CONTENTS

6.1. Introduction
6.2. Myxomycota
6.3. Mastigomycotina
6.4. Summary
6.5. Model questions
6.6. Reference books.

6.1. INTRODUCTION

The fungi belonging to the division Myxomycota and the fungi belonging to subdivision Mastigomycotina of the division Eumycota are having one common character, that is, production of motile spores but myxomycota essentially differ from eumycota fungi in lacking cell wall. These fungi show variation in vegetative structure, asexual reproduction and sexual reproduction. The general characters of the fungi of these groups are explained in this lesson.

6.2. MYXOMYCOTA: The division Myxomycota includes organisms generally termed as 'slime moulds', with a few exceptions. They essentially differ from Eumycota in having a naked protoplast termed 'plasmodium' as vegetative phase in contrast to the walled structures in true fungi. Whether the slime moulds are closely related to fungi is doubtful. They may be closely related to protozoa. Debary (1887) used the term 'mycetozoa' to describe these organisms, indicating a relationship with animals. Olive (1975) also treated them 'mycetozoans' in his monograph on this group. Ainsworth (1973) treated them along with fungi and proposed a separate division 'myxomycota' for them. The division Myxomycota has been divided into 4 classes viz. 1. Acrasiomycetes 2. Hydromyxomycetes 3. Myxomycetes and 4. Plasmodiophoromycetes.

The ecology, structure and reproduction of various groups are so different that no general account is simple to give and can be treated along the taxonomic lines.

6.2.1. ACRASIOMYCETES:

The organisms constituting the class acrasiomyctes may not represent a natural group, but they have in common a trophic stage consisting of myxamoebae or in a few cases a minute plasmodium. The vegetative cells are called myxamoebae because they resemble amoebae of protozoa but produced by slime moulds. In general, trophic stage is represented by myxamoebae, and before formation of fructifications they aggregate to form a structure described as pseudoplasmodium. Since the individual cells in a pseudoplasmodium retain their individuality, acrasionmycetes are also referred to as communal slime moulds or cellular slime moulds. The fructifications arise from pseudoplasmodia may be sporocarps (delicate tubular stalks bearing one or a few spores) or sporocarps (multi cellular fruiting structures which bear a sorus of spores of at the tip). The genus *Dictyostelium* is the well studied member of the group, and the life cycle of *Dictyostelium discoideum* is shown in the figure 6.1.

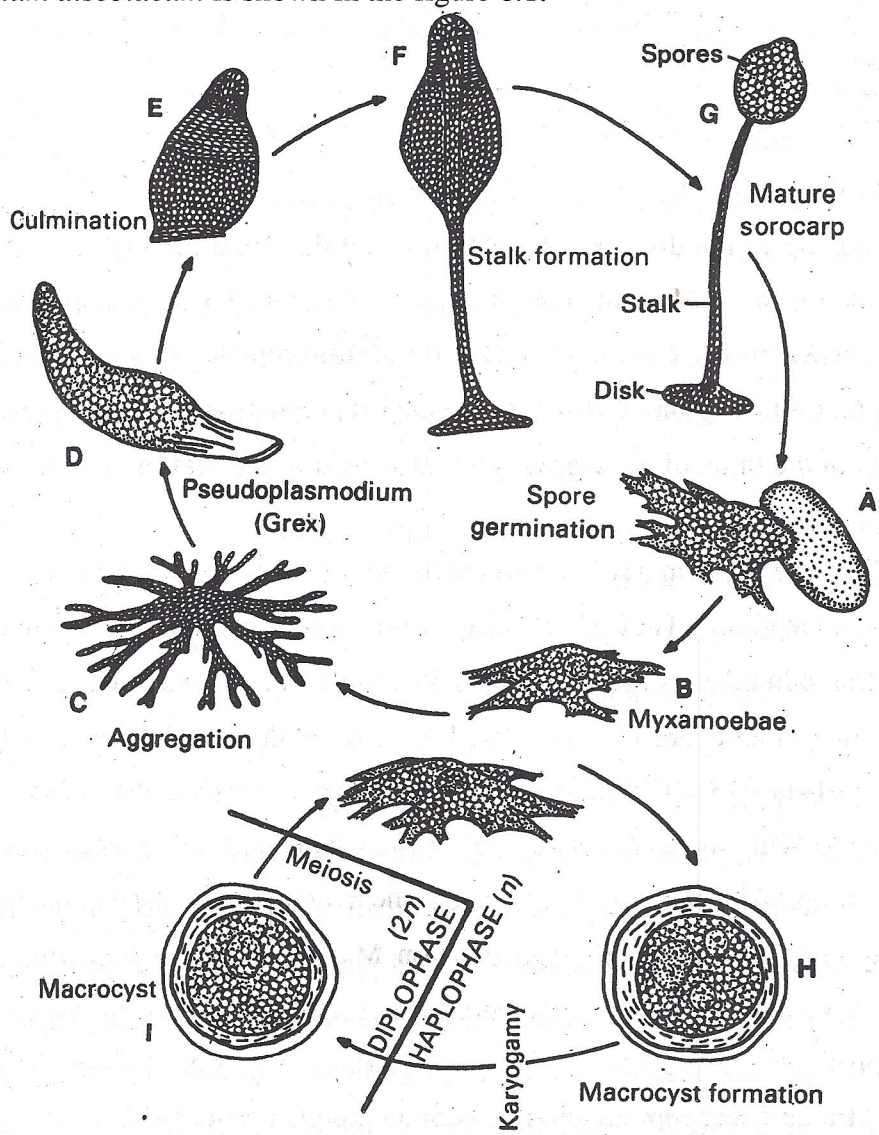

Figure 6.1. Life cycle of *Dictyostelium discoideum*

The class Acrasiomycetes is divided into 3 orders viz. Protosteliales, Acrasiales and Dictyosteliales.

6.2.1.1 Protosteliales: The trophic stage is uninucleate or multinucleate myxamoebae, they do not form pseudoplasmodium, but form sporocarps individuvally. Two families are recognized in this order.

1. protosteliaceae – Flagellated cells absent
2. Cavosteliaceae – Myxamoebae are flagellated.

6.2.1.2. Acrasiales: In this group, myxamoebae aggregate to form loose multicellular aggregations called pseudoplasmodia. Fruit bodies formed from pseudoplasmodia bear chains of spores at the top or a sorus may two families.

The order comprise two families.

1. Acrasiaceae : Spores formed in simple or branched chains.
2. Guttulinaceae : Spores formed in a sorus.

6.2.1.3. Dictyosteliales: They are well developed forms. Myxamoebae represent trophic stage. Before fruiting myxamoebae in a colony stop feeding, set up centres of aggregation towards which the myxamoebae stream in lines across a chemical gradient. The chemical that stimulate formation of aggregation is called acrasin and identified as 3' – 5' cyclic AMP. The pseudoplasmodium formed by myxamoebae produce well developed sorocarps.

The order comprises two families.

1. Acytosteliaceae: Sorocarps are delicate, small
2. Dictyosteliaceae: Sorocarps well developed.

6.2.2. HYDROMYXOMYCETES:

The members of this group are acquatic forms occurring in marine and estuarine environments, found growing on red algae, diatoms and marine angiosperms. The thallus is made of a network of branched tubes in which spindle shaped plasmodia crawl. Hence these are also called net slime moulds. After a period of growth, vegetative cells accumulate in groups within the slimenet, become spherical and secrete thin walls. A pellicle is formed around each group of encysted cells and delimits a kind of sorus. Each cell of the sorus functions as a sporocyte, its contents dividing into several cells that emerge as laterally biflagellate zoospores. The flagella are

heterokont, one whiplash type and the other tinsel type. Meiosis precedes the formation of zoospores. Hence zoospores are haploid, but at what stage diploidization takes place is not known. The zoospores are capable of swimming for about 24 hours. Upon settling on a substratum, the zoospores lose their flagella and differentiate into non motile spindle shaped cells. These cells secrete slime tubes, within which they crawl (fig. 6.2).

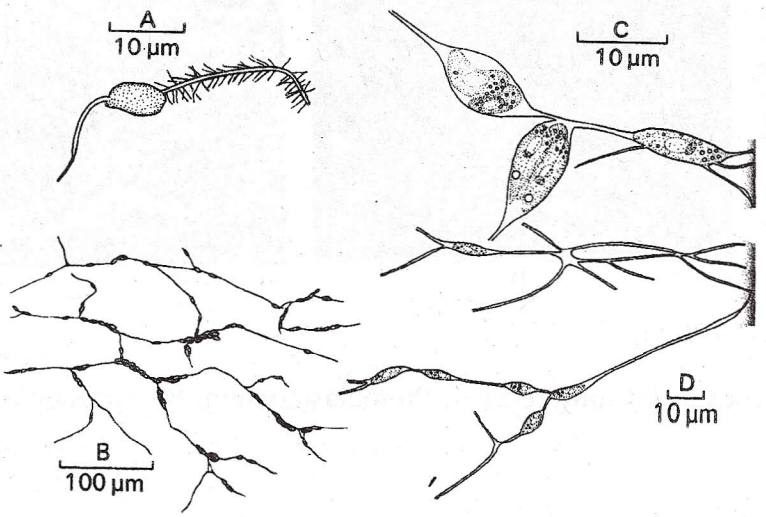

Figure 6.2. Characters of hydromyxomycetes. Zoospores (A), and slime net with plasmodia (B,C,D)

The class Hydromyxomycetes comprise a single order labyrinthulales and a single family labyrinthulaceae. The important genera are *Labyrinthula* and *Labyrinthulina.*

6.2.3. MYXOMYCETES:

The organisms comprising this class are termed "true slime moulds". About 500 species are known and fruit bodies (sporocarps) of most of them can be seen with maked eye. They commonly occur on decaying logs, humus, soil, bark, dung etc. They occur even on living leaves but are not parasitic. They feed on particulate organic matter, bacteria, yeasts etc.

6.2.3.1. Vegetative phase: The spores of myxomycetes are unicellular, globose with smooth spiny or reticulately thick walls. They germinate by giving rise to one or two uninucleate amoeboid or flagellated cells. Flagella usually form on moist surfaces and two flagella are usually formed at anterior end. Both flagella are of whiplash type but one longer than the other. Myxamoebae or

swarmers multiply in number and compatible mating types usually fuse to form a binucleate cell, in which karyogamy soon occurs. This zygotic cell is the beginning of the plasmodium stage. They begin to feed and enlarge. Basing on the structural features, plasmodia of slime moulds are recognized as three types (Fig 6.3).

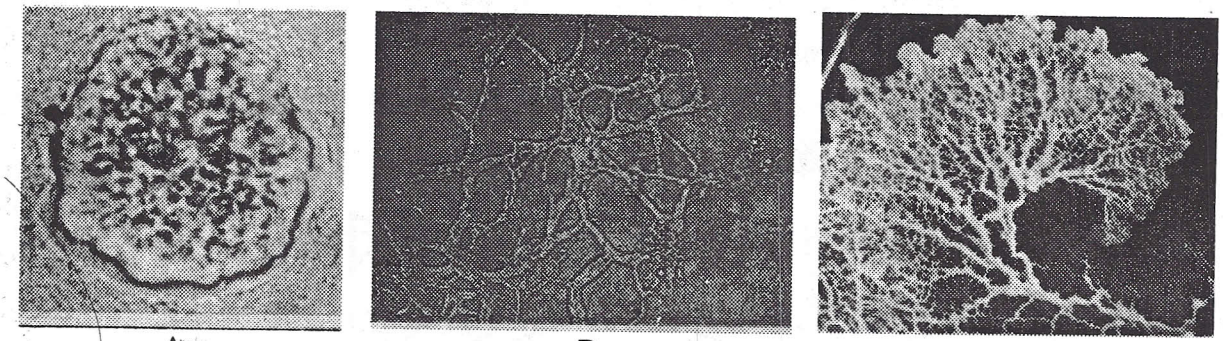

Figure 6.3. Plasmodium types in myxomycetes A. Protoplasmodium, B. Aphanoplasmodium and C. Phaneroplasmodium

Protoplasmodium (proto = primitive): The plasmodium remains microscopic throughout its existence. It is more or less homogenous and protoplasm may show very slow, irregular streaming. It gives rise to a single sporangium. Eg. *Echinostelium.*

Aphanoplasmodium(aphano= inconspicuous): Plasmodim is inconspicuosus, but as it grows forms a network of very fine transparent strands or branches. The protoplasm shows rapid rhythmically reversible streaming. It remains colourless up to a stage and become yellowish in colour shortly before sporulation. Eg. *Stemonitis.*

Phaneroplasmodium (phanero=obvious): They are typically organized into a fan shaped reticulate masses. In the branches of plasmodium, outer portion of protoplasm is thick and gelified and inner position remains as fluid in the center. Rhythmic, reversible streaming of protoplasm is very conspicuous. Eg. *Physarum.*

6.2.3.2. Sporulation: After a period of feeding plasmodium is converted into fruit bodies. Usually the whole of plasmodium is involved in fruit body formation. Three types of fruit bodies are recognized in myxomycetes (Fig. 6.4).

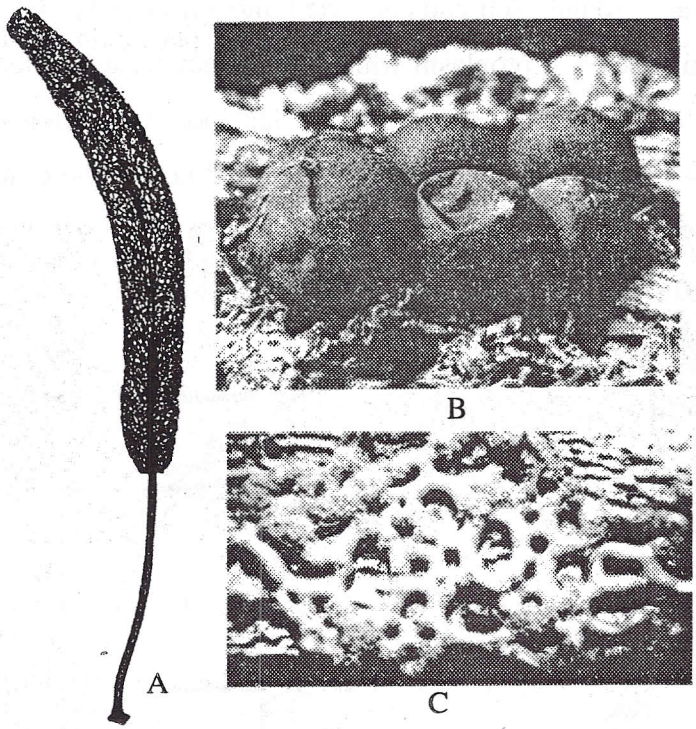

Figure 6.4. Different types of fruit bodies in myxomycetes A. Sporangium B Aethalia and C. Plasmodiocarp.

Sporangium: Sporangium may be stalked or sessile, globose, cylindrical or cup shaped. The plasmodium usually forms a membranous layer on the substratum. It is called hypothallus. On this membrane hypothallus, fruit bodies are formed. In young sporangium, the spores are encased in a peridium. In stalked forms, the stalk may extend into the sporangium as columella. Within the fruit body, a branched network of capillaries or sterile threads extend from all around the columella to the peridium and it is called capillitium. Eg. *Stemonitis*.

Aethalium: They are large masses, probably represent a mass of fused sporangia. They are usually sessile. Eg. *Reticularia*.

Plasmodiocarp: It is similar to a stalkless sporangium, but retains the branching habit of the plasmodium. Usually phaneroplasmodium type of thallus forms these fructifications. In the

formation of the plasmodiocarp, the protoplasm concentrates around some of the main branches of plasmodium, secrete a membrane around itself and converted into a fruit body. Eg. *Hemitrichia.*

In all the types of fruit bodies, protoplasm which is coenocytic, uninucleate portions are formed and eventually separate as a spore by developing a thick spore wall. Meiosis occurs in the maturing spore and 3 out of 4 resultant nuclei degenerate by the time the spore matures. The life cycle of a typical myxomycete is shown in the figure 6.5.

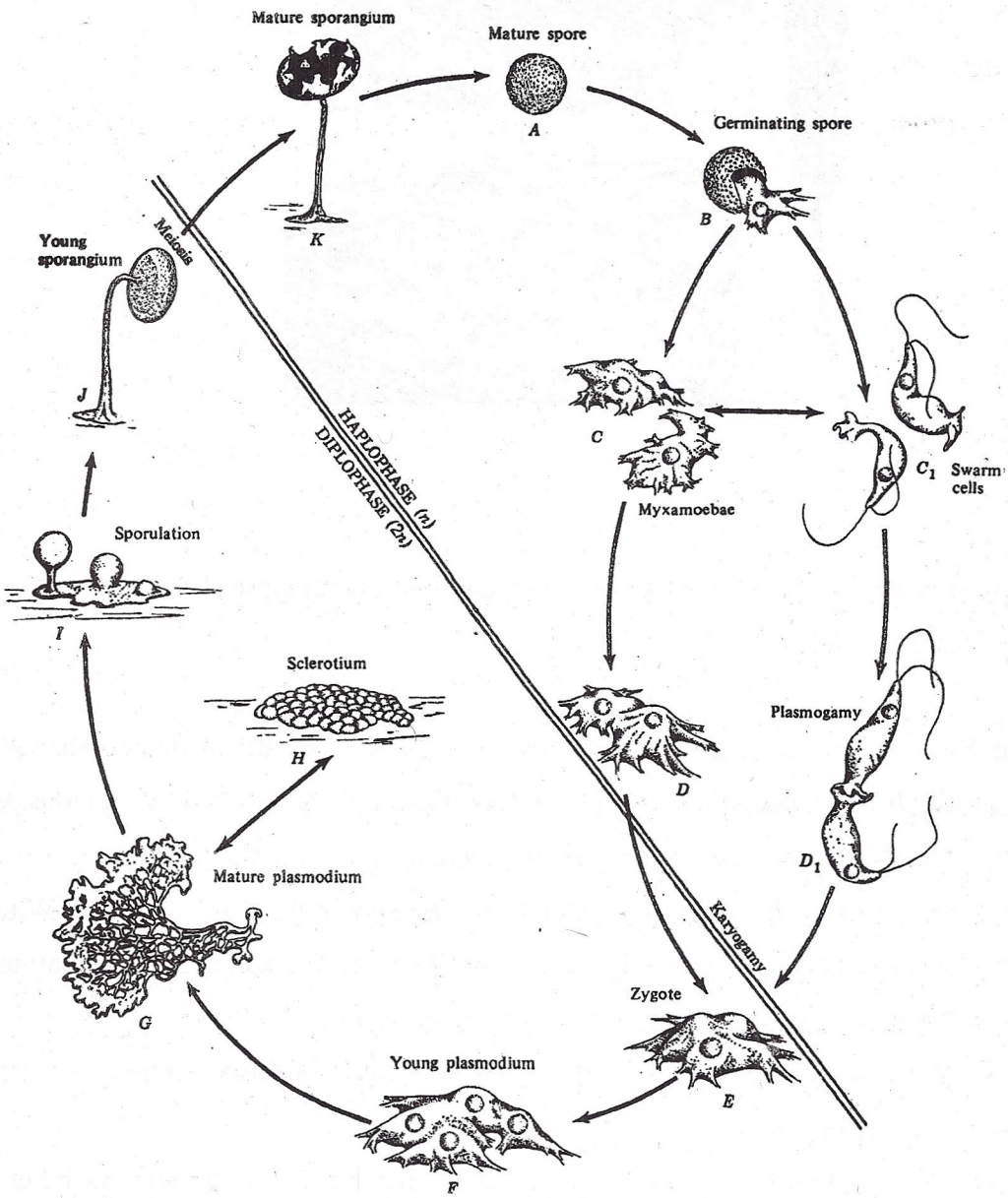

Figure 6.5. The life cycle pattern of a typical myxomycete fungus

6.2.3.3. Classification: The class myxomycetes is divided into 3 subclasses. 1) Ceratiomyxomycetidae, 2) Myxogastromycetidae and 3) Stemonitomycetidae.

6.2.3.3.1. Ceratiomyxomycetidae: This subclass comprises a single order ceratiomyxales with a single family ceratiomyxaceae and a single geus *Ceratiomyxa*. This group is characterized by formation of spores externally on a columnar structure, and hence is called exosporae.

6.2.3.3.2. Myxogastromycetidae: Spores are borne internally. Plasmodium may be any one of the three types. This subclass comprises 4 orders.

Echinosteliales: Plasmodium is a minute protoplasmodium. Spores are produced in stalked sporangia. The order comprises two families Viz. Echinosteliaceae and Clastodermataceae.

Trichiales: Fructifications are sporangiate or plasmodiocarpous type. They are non columellate but possess abundant capillitium. The order comprises two families viz. Trichiaceae and Dianemaceae.

Liceales: Fruit bodies any one of the three types and spores typically light coloured; Capillitium absent; the order comprises three families viz. Liceaeae, Reticulariaceae and Cribrariaceae.

Physarales: This is a large order and deposition of lime on peridium is the distinguishing feature. Plasmodium is a phaneroplasmodium. Spores are dark brown to black. The order comprises two families viz. Physaraceae and Didymiaceae.

6.2.3.3.3. Stemonitomycetidae: This subclass comprises a single order stemonitales and a single family stemonitaceae. Chief characters are plasmodium usually an aphanoplasmodium and fructifications sporangium type.

6.2.4. PLASMODIOPHAROMYCETES

This class comprises a group of obligate, endobiotic parasites whose thallus is a naked holocarpic plasmodium. They are fairly well distributed throughout the world; both in aquatic environment and in soil. Some are parasitic on algae, some on pteridophytes and majority on higher plants causing hypertrophy of the infected parts. Two most important species of the group are *Plasmodiophora brassicae*, which cause club root disease of cruciferous plants, and *Spongospora subterranea* which cause powdery scab of potatoes.

The vegetative stage represented by plasmodium is always internal and intracellular in the host. Initial infections result in plasmodia forming zoosporangia and zoospores. The zoospores are

biflagellate, both whiplash type but one longer than the other. After a period of growth, plasmodium produces zoospores, which act as gametes and fuse to form diploid zygote. The infections by zygotes result in diploid plasmodium which under goes divisions to form resting spores. The division before formation of resting spores is supposed to be meiotic. The resting spores on germination give rise to zoospores which initiate infection of the host.

In this class, a single order plasmodiophorales and a single family plasmodio phoraceae are recognized. Basing on the aggregation of resting spores various genera are recognized.

6.3. SUB DIVISION : MASTIGOMYCOTINA (OF DIVISION EUMYCOTA)

Mastigomycotina is the first subdivision in the division Eumycota or true fungi, i.e. the fungi having rigid cell walls. Most of the members of this subdivision are aquatic, some are soil borne and a few are obligately parasitic on higher plants.

The most important character of the fungi included in this group is the production of zoospores. Hence, these are also called zoosporic fungi. Amongst the Eumycota, three distinct types of zoospores are present (fig. 6.6).

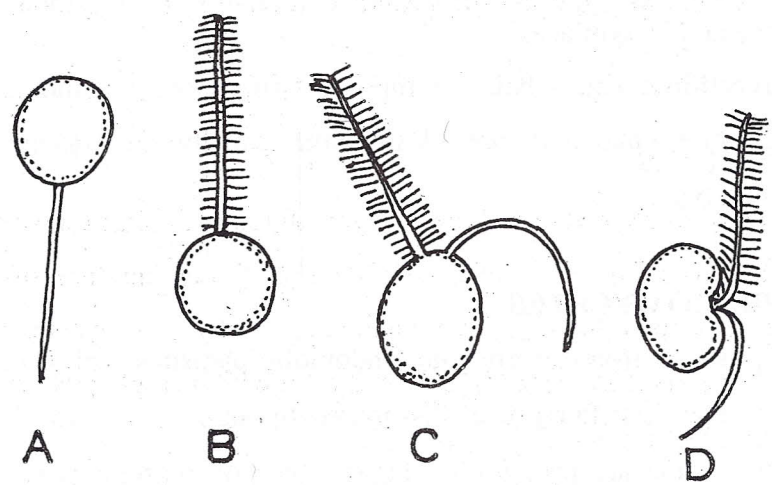

Figure 6.6. Different types of zoospores found in mastigomycotina A. Posteriorly uniflagellate zoopore, B. Anteriorly uniflagellate zoospore and C & D. Anteriorly and laterally biflagellate zoospores

1. Posteriorly uniflagellate zoospores with flagellum of whiplash type.

2. Anteriorly uniflagellate zoospores with the flagellum bearing fine lateral appendages i.e. tinsel type.
3. Biflagellate zoospores, with two flagella attached to the zoospore either anteriorly or laterally. Among the two flagella, one projecting upwards is tinsel type and the other projecting downwards is whiplash type. Hence these zoospores are described as 'heterokont'.

Basing on the structure of flagella, and associated vegetative and reproductive characters, Mastigomycotina is divided into three classes.

1. Chytridiomycetes: Zoospores posteriorly uniflagellate, thallus unicellular holocarpic or coenocytic eukarpic with rhizoidal system. Sexual reproduction varies from planoisogamy, planoanisogamy to heterogamy in which motile male gamete fertilize nonmotile oosphere.

2. Hyphochytridiomycetes: Zoospores anteriorly uniflagellate tinsel type; Thallus unicellular holocarpic to eukarpic with rhizoidal system; sexual reproduction isoplanogamy to anisoplanogamy.

3. Oomycetes: Zoospores anteriorly or laterally biflagellate heterokont type; thallus unicellular holocarpic to extensive coenocytic mycelium; sexual reproduction is oogamous type producing oospores.

6.3.1. CHYTRIDIOMYCETES:

The fungi belonging to chytridiomycetes are typically aquatic, but a few may be found in moist soil. Most of the members are saprophytic or free living and are of little economic importance. The one characteristic feature which distinguishes chytridiomycetes form other fungi is the production of motile cells (zoospores or gametes) each with a single posterior whiplash type flagellum. Other important features are

1. Coenocytic thallus, which may be unicellular holocarpic or eukarpic hyphae with rhizoidal system.
2. Chitin is the chief constituent of cell wall.
3. Sexual reproduction is isogamy, anisogamy or heterogamy.
4. Zygote is converted into a resting spore or resting sporangium

The class chytridiomycetes divided into 4 orders viz. chytridiales, harpochytriales, blastocladiales, and Monoblepharidales. The characters of these fungal groups are very much varied and described on taxonomic lines.

6.3.1.1. Chytridiales: Members of this group are mostly aquatic. Vegetative thallus shows much variation in different members. In morphologically simpler forms such as *Olpidium* and *Synchytrium*, it is holocarpic. i.e. whole structure is reproductive in function. Holocarpic forms are usually intracellular in the host i.e. endobiotic. In many chytrids, the thallus is differentiated into a vegetative part concerned with collection of nutrients and a reproductive part which produce zoospores or gametes; and it is described eucarpic. The vegetative part in chytrids mainly comprise of rhizoids, which help the fungi in anchoring and absorption of nutrients from substratum. In some eucarpic forms, only a single spore bearing structure, zoosporangium or gametangium is formed and such forms are called monocentric forms. Monocentric forms may be endobiotic or epibiotic. In chytrids, a number of zoosporangia are formed on rhizoidal system, and they are described as polycentric forms. In highly developed polycentric forms, rhizoidal system develops extensively and is described as 'rhizomycelium'. It differs from true mycelium in lacking nuclei. Different forms of thalli in chytridiales are shown in the figure 6.7.

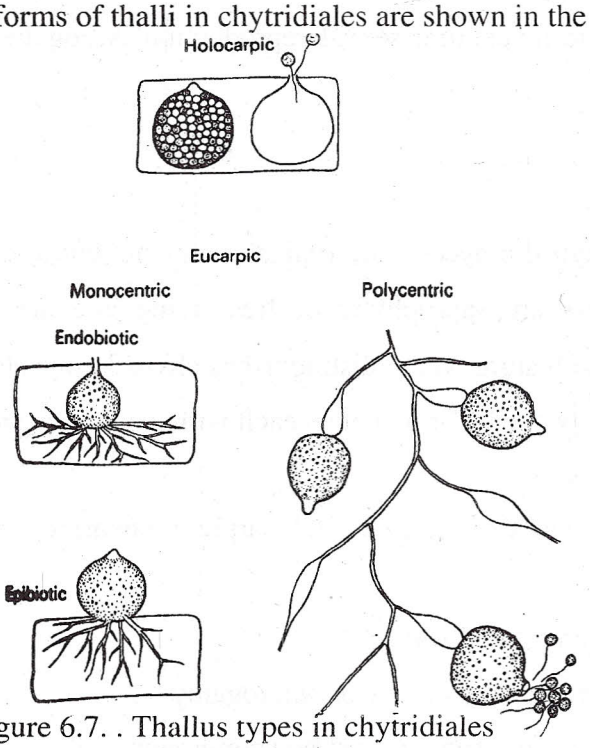

Figure 6.7. . Thallus types in chytridiales

In chytridiales, asexual reproduction is by means of production of zoospores in a zoosporangium. The zoosporangium is usually a spherical or pear shaped sac bearing one or more discharge tubes or exit papillae. The zoospores are released by dissolution of the tip of exit papilla. In some forms, on the tip of the papilla a shield like structure is present and it is described as operculum. In operculate forms, zoospores are released by throwing out the operculum.

The zoospore is a spherical or oval body with a long trailing whiplash type flagellum. The size of the zoospore is variable but roughly constant for a given species. After a period of motility they encyst and on germination, protoplast directly enters host cells in holocarpic forms, while in eucarpic forms, it germinate by giving rise to rhizoids.

Sexual reproduction in chytridiales is isogamous type. Two motile gametes unite to forms a zygote. The zygote usually forms thick walled resting sporangium.

The order chytridiales comprises of 9 families. Three families viz. Olpidiaceae, Achlyogetanaceae and Synchytriaceae the fungi are simple, holocarpic, endobiotic forms.
In three families viz. Phlyctidiaceae, Rhizidiaceae and Chytridiaceae, the fungi are eucarpic, monocentric forms. In three other families viz. Physodermataceae, Cladochytriaceae and Megachytriaceae, the fungi are well developed polycentric forms with extensive rhizomycelium

6.3.1.2. Harpochytriales: The fungi included in this order are characterized by eucarpic, uniaxial thallus with a basal disc like hold fast on the surface of the substratum and distal part composed of upper sporogenous and nucleated vegetative region, which persists after sporulation.

A single family Harpochyriaceae is recognized in the family with two genera viz. *Harpochytrium* and *Oedogoniomyces*.

6.3.1.3. Blastocladiales: The fungi belonging to this order are chiefly water moulds or soil inhabitant characterized by production of thick walled, resistant resting sporangia. The order is divided into three families viz. Coelomomycetaceae, Catenariaceae and Blastocladiaceae. The characters of the families are distinctly different.

The members of coelomomycetaceae are obligate parasites in the body cavities of mosquito larvae. The hyphae are naked i.e. with out cell walls and some what resemble the plasmodium of myxomycetes. The entire mycelium is converted into a thick walled resting

sporangium, which germinate releasin a mass of zoospores. Sexual reproduction of the fungus occurs in another host i.e. a copepod *C clops venalis*. Thus it shows heteroecious life cycle.

In Catenariaceae, thick walled sporangia are produced in chains i.e. sporangia are formed in catenate fashion.

In Blastocladiaceae, the members show alternation of generations between diploid sporothallus and haploid gametothallus. *Allomyces* is the best studied genus in this group. The life cycle of *Allomyces* is shown in the figure 6.8.

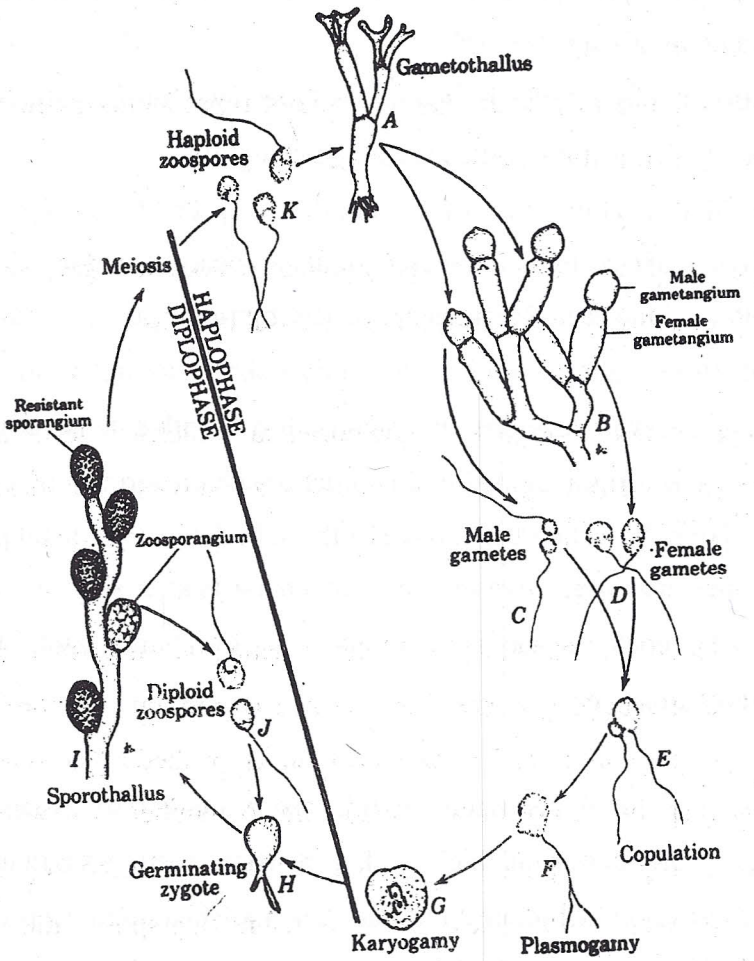

Figure 6.8. Life cycle pattern of of *Allomyces*

In many species of *Allomyces*, diploid thallus produces two types of sporangia viz. zoosporangia or mitosporangia, and resting sporangia or meiosporangia. In zoosporangia the nuclei divide

mitotically and produce diploid zoospores, which give rise to diploid sporothallus. In resting sporangia, at the time of germination meiosis occurs and release haploid zoospores which give rise to haploid gametothallus. Both sporothallus and gametothallus are identical morphologically except for the spore producing structures they bear. On gametothalli, smaller male gametangium and bigger female gametangium are produced in a chain and gametes they release are dissimilar in size but both are motile. Sexual reproduction occurs by fusion of planoanisogametes and zygote gives rise to diploid sporothallus.

6.3.1.4. Monoblepharidales:

This group is characterized by heterogamous type of reproduction which is unique in that the non motile oosphere is fertilized by a motile male gamete.

The thallus is well developed mycelial type; hyphae coenocytic highly vacuolate and much branched, the hyphae are attached to the substratum by a basal holdfast.

Asexual reproduction is by forming of zoosporangia, which are terminal and elongated. They are not well distinguished from vegetative hypae. The zoospores are released through the tip of the sporangium.

The same thallus produces gametangia. Male gametangia, antheridia, are small, narrow elongated and terminal. The female gametangium, termed oogonium, is round, big and formed below antheridium. As oogonium develops, antheridium is pushed to a side and the mature antheridium appears as an appendage of mature oogonium. A number of motile antherozoids are formed in antheridium. A single oosphere or egg is differentiated in the oogonium. The motile antherozoid fertilize the oosphere and zygote develops into a resting spore called oospore.

The order comprises two families viz. Monoblepharidaceae with a single genus *Monoblepharis* and *Gonapodyaceae* with two genera. *Monoblepharella* and *Gonapodya*. The vegetative and reproductive structures of Monoblepharis are shown in the figure 6.9.

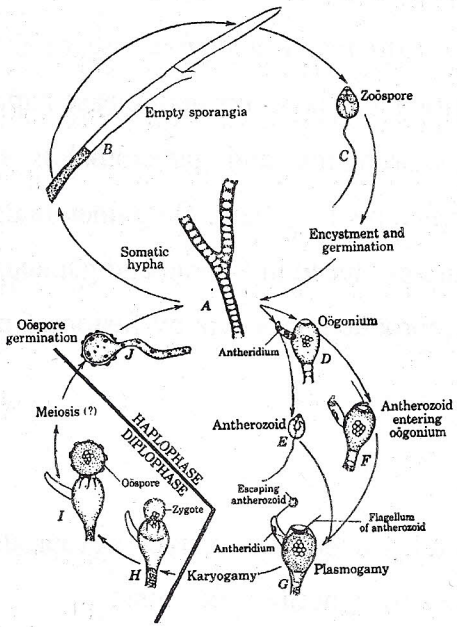

Figure 6.9. The vegetative and reproductive structures of *Monoblepharis*

6.3.2. HYPHOCHYTRIDIOMYCETES:

The hyphochytridiomycetes are aquatic, fresh water or marine chytrid like fungi, whose motile cells are anteriorly uniflagellate possessing a flagellum of tinsel type. They are parasitic on algae and other fungi or saprobic on plant and insect debris in water.

This is a small class comprising a single order hyphochytriales with 3 families 1. Hyphochytriaceae 2. Anisolpidiaceae, and 3. Rhizidiomycetaceae

The thallus closely resembles that of chytridiales. It may be holocarpic or eucarpic. Holocarpic forms are endobiotic. Eucarpic forms may be monocentric or polycentric with rhizoidal system.

The zoosporangia are inoperculate and zoospores are released by dissolution of exit tubes. The zoospores are characteristic with anterior tinsel flagellum

Sexual reproduction is found only in a few species and incompletely known.

6.3.3. OOMYCETES:

The members of the class are characterized by the presence of biflagellate zoospores, each bearing one tinsel flagellum directed upwards and one whiplash type flagellum directed backwards. Sexual reproduction is characterized by oogamous type and oospores are the resting structures.

The cell wall of oomycetes fungi are composed of cellulose with little or no chitin. Meiosis occurs in the gametangia rather than at the time of germination of zygote, as in other fungi. Hence, the vegetative mycelium is diploid. Basing on the cell wall composition and gametangial meiosis, oomycetes are considered as distinct from other fungi.

The vegetative thallus of oomycetes range from unicellular holocarpic forms to well developed mycelial type and reduced biotrophic mycelium in highly developed parasitic forms.

The class oomycetes is divided into 4 orders viz. 1. Lagenidiales. 2. Saprolegniales. 3. Leptomitales and 4. Peronosporales.

6.3.3.1. Lagenidiales:

The members of this order are a small group of aquatic fungi, which are all holocarpic forms and parasitic on algae, small animals and other forms of aquatic life. The zoospores are anteriorly biflagellate and the flagella are heterokont. The vegetative structures range from unicellular to branched filamentous forms. Sexual reproduction is by gametangial copulation, which is unique among the zoosporic fungi. The asexual and sexual reproductive structures of *Lagenidium* are shown in the figure 6.10.

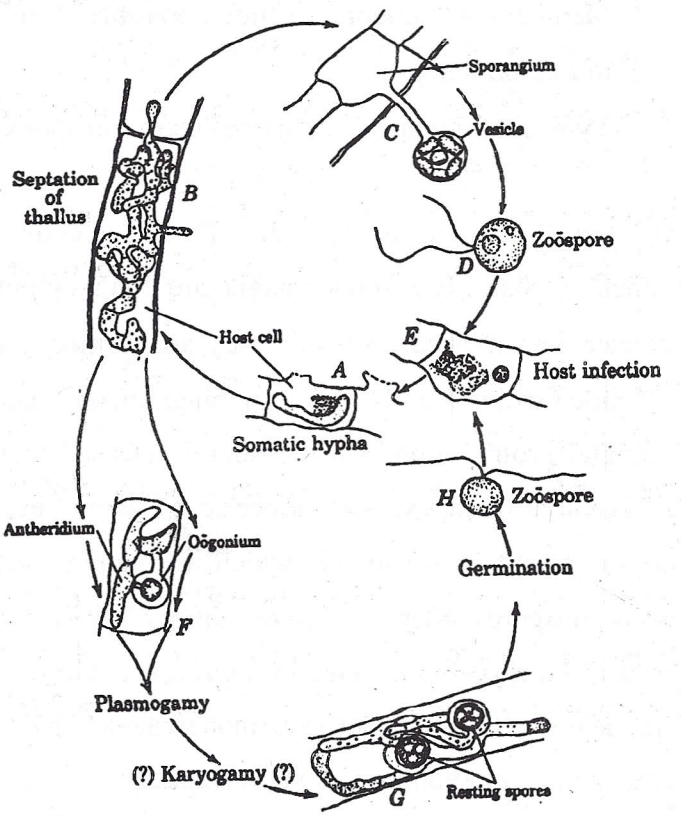

Figure 6.10. Asexual and sexual reproductive structures in *Lagenidium*

The order comprises three families viz. 1. Olpidiopsidaceae 2. Sirolpidiaceae and 3. Lagenidiaceae.

The members of olpidiopsidaceae are unicellular holocarpic forms. In the other two families the vegetative thallus is very limited, intracellular and holocarpic.

6.3.3.2. Saprolegniales:

The term 'water moulds', though applicable to a number of other fungal groups as well, is customarily used to designate the members of saprolegniales because most of them occur abundantly in clear waters and are easily isolated. Many species are soil inhabiting also.

The order saprolegniales is divided into 5 families.

Ectrogellaceae	– include unicellular holocarpic forms.
Thraustochytriaceae	– include chytrid like fungi with rhizoids but produce typically biflagellate zoospores.
Haliphthoraceae	– Marine zoosporic fungi.
Leptolegniellaceae	– Members with doubtful affinity, possibly more closely related to Leptomitales.
Saprolegniaceae	– Typical of the order, comprises many zoosporic aquatic fungi.

Saprolegnia is a typical member of the order. Thallus is composed of coenocytic, much branched, relatively thick hyphae. The zoosporangia are long cylindrical, terminal and only slightly bigger in diameter than hyphae on which they are formed. After release of zoospores, another sporangium is formed within the old empty sporangium; and such a process is called sporangial proliferation. The zoospores released from the sporangia are pear shaped and bear two flagella at the apex. After a period of swimming, they encyst. The cyst on germination gives rise to secondary zoospores, which are kidney shaped and bear two lateral flagella. These secondary zoospores, after a period of motility, encyst and germinate to give rise to a germ tube which grows into mycelium. Thus, in *Saprolgnia*, the zoospores are dimorphic and diplanetic. In other members of saprolegniaceae, monoplanetic and aplanetic spores are also formed and it is considered to be of evolutionary significance.

Sexual reproduction is typical oogamous type. Oogonium is globose and contains more than one egg or oosphere without periplasm Antheridia are elongate multinucleate structures. They attach to oogonium and sends in fertilization tube, which branch with in the oogonium to release one functional male nucleus into each egg contained in the oogonium. After fertilization each fertilized egg develops into oospore. The life cycle of *Saprolegnia* is shown in the figure 6.11.

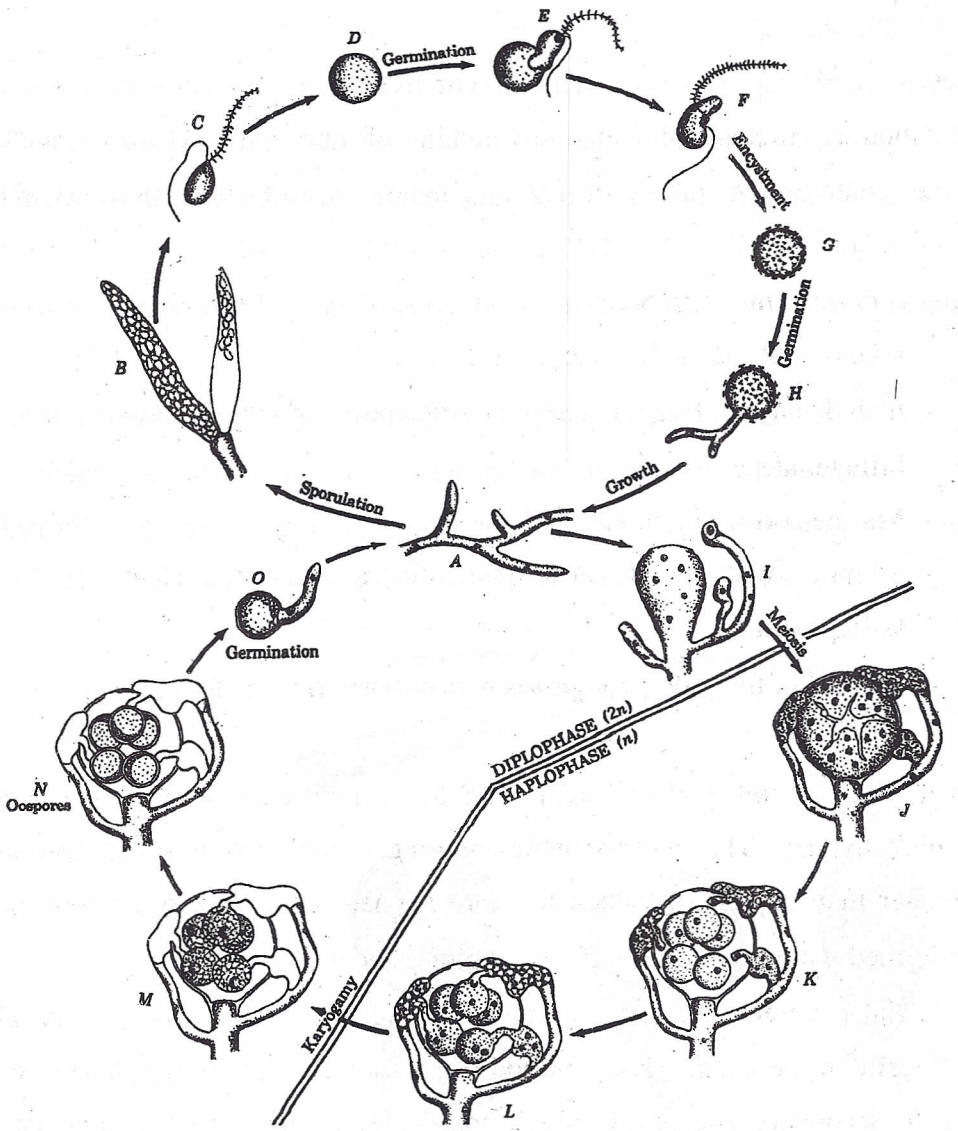

Figure 6.11. Life cycle of *Saprolegnia*

Some members of saprolegiaceae such as species of *Achlya* are heterothallic, and when two compatible mycelia grow near by produce a series of at least 4 hormones (ABCD) which are involved in formation of sex organs.

6.3.3.3. Leptomitales:

Leptomitales is small order of aquatic, saprobic fungi classified into two families 1) Leptomitaceae and 2) Rhipidiaceae.

In general they resemble saprolegniales. The somatic hyphae, are tubular coenocytic, truly aseptate, but constricted at regular intervals. The constrictions are often plugged with granules of cellulin, giving the false appearance of septa; and this feature distinguishes this group from saprolegniales.

Asexual reproduction is by means of terminal zoosporangia from which biflagellate zoospores are formed.

Sexual reproduction is by gametangial contact. Oogonium is globose, usually terminal, with central oosphere surrounded by a small amount of periplasm. Antheridia are smaller, club shaped, borne an same or different hyphae bearing oogonium. A single oospore is formed per oogonium. The vegetative hyphae and reproductive structures of leptomitales are shown in the figure 6.12.

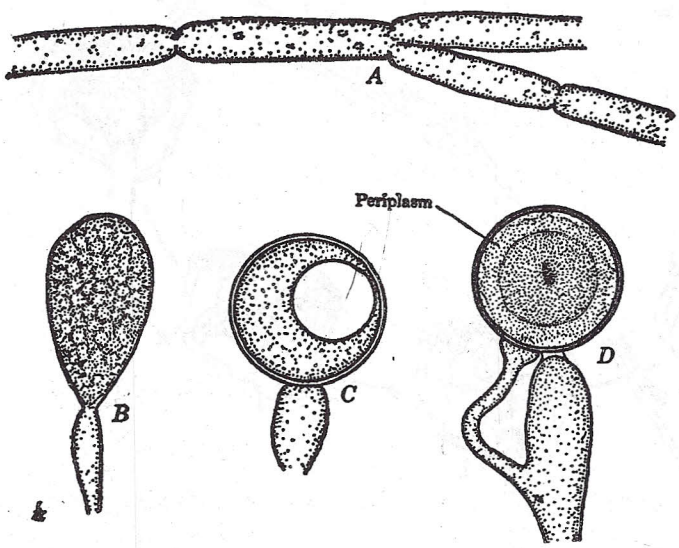

Figure 6.12. The vegetative hyphae (A), sporangium (B), oospore (C) and sexual reproductive structures (D) of the members of Leptomitales

6.3.3.4. Peronosporales:

The peronosporales represent the highest development of the class oomycetes. This large order of fungi includes aquatic, amphibious and terrestrial forms, culminating in a group of highly specialized obligate parasites. The damping off fungi; white rust fungi, downy mildew fungi; all belong to this order which includes several hundred species. This makes the group economically very important

In peronosporales, mycelium is well developed consisting of coenocytic, stout hyphae which branch freely. A large number of species produce haustoria which may be knob like, elongated or branched within the host cells. The hyphae of the parasitic species may be intercellular or intracellular.

Asexual reproduction is by production of sporangia. The sporangia are produced on undifferentiated somatic hyphae in lower peronosporales while in higher forms they are produced on specialized stalks called sporangiophores. The sporangium in most cases germinate to give rise to a number of laterally biflagellate, reniform zoospores; while in highly specialized forms it germinate by germ tube, thus act as a conidium.

Sexual reproduction is by means of well differentiated oogonia and antheridia borne on same or on different hyphae. The oogonium is generally globose, contain uni-or multinucleate oosphere surrounded by a layer of periplasm. The antheridium is usually elongated, uni-or multi nucleate. Fertilization is achieved by gametangial contact and formation of fertilization tube. After fertilization, zygote develops a thick wall and becomes oospore. The oospore wall is three layered. The outer layer is smooth or variously sculptured or ornamented, it may be spiny, warty, wavy, ridged or variously marked. The mature oospore generally lies free within the oogonial wall, but in many species it adheres to the oospore so closely that it appears to be united with it. After overwintering, the oospore germinates by giving rise to zoospores or by germ tubes.

The order peronosporales is divided into 4 families.

1. Pythiaceae: Sporangiophores similar to hyphae (*pythium*) or slightly different (*Phytopthora*). The sporangiophore continue to grow indefinitely producing sporangia as it grows resulting in

sporangia of different ages from immature to mature on same sporangiophore. Important genera of the family are *Pythium*, and *Phytophthora*. The life cycle of *Pythium debaryanum* is shown in the figure 6.13.

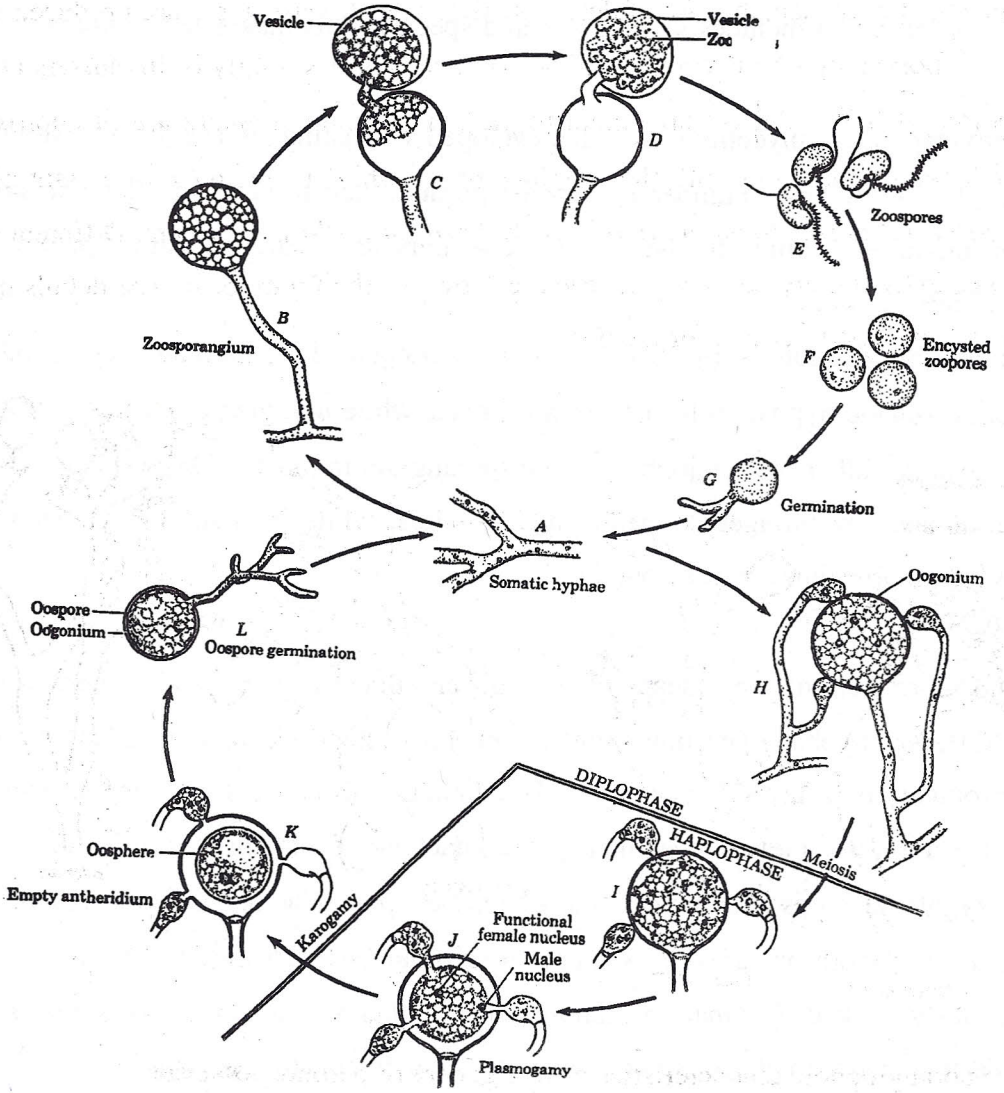

Figure 6.13 The life cycle of *Pythium debaryanum*

2. Peronophythoraceae: This is a small family comprising a single genus *Peronophythora.* It resembles pythiaceae members in most characters, but the sporangiophores are of determinate growth.

3. Peronosporaceae: All the members of the family are highly developed obligate plant pathogens producing a disease described by downy mildew. In this family, sporangiophores are distinctly different from somatic hyphae, and are of determinative type. No sporangia are produced until the development of sporangiophore is complete. Sporangia are formed singly or in clusters at the tips of mature sporangiophores. All the sporangia formed on a sporangiophore are of approximately same age. After the sporangia fall off, the sporangiophore whithers and dies. Important genera of the family are *Peronospora*, *Pseudoperonospora*, *Sclerospora*, *Plasmopara* etc. Different types of sporangiophores in the family peronosporaceae are shown in the figure 6.14. The details of sexual reproduction are same as described for the order.

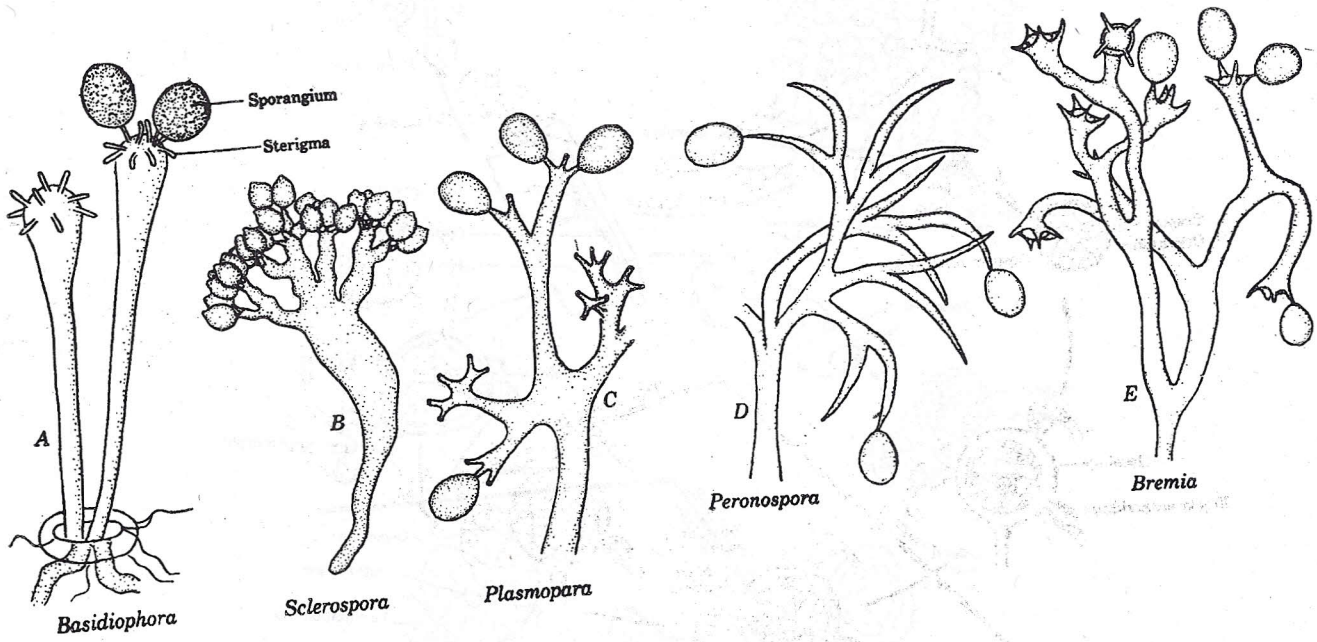

Figure 6.14. Sporangiophore characteristics of five genera of peronosporaceae

4. Albuginaceae: This family contains a single genus *Albugo* with about 30 species. All the species are pathogenic to higher plants producing a disease described as white blisters or white rust. The sporangiophores are distinct and sporangia are formed in chains at the tips of short stout club shaped sporangiophores. The sporangiophores are of indefinite in growth but constant in its morphology and structure. The sexual reproduction is typically oogamous type. The life cycle of *Albugo candida* is shown in the figure 6.15.

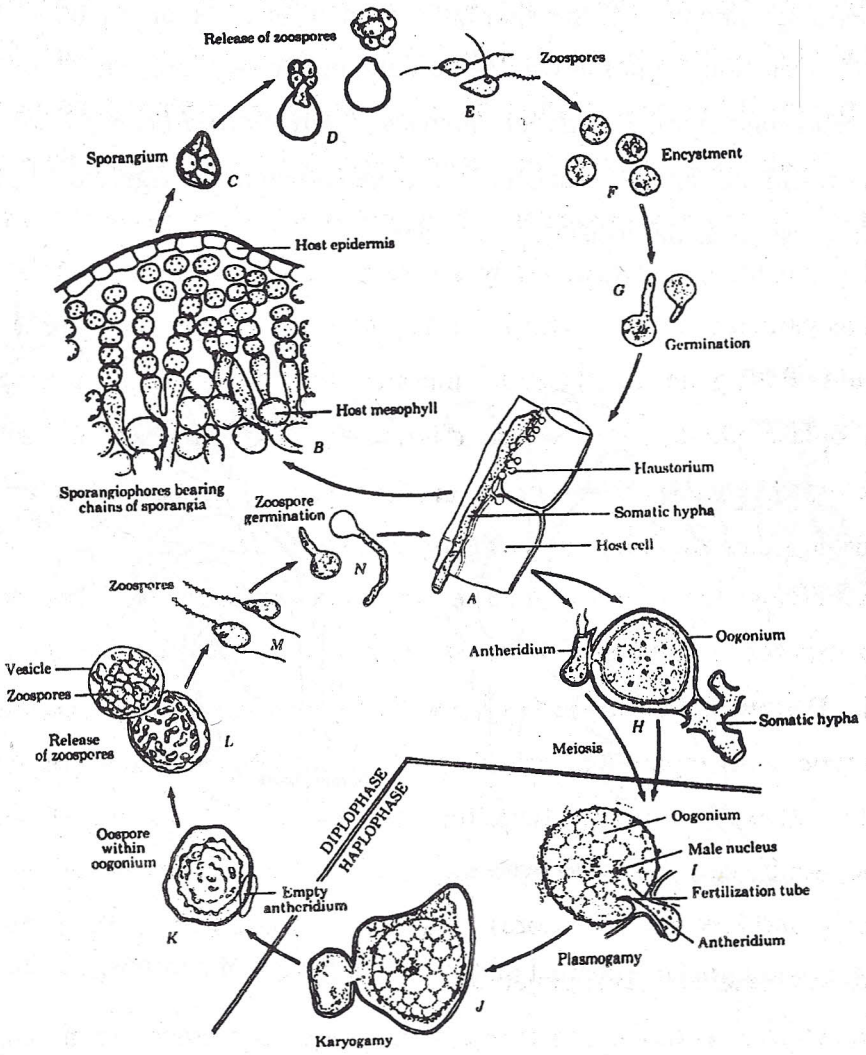

Figure 6.15. Life cycle of *Albugo candida*

6.4. SUMMARY

Ainsworth (1973) divided the fungi into two divisions viz. Myxomycota and Eumycota. The fundamental difference between the two groups is presence or absence of cell wall. The fungi without cell wall are placed in Myxomycota and those with a rigid cell wall are treated as Eumycota or true fungi.

The division myxomycota is a relatively small group of organisms commonly called slime moulds. The class Myxomycota is divided into four Classes viz. Acrasiomycetes, Hydromyxomycetes, Myxomycetes and Plasmodiophoromycetes. The vegetative stage of all the members is called plasmodium; but it differs in structure in various groups. In Acrasiomycetes, myxamoebae represent the trophic stage. They aggregate to form a composite structure in which individual cells retain their identity, and hence it is called pseudoplasmodium and it gives rise to fruiting structure. In hydromyxomycetes, which are aquatic slime moulds, the plasmodia crawl in a network of slime tubes and hence they are called net slime moulds. The amoeboid cells or swarmers act as gametes and unite to form a zygote, which is the starting paint of trophic stage, the Plasmodium. Basing on the structure, plasmodium in myxomycetes is recognized as three types viz. protoplasmodium, aphanoplasmodium and phaneroplamodium. Fruit bodies formed in myxomycetes are three types viz. sporangium aethallium and plasmodiocarp. The members of plasmodiophoromycetes are obligately parasitic, endobiotic forms.

The sub division Mastigomycotina of Eumycota are characterized by production of motile cells, either zoospores or gametes. The zoospores formed in Mastigomycotina are fundamentally three types viz. Posteriorly uniflagellate type with single long whiplash type flagellum, Anteriorly uni flagellate type with single tinsel type flagellum and biflagellate zoospores with one whiplash type flagellum and one tinsel type flagellum. Basing on the structure of zoospores and other characters, the sub division Mastigomycotina is divided into three classes viz. Chytridiomycetes with posteriorly uniflagellate zoospores, Hypochytridiomycetes with anteriorly uniflagellate zoospores and oomycetes with biflagellate zoospores.

The vegetative thallus of zoosporic fungi vary very much from unicellular holocarpic forms to highly developed mycelial type with coenocytic, tubular, much branched hyphae without cross walls. Asexual reproduction is by producing zoospores in zoosporangia. Sexual reproduction

varies from planoisogamy to planoanisogamy to oogomous type the characters of various groups vary very widely and described on taxonomic lines.

6.5. MODEL QUESTIONS

Essay type questions

1. Give a general account of slime moulds.
2. Discuss the structure and reproduction in different groups of slime moulds
3. Discuss the thallus organization and reproduction in Mastigomycotina.
4. Give an account asexual and sexual reproduction in zoosporic fungi.
5. Discuss the classification of Mastigomycotina giving salient features of different groups.

Short answer type questions

6. Slime moulds
7. Classification of Myxomycota
8. Types of zoospores in Mastigomycotina
9. Chytridiales
10. Peronosporales.

6.6. REFERENCE BOOKS

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LESSON – 7. GENERAL ACCOUNT OF ZYGOMYCOTINA AND ASCOMYCOTINA.

OBJECTIVE: To explain the general characters, reproduction and classification of fungi belonging to the subdivisions zygomycotina and ascomycotina of Division Eumycota.

CONTENTS

7.1. Introduction
7.2. Zygomycotina
7.2.1. Zygomycetes
7.2.2. Trichomycetes
7.3. Ascomycotina
7.3.1. Hemiascomycetes
7.3.2. Plectomycetes
7.3.3. Pyrenomycetes
7.3.4. Discomycetes
7.3.5. Loculoascomycetes
7.3.6. Laboulbeniomycetes
7.4. Summary
7.5. Model questions
7.6. Reference books

7.1. INTRODUCTION

The fungi that produce coenocytic mycelium and reproduce asexually by aplanospores produced in sporangia, and sexually by producing zygospores are placed in the subdivision zygomycotina. The fungi that produce septate mycelium and reproduce asexually by conidia and sexually by producing ascospores are placed in the subdivision Ascomycotina. The details of the fungi placed in these two subdivisions of Eumycota are explained in this lesson

7.2. SUBDIVISION: ZYGOMYCOTINA

The fungi belonging to zygomycotina usually produce mycelium, which is coenocytic and aseptate like in members of Mastigomycotina, but differ from them by complete absence of motile spores either in asexual or sexual reproduction.

Two important characters of these fungi are

1. Production of non motile aplanospores that are produced in specialized sac like structures sporangia in a sexual reproduction, and

2. Production of zygospores in sexual reproduction, which are formed by gamentangial copulation.

This subdivision includes more than 145 genera 765 species. Most of them are saprophytes and a few are facultative or weak parasites of plants, animals and other fungi. Some are associated with arthropods.

The subdivision comprises two classes viz. Zygomycetes and Trichomycets. The fungi included in the class Zygomycetes are mo[illegible]y saprophytes, though some are parasitic on plants and animals. The class Trichomycetes is a [illegible]up of uncertain affinity and are mostly associated arthropods, living in their guts.

7.2.1. Class: ZYGOMYCETES

The fungi included in zygomycetes possess typically coenocytic tubular mycelium; reproduce asexually by sporangiospores and sexually by zygospores.

The class is divided into 3 orders viz. Mucorales, Entomophthorales and Zoopagales.

Mucorales include mainly saprophytic fungi. Entomophthorales include insect parasites and zoopagales include fungi either parasitic or predaceous on protozoa and nematodes occurring in soil.

7.2.1.1. Order MUCORALES

Members of the order mucorales are abundant in soil, on dung and on moist fresh organic matter. Most of them are free living saprophytic and play an important role in early colonization of various substrata in soil. Species of *Mucor* and *Rhizopus* which cause spoilage of bread and other exposed foodstuffs are common ubiquitous fungi of the group. The fungi which are symbiotically

associated with roots of most of the land plants producing vesicular-arbuscular mycorrhizal association also belong to this group.

7.2.1.1.1. Mycelium: In Mucorales, mycelium is stout, well developed, much branched and coenocytic. Septa may appear in older mycelium and also to differentiate reproductive organs.

The mycelium in some species bears rhizoids Eg. *Rhizopus*. The rhizoids are formed at points where the mycelium contacts a hard surface. The rhizoids adhere to the substratum and anchor the fungus securely. The hyphae that connects two groups of rhizoids is called stolon. In rhizoidal species, sporangiophores usually develop opposite to rhizoids.

Some fungi produce normal mycelium and also can exist as unicellular yeast like forms. The phenomenon is known as dimorphism. It is quite common in the genus *Mucor*; E.g. *M. rouxii*.

7.2.1.1.2. Asexual Reproduction: The members of mucorales reproduce asexually by aplanospores produced in sporangia. The sporangia are produced terminally on simple or branched specialized stalks called sporangiophores. A portion of the contents of hyphae pass into tip region which enlarges and then cut off from the hyphae by a septum. The multinuclear contents of the sporangium are cleaved into segments containing one or more nuclei. Then these round up, encyst and develop into spores. The spores are released by the rupture or dissolution of the sporangium wall.

The morphology of sporangiophores and sporangia show much variation, and it is based on these variations the order is divided into various families.

In *Mucor*, typical sporangium develops at the tip of a sporangiophore as a globose swelling. A dome shaped septum is laid down cutting a distal soporiferous region from basal cylindrical or sub globose spore free region, the columella. The columellate sporangia of *Mucor* is considered as typical sporangium.

In the genus *Saksenaea*, the sporangium is typically columelate but distal part of the sporangium becomes elongate and forms a long beak

In the genus *Pilobolus*, the sporangia are thick walled, dark brown to black, sub globose and are formed terminally on a long sporangiophore. The sporangiophore is differentiated into an enlarged trophocyst at the base and thin sub sporangial vesicle below the sporangium. The sporangia are shot off forcibly by bursting of the sub sporangial vesicle.

In the genus *Chaonephora*, two types of sporangia are formed 1) Mucor type sporangium with columella and 2) non columellate small sporangiola on a vesicle at the tip of sporangiophore.

In the genus *Thamnidium*, two types of sporangia viz. columellate Mucor type sporangium, and non-columellate sporangiola are formed on the same sporangiophore. The columellate sporangium is formed at the tip of the sporangiophore while non-columellate sporangiola are formed on the branches of sporangiophore lower down.

In the genus *Cunnighamella*, the terminal portion of the sporangiophore enlarges to form a vesicle, and on the vesicle single spored sporangiola are formed in large numbers.

In the genus *Syncephalastrum*, sporangiophore forms a globose vesicle at the tip. From this vesicle, large number of elongated, cylindrical, non-columellate sporangia with spores formed in uniseriate manner are produced. Such sporangia are described as merosporangia. In the merosporangia of *Syncephalastrum* a number of spores are formed.

In the genus *Dimargaris* two spored merosporangia are produced.

In the genus *Kickxella*, single spored merosporangia are produced on fertile branches called sporocladia.

Various types of asexual spore forming structures seen in the order Mucorales are shown in the figure 7.1.

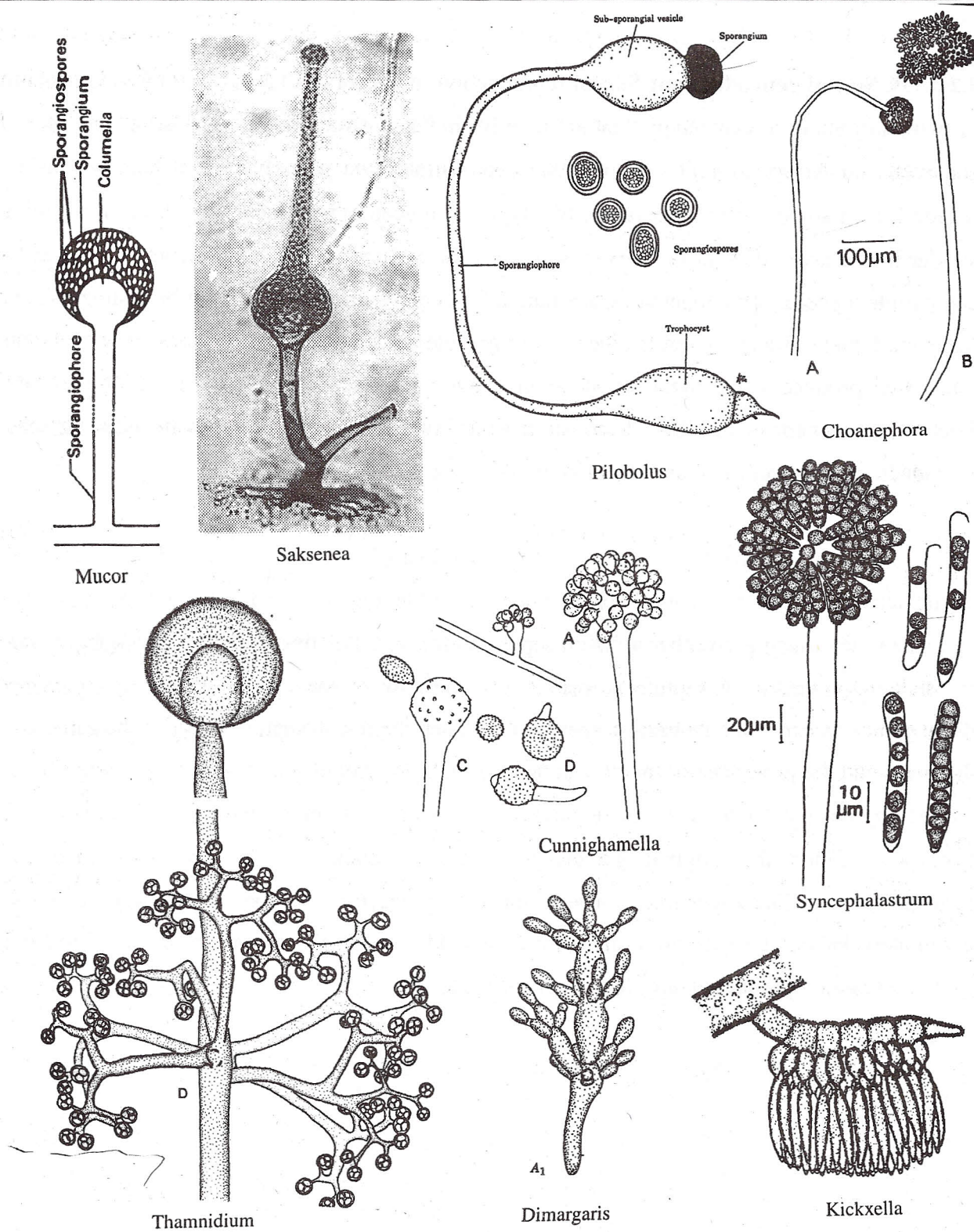

Figure 7.1. Sporangiophore and sporangia in A. Mucor B. Saksenea C. Pilobolus D. Choanephora. E. Thamnidium F. Cunninghamella G. Syncephalastrum H. Dimargaris I. Kickxella .

7.2.1.1.3. Sexual reproduction: Sexual reproduction in Mucorales takes place by the copulation of two multi nucleate gametangia that are mainly similar in structure, but may differ in size. A.F. Blakeslee, an American geneticist, in 1904 demonstrated the phenomenon of heterothallism in mucorales. In some mucoralean fungi, two types of mycelia are present, which are morphology similar but behave differently in sexual reproduction. Sexual reproduction occurs only when two compatible mycelia come together. Gametangia formed are similar and cannot be distinguished as male and female, Blakeslee termed the two compatible mycelia as + and – strains. However, some mucorales produce gametangia on same mycelium and such species are called homothallic. Burgeff, a biochemist, in 1924, demonstrated the involvement of hormones in inducing sexual reproduction. The hormones are identified as Trisporic acid A, B, C.

When two compatible hyphae come together, zygophores are induced to form near the tips of the actively growing somatic hyphae. The compatible zygohores are attracted to one another, and when they touch each other a fusion septum is formed. The tips of the two zygophores swell to form progmetangia. A septum is formed near the base of each gametangium to separate the distal gametangium from the basal suspensor cell. Then the fusion septum between the gametangia dissolves and the protoplast of the two gametangia mix. Nuclei of + and –strains pair together and karyogamy occurs. Then the zygote enlarges, develops a thick multi layered wall and becomes a zygospore. Since the original gametangial wall is retained it is often described as a zygosporangium. The zygospore is a resistant resting structure. Meiosis occurs when zygospore germinates. Hence the vegetative mycelim is haploid. The details of the life cycle of *Rhizopus*, showing sexual reproduction are shown in the figure 7.2

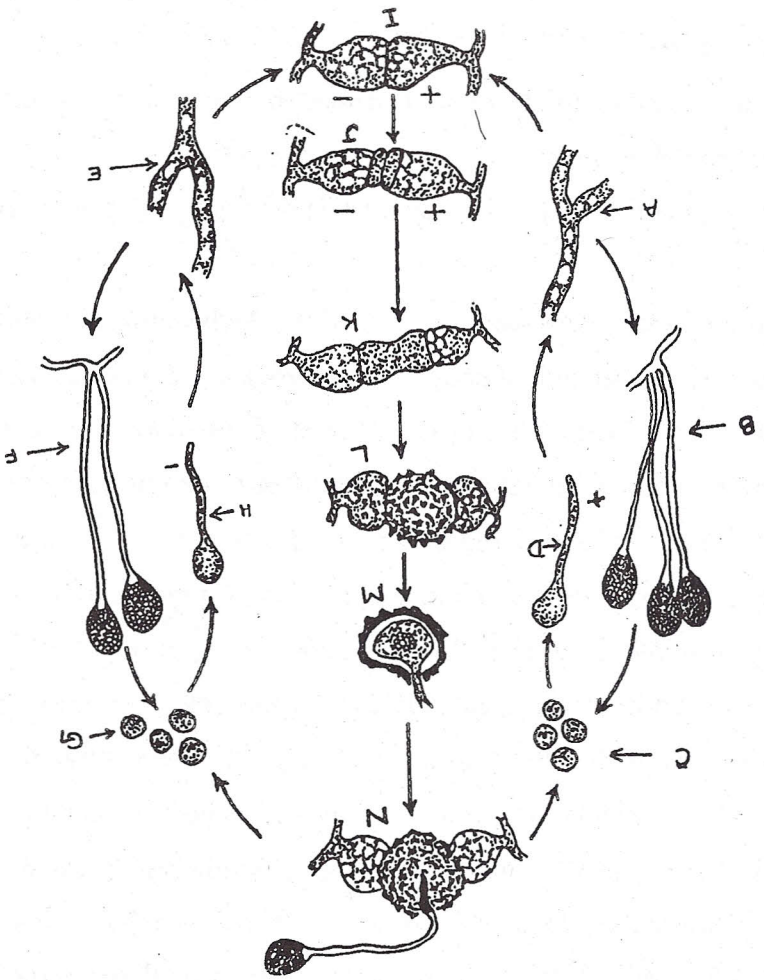

Figure 7.2. Life cycle of *Rhizopus stolonifer*

7.2.1.1.4. Classification: The order Mucorales is divided into 14 families, mainly basing on asexual reproductive structures.

1. Mucoraceae: Produce typical columellate sporangia. Eg. *Mucor*

2. Pilobolaceae: Sporangium is released with force from sporangiophore. Eg. *Pilobus*

3. Choanephoraceae: Produce two types of sporangia 1. Typical Mucor type columellate sporangia and 2. Sporangiola on different sporangiophores. Eg. *Choanephora*

4. Thamnidiaceae: Columellate sporangia and sporangiola are formed on same sporaniophore. Eg: *Thamnidium, Helicostylum.*

5. Cunninghamellaceae: Produce single spored sporangiola. E.g. *Cunnighamella*

6. Syncephalastraceae: Produce spores in merosporangia, occur in soil. Single genus *Syncephalastrum* is recognized.

7. Piptocephalidaceae: Similar to *Syncephalastrum*, but parasitic on other fungi mostly mucorales. E.g: *Piptocephalis, Syncephalis*

8. Dimargaritaceae: A small family in which two spored merosporangia are produced. E.g. *Dimargaris*.

9. Kickxellaceae: Sporangia are indehiscent, one celled in which sporangial wall and sore wall are fused. Spores are produced on special branches called sporocladia. Eg: *Kickxella*.

10. Mortierellaceae: The members produce non-columellate sporangia on tapering sporangiophores. The peridium of sporangium is evanescet quite early and maturity a group of spores formed attached to the tip of sporangiophore. In addition, they also produce single spored sporangiophore. One peculiarity of the members in sexual reproduction is that a group of zygospores are loosely surrounded by sterile hyphae, giving the appearance of a sporocarp.

11. Endogonaceae: The family is of great interest because the members of the family form endomycorrhizal association with almost 85% of land plants. The fungi produce thin walled arbuscules and thick walled vesicles in the host roots and hence are called vesicular-arbusclar mycorrhizae or simply VAM. The VAM fungi are obligate symbionts and cannot be cultured.

The VAM fungi do not produce any sporangia. Zygospores are produced in clusters and are surrounded by thick well of sterile hyphae forming specialized structure described as sporocarp.

All fungi do not produce zygpspores. Those that produce zygospores are placed in the genus *Endogone*.

VAM fungi produce very large, conspicuosus chlamydospores or VAM spores, and basing on the characters of spores different genera like *Glomus*, *Gigaspora*, *Acaulospora*, *Sclerocystis* etc. are recognized.

A part from the 11 families described above, 3 more families are recently described basing on specialized asexual reproduction structures. They are as follows.

12. Sakseneaceae: It includes two genera viz. *Saksenea* and *Ehinosporangium*. They produce columellate sporangia and the upper portion of the sporangium becomes elongated into a long

neck, giving flask shaped appearance to the sporangium. No sexual reproduction has been observed.

13. Radiomycetaceae: Two genera viz *Radiomyces* and *Hesseltinella* are recognized.

In the fungi, the tip of the sporangiophore bulges to form a globose vesicle. It is described as primary vesicle. On this a number secondary vesicles are formed and on secondary vesicles, small sporangiola are produced.

14. Helicocephalidaceae: Two genera *Rhopalomyces* and *Helicocephalum* are recognized. They are pathogenic to soil nematodes and eggs.

They produce globose spores in chains at the tip of the sporangiophores. Some Mycologists prefer to place the genus in zoopagales.

7.2.1.2. Order Entomophthorales:

The order Entomophthorales is an interesting group of fungi parasitic on both plants and animals or saprobic. There are 6 genera in the group and all of them are placed in a single family Entomophthoraceae. The best-known genus is *Entomphthora*, the species of which are insect parasites.

The mycelium in *Entomophthora* is limited, and is divided by septa into uninucleates segments. There is a tendeny for the mycelium to fragment into forms called 'hyphal bodies'.

From the hyphal bodies, a sporangiophore develops which may be undivided or divided at the upper part to form a number of upright branches. At the tip of the sporangiophore or its branches a single spored sporangium is formed and it is forcibly discharged.

In sexual reproduction, hyphal bodies act as gametangia and copulate to develop a zygosporangium containing a zygospore. The zygosporangium develops from as a lateral growth or bud arising from between the two fusing cells. The details of asexual and sexual reproduction in *Entomophthora* are shown in the figure 7.3.

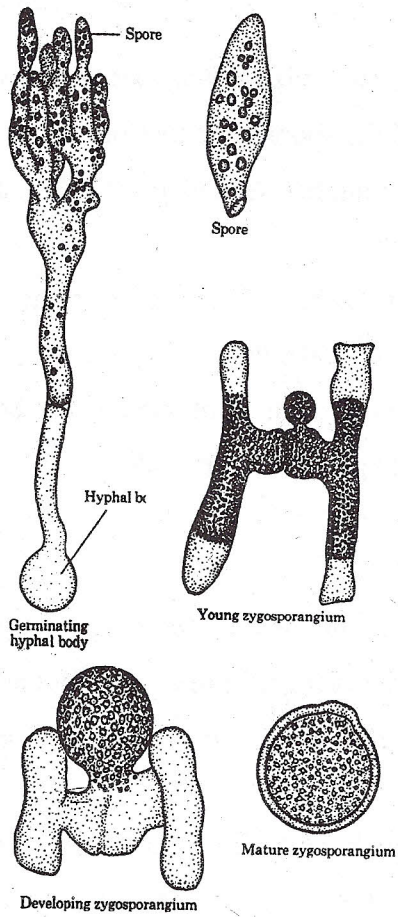

Figure 7.3. Asexual and sexual reproductive structures in *Entomophthora sepulchralis*

7.2.1.3. Order Zoopagales:

The members of zoopagales are soil borne, predacious or parasitic on amoebae, rhizopods or nematodes. The fungi are mainly predacious on nematodes and parasitic either ecto or endo, on amoebae. The order comprises two families.

The fungi predaceous on nematodes are placed in the family. Zoopagaceae while the parasitic forms on amoebae are placed in the family cochlonemaceae.

In predacious forms, mycelium is extensive, aseptate and branched irregularly. The mycelium is coated with a sticky substance to which the prey adheres. The animal thus captured is invaded by fine variously branched haustoria and eventually die. The fungus absorbs nutrients from dead or dying animal. *Zoopage* and *Cystopage* are important predaceous genera.

In endoparasitic forms, mycelim consists of short thick, branched or unbranched hyphae that may be spirally coiled.

In ectoparasitic forms, infecting spore that adheres externally to the host functions as the thallus. Nutrients are absorbed by a branched haustorium. The genus *Cochlonema* is an important endoparasite. The genera *Bdellospora* and *Amoebophilous* are ectoparasites.

Asexual reproduction is by means of thread like spindle shaped or globose spores that are borne singly, in chains or in loose beads. The spores are not forcibly discharged.

Sexual reproduction, where known, is by production of zygospores formed following fusion of equal or unequal gametangia.

The characters of ecto and endoparasitic zoopagales are shown in the figure 7.4

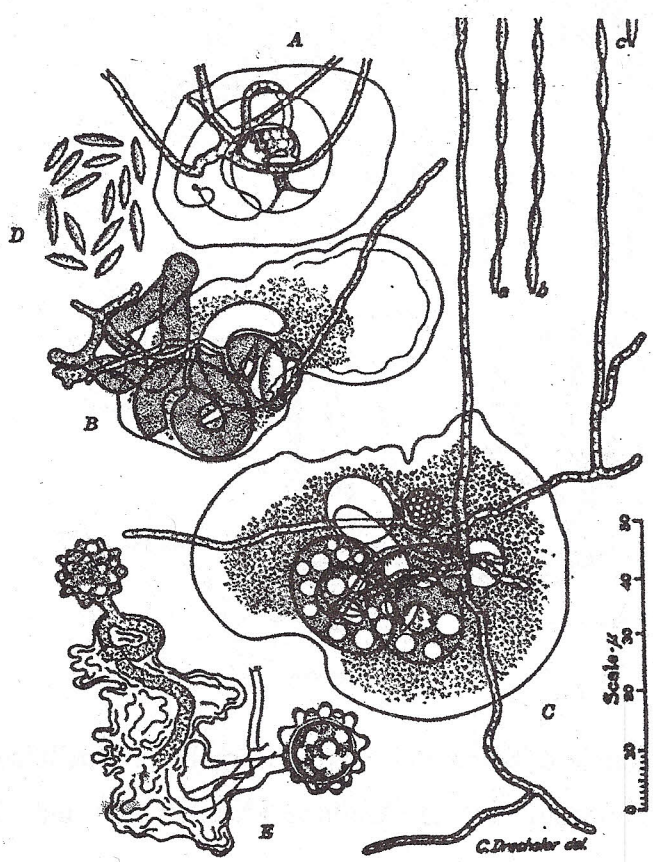

Figure 7.4. Characters of ecto and endoparasitic zoopagales

7.2.2. TRICHOMYCETES:

The class Trichomycetes is comprised of forms obligately associated with living arthropods – that is, insects, millipedes and crustaceans. The word associated is used because the exact nature of the relationship between these fungi and their hosts is not fully understood.

Trichomycetes mainly grow in the hind gut of the host, where they attach to chitinous gut lining by means of a special structure called hold fast. The mycelium does not penetrate the host tissue and apparently obtain nutrients from the contents of the gut lumen. More than one species may occur in the same host and specificity is not high. They should be teased out of their hosts for study. Mycelium is very limited, may be branched or unbranched. Some are septate while others are aseptate. Asexual reproduction varies much in different groups while sexual reproduction is not confirmed.

The characteristic features of some representative genera of Trichomycetes are shown in the figure 7.5.

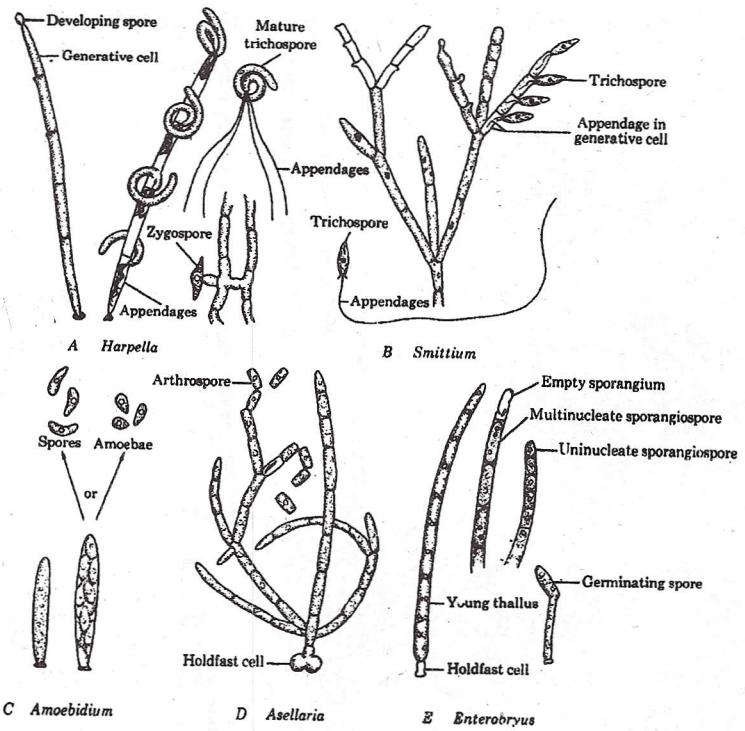

Figure 7.5. Some representative genera of Trichomycetes A. Harpella (Harpellales), B. Smittium (Harpellales), C. Amoebidium (Amoebidiales) D. Asellaria (Asellariales) and E. Enterobotrys (Eccrinales).

The class Trichomycetes is divided into 4 orders.

Order Harpellales: Thallus filamentous, septate, branched or unbranched. Asexual reproduction is by means of producing trichospores. A trichospore is an exogenous, dehiscent, usually elongate sporangium containing single uninucleate sporangiospore and having one to several basally attached filamentous appendages contiguous with the sporangium wall.

The order comprises two families viz. Harpellaeae and Genistellaceae.

Order Asllariales: The mycelium comprises branched, septate hyphae. The cells of the branches transform into spores and detach. Hence, they are called arthrospores.

The order comprises a single family Asellariaceae.

Order Amoebidiales: The mycelium is unbranched and coenocytic. The contents of the mycelium cleave to produce amoeboid spores. The order comprises a single family Amoebidaceae.

Order Eccrinales: Mycelium is at first a septate but later become septate. The contents of each cell transforms into a single sporangiospore. The spores are formed first in the top cell and proceed down wards.

The order comprises three families 1. Eccrinaceae 2. Palvasciaceae 3. Paratieniellaceae.

Sexual reproduction has not been confirmed in trichomycetes but structures interpreted as zygospores are formed in some members of Harpellales. Two comatible hyphal cells, when come together a conjugation tube is formed between them and contents of one cell pass into another cell. From the cell, with composite protoplast, a stalk described as zygophore develop and bear a semicircular spore at the tip. It is called zygspore.

7.3. SUB DIVISION ASCOMYCOTINA

This is the largest group of fungi with more than 2700 genera and 28,000 species. They are widely distributed in nature. The one characteristic feature of all these fungi is production of sexual spores, called ascospores, in a specialized sac like structure called ascus.

The vegetative thallus of ascomycetes is mycelium composed of septate hyphae, except in unicellular yeasts. The hyphae are well developed, septate and profusely branched. The septa are perforated by a simple pore (Fig. 7.6). The cells are uninucleates or may become multinucleate.

The hyphae often aggregate to form fungal tissues such as sclerotia or stroma. Sclerotium is a resting a structure. The fungal tissues associated with sporulating structures is called stroma.

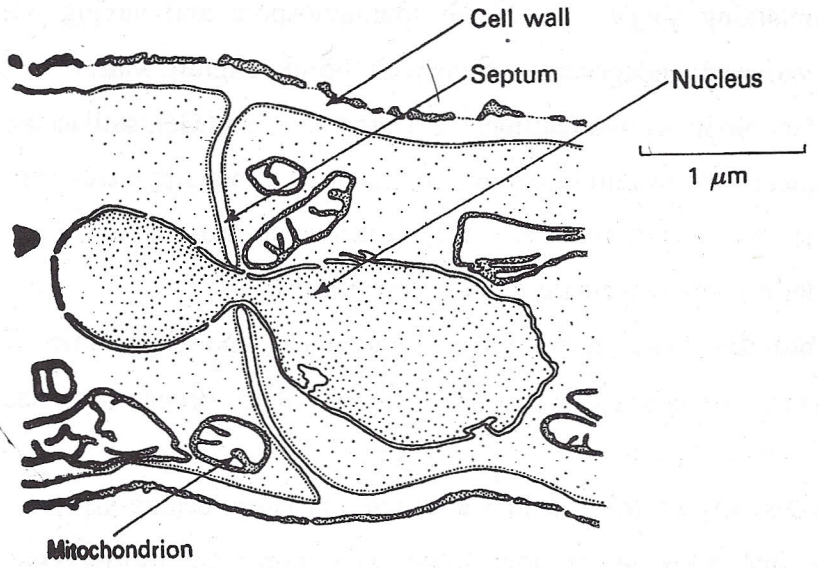

Figure 7.6. Simple septal pore of ascomycete hypha through which nucleus is passing

Asexual reproduction in ascomycetes is essentially by means of producing conidia; which are formed externally on conidiogenous cells. The branches that bear conidiophores may be simple and free or may be organized into specialized structures like synnemata, sporodochium, acervulus or pycnidium. The conidia also vary from small single celled structures to very large multicultural spores. Asexual reproduction is also brought out by vegetative means like fragmentation of hyphae, chlamydospore formation and budding.

Sexual reproduction is by formation ascospores in ascus. In this group of fungi plasmogamy is not immediately followed by karyogamy, but a short dikaryotic phase intervenes But karyogamy is immediately followed by meiosis and ascospore formation.

Sexual reproduction in ascomyceties occurs by various ways.

Gametangial copulation: In yeasts and other members of hemiascomycetes, two cells acting as gametangia completely fuse. The fusion cell develops into an ascus. No dikaryotic stage develops because karyogamy takes place immediately after plasmogamy.

Gametangial contact: In higher ascomyceties two morphologically distinct gametangia are formed. Female gametangium is called ascogonium and male one is Antheridium. On making contact, antheridium transfers male nuclei to ascogonium. Male and female nuclei pair together to form dikaryotic phase.

Spermatization: In some genera ascogonium is formed but no antheridium is formed. Small unicellar spores called spermatia formed in a spermagnium, unite with ascogonium to contribute male nuclei.

Somatogamy: In some ascomycetes, fusion of somatic hyphae of two compatible mycelia takes place and nuclei migrate throughout mycelium through septal pores to produce dikaryotic stage.

Compatibility:

About 25% of ascomycetes are heterothallic while others are homothallic. In homothallic forms compatible gametangia are produced on the same thallus. In hetero thallic forms, compatibility is determined by a pair of genus or alleles Aa which segregate during meiosis. As a result four ascospores in each ascus contain allele A and other four allele 'a'. Both types of sex organs are formed on mycelium from each ascospore but gametangia containing same allele cannot mate. The one locus, two allele type of compatibity is called bipolar heterothallism.

Development of asci:

The stimulus of the sexual act causes the ascogonium to produce a number of papillae just opposite groups of nuclei located in the periphery of the ascogonium. These papillae enlarge into ascogenous hyphae, and pairs of nuclei enter it. Septa are formed in such away that each cell contain two non sister nuclei. In many ascomycetes the binucleate cells of the ascogenous hyphae at the tip elongates and bends over to form a hook or crozier. The two nuclei in the hook cell divide in such a way that their spindles are oriented more or less vertically and parallel to one another, so that two of the daughter nuclei – one from each spindle – are close together in the hook cell, while one of the other two nuclei is located at the tip and one near the basal septum of hook. Two septa are laid down separating the hook into three cells, and of these, crook cell is binucleate and it is ascus mother cell. Karyogamy occurs in the ascus mother cell. Soon zygote nucleus undergoes meiosis resulting in four haploid nuclei, each of which under go one mitotic division to

form eight nuclei. Then each nucleus surrounded by a bit of cytoplasm and develops into an ascospore by a process described as free cell formation. The portion of cytoplasm left out side the spore walls is called epiplasm and it serves to nourish the developing ascospores. The method of development of ascus is shown in the figure 7.7.

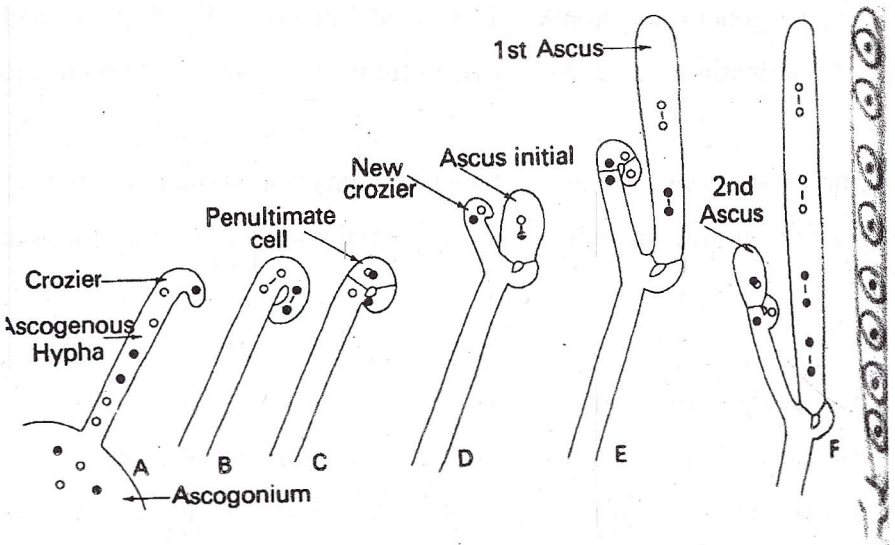

Figure 7.7. Different stages in the development of ascus

Ascus: With formation of ascospores, the ascus development is complete. The asci may be globose, oval, clavate or cylindrical (Fig. 7.8)

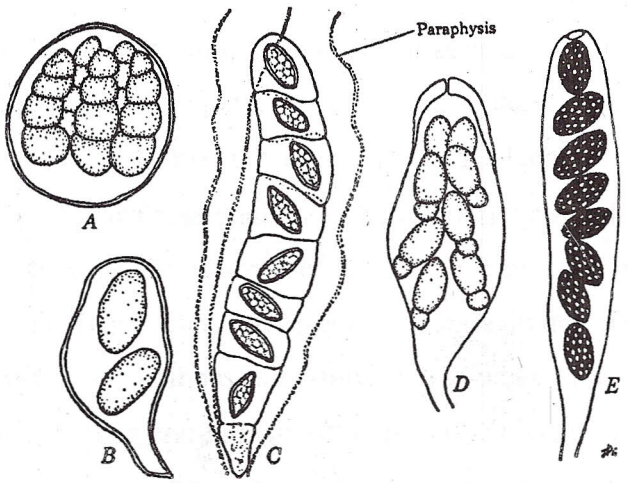

Figure 7.8. Various types of asci. A. Globose ascus B. Broadly ovate ascus with stalk, C. Septate ascus, D. Clavate ascus, E. Cylindrical ascus.

The cell wall of all asci is made up of two layers described as endotunica and exotunica. Basing on the behaviour the wall layers the asci may be categorized into 3 types.

1. Prototunicate asci – in this type the wall layers become evanescent quite early in the developmental stages, and mature ascrpores lie free in the ascocarp cavity.

2. Unitunicate asci – in this type the two wall layers are persistent and behave as a single unit throughout.

3. Bitunicate asci – in this type the wall layers are persistent, but behave differently. The outer exotunica is rigid while inner endotunica is elastic. At the time of spore release, the outer layer ruptures and inner layer balloons out.

Ascospores: The ascospores vary greatly in size, shape, colour, wall ornamentation and other characteristics. In size they vary from minute to more than 1000 μm length; in shape from globose to thread like; in colour from colour less to black; in number of cells, from one to many. Various types of ascospores are shown in the figure 7.9.

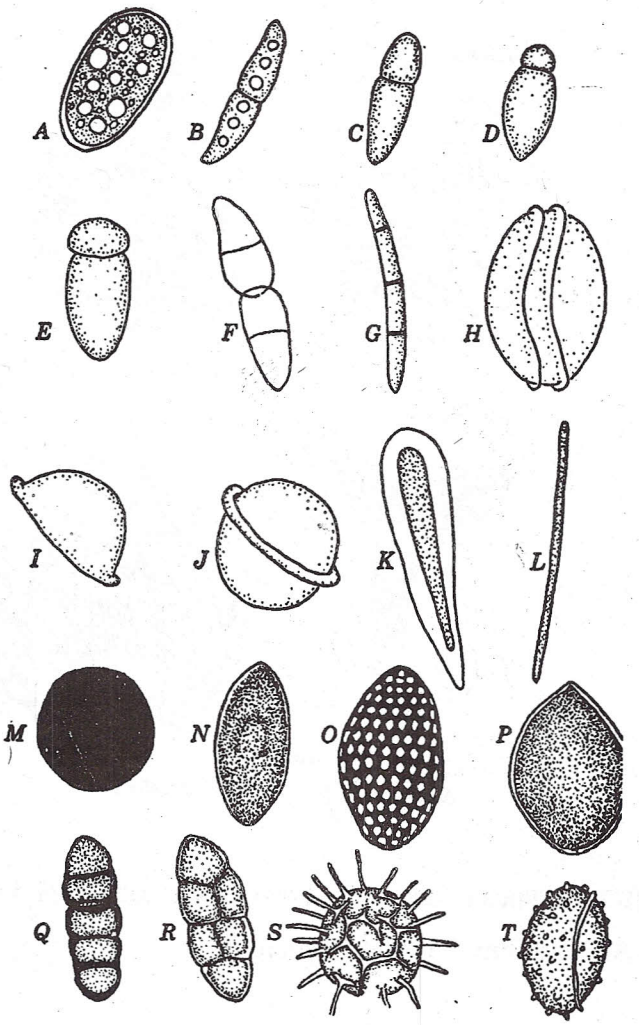

Figure 7.9. Various types of ascospores.

Ascocarps: As asci are developing, sterile mycelium is stimulated to form a thick cover around the asci, and the entire structure is called ascocarp. Except in primitive members of the group called hemiascomycetes, all other fungi develop distinct ascocarps. According to the structure, the ascocarps are mainly four types. In earlier literature, the ascocarps are categorized basing on gross morphology, but now centrum type is considered as important. The totality of the structures that makeup an ascocarp is called its centrum. Three main types of ascocarps viz. cleisothecium, perithecium and apothecium are recognized A fourth type of ascocarp described as pseudothecium is recognized in which peridium is absent and asci are produced in the locules formed in fungal stroma. Usually an ostiole is formed on the group of asci, giving the ascocarp the appearance of a perithecium. Hence, it is also called pseudoperithecium. Since it is formed stroma it is also called ascostroma (Fig. 7.10).

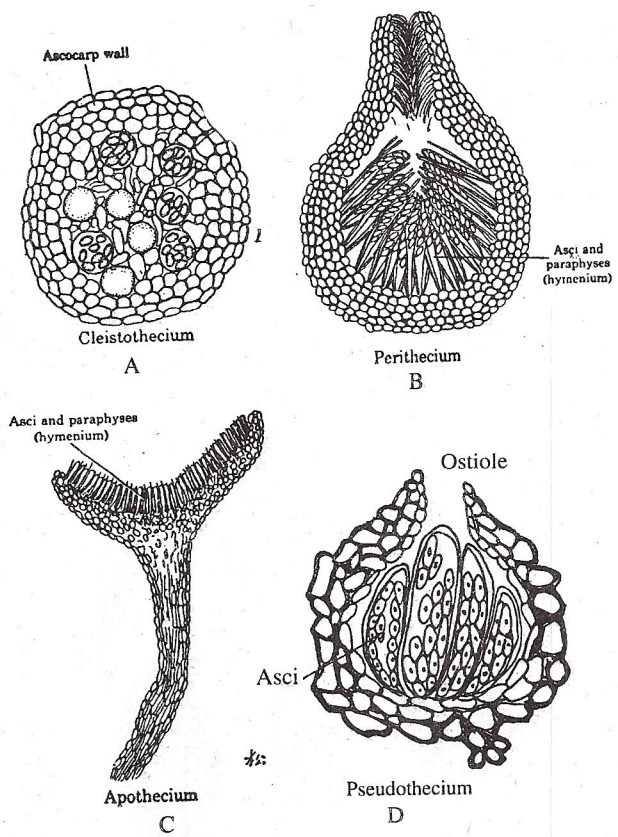

Figure 7.10. Different types of ascocarps. A. Cleistothecium B. Perithecium C. Apothecium D. Pseudothecium

Cleisothecium: The ascocarp in which **asci are globose**, evanescent and do not form a hymenium. Usually peridium completely envelop without an ostiole.

Perithecium: *The ascocarp in* which unitunicate asci are arranged in a basal hymenium is called perithecium. Usually an ostiole is present in peridium and ascocarp appears flask shaped.

Apothecium: The unitunicate asci are arranged in an open hymenium.

Pseudothecium or ascostroma: An ascocarp in which the bitunicate asci are produced in a locule formed in a fungal stroma; without a proper peridium. Usually an opening or ostiole develops in mature ascocarp. Since asci are formed in a stroma, the fruit body is called ascostroma, and as it appears like perithecium, it is described as a pseudoperithecium or simply pseudothecium.

Classification: The type of asco carp formed is the major character to distinguish various classes in ascomycetes. The characters of asci and ascospores are taken into consideration for identification of genera and species.

The subdivision ascomycotina is divided into 6 classes.

1. Hemiascomycetes: No ascocarps, no ascogenous hyphae, Asci formed without ascogenous hyphae.

2. Plctomycetes: These are cleistothecial fungi.

3. Pyrenomycetes: These are perithecial fungi.

4. Discomycetes: These are apothecial fungi.

5. Loculoascomycetes: Asco carps are formed in locules in stroma and hence these are called pseudothecial fungi.

6. Lobulbeniomycetes: Perithecial fungi associated with insects and red algae.

7.3.1. CLASS HEMIASCOMYCETES:

These are considered primitive ascomycetes and are characterized by the absence of ascocarp. The asci are formed singly, usually following karyogamy, and are not borne on ascogenous hyphae. Three orders are recognized in this class. 1. Protomycetales 2. Endomycetales and 3. Taphrinales.

7.3.1.1. Order Protomycetales: This is a small group of fungi, which are parasitic on higher plants producing galls and extensive colour changes. There are four genera in the group,

Protomyces, *Protomycopsis*, *Mixia* and *Taphridium*. All are placed in a single-family protomycetaceae.

Mycelium is internal, intercellular and septa are formed at intervals that divide mycelium into multinucleate compartments. Some of the hyphal segments become thick walled resting structures called chlamydospores. A resting cell germinates with the inner wall pusing through the outer wall and forms an elongated, cylindrical sac, which is considered by some mycologists as sporangium and others as synascus. Nuclear divisions that are interpreted as meiotic take place in spore sac and the resulting nuclei become incorporated into spores considered as ascospores. These are forcibly discharged from the spore sac in a single mass. How diploidization occurs has not been demonstrated.

7.3.1.2 Endomycetales: Unicellular yeasts belong to this group and hence, often called saccharomycetales. However, other fungi belonging to the group are mycetial forms. Four families are recognized in this order. Spermophthoraceae, Ascoideaceae, Endomycetaceae and Saccharomycetaceae.

In the family spermophthoraceae, all the members are plant parasities and mycelium is coenocytic and details of life cycle are not clearly established.

In Ascoidaceae, mycelium is septate and the tips cells of sexually active hyphae slightly bulge and unite. After plasmogamy, karyogamy occurs and the structure transforms into an ascus, with large number of spores.

In Endomycetaceae, mycelium is well developed as in ascoideaceae, and ascus is only 8 spored but not multispored.

The members of the family sacchromyetaceae are unicellular and called yeasts.

7.3.1.3. Order Taphrinales: A single family Taphrinaceae with a single genus *Taphrina* with about 30 species, is recognized in this order. All the species of *Taphrina* are parasitic on vascular plants causing leaf spots, blisters, malformations etc. The mycelium is confined to epidermal cells or occurs between epidermis and cuticle. The hyphae are septate and cells are dikaryotic. At maturity the cells under go transverse division and the nuclei in the upper cell fuse to form diploid nucleus and the cell transform into ascus, the nucleus divide meiotically and produce 8 ascospores. The ascospores in the ascus may give rise to more cells by budding and the asci often appears multispored. The asci may be produced in a layer but there is no ascocarp formation.

7.3.2. CLASS PLECTOMYCETES:

The class Plectomycetes includes the fungi that produce simple cleistothecium type ascocarp. They share the following general characters.

1. Asci are typically thin walled, globose to pyriform and evanescent i.e. prototunicate.
2. Asci are scattered at various levels not forming a hymenium; they arise from ascogenous hyphae of varying lengths that ramify through ascocarp centrum.
3. Ascospores are unicellular.
4. Ascocarp peridium varies from none to thin wefts of hyphae forming a covering over asci to a definite pseudoparenchymatous peridium.
5. Cleistothecium usually does not possess an ostiole.
6. Paraphyses are completely absent.

Many plectomycetes produce a conidial stage, and this anamorph stage is denoted by the conidial genus. The anamorph genera like *Aspergillus*, *Pencillium*, *Cladosporium* etc. are ubiquitous and widely distributed throughout the world in many habitats. Most of them are saprophytes.

A single order Eurtiales was recognized with 9 families.

1. Gymnoascaceae: Produce a cluster of asci without peridium.

2. Eurotiaceae: Produce ascospores with bivalve construction. This is the most important family comprising *Eurotium* and *Talaromyces*, the perfect stages of *Aspergillus* and *Penicillium* respectively.

3. Pseudoeurotiaceae: Ascorpores are hyaline to dark coloured, never show bivalve construction.

4. Amorphothecaceae: It is a monogeneric family. Peridium of ascocarp is an amorphous melanoid membrane. The conidial stage genus is *Cladosporium.*

5. Monoascaceae: These are called pink moulds. The mature peridium consists of one or two layers of cortical cells around the cavity enclosing numerous ascospores. The asci are eight spored, but the asci wall dissolve quite early releasing the spores into peridial cavity. This was first interpreted as a single ascus with many spores. Hence the name.

6. Onygenaceae: The ascus wall burst and release the spores into the ascocarp cavity. The ascus walls do not completely disintegrate but form a capillitium.

7. Trichocomataceae: The ascocarp cavity is separated into locules by interthecial sterile hyphae. The spore mass with capillium is formed.

8. Thermoascaceae: Thermophilic plectomycetous fungi.

9. Cephalothecaceae: Sutures or definite lines of dehiscence in peridium are conspicuous.

7.3.3. CLASS: PYRENOMYCETES:

The pyrenomycetes are defined as ascomycetes with ascocarp entirely surrounded by a peridial wall, containing unitunicate asci, which are arranged in a hymenial layer. In general, the ascocarp is provided with an apical opening (ostiole), which is covered with periphyses.

The class pyrenomycetes is divided into 4 orders. 1.Erysiphales 2. Meliolates 3. Coronophorales and 4. Sphaeriales. The first three orders are small-specialized groups with atypical perithecia, while majority of typical perithecia are placed in the single order sphaeriales, because the classification of these fungi is still in fluid state. Many groups of fungi recognized as families in Ainsworth system, were elevated to the rank of orders by later workers.

7.3.3.1. Order Erysiphales: It includes a single family Eysiphaceae the members of which cause conspicuous plant diseases called powdery mildews. They are all ectoparasites on aerial parts of the host plants. The important genera are *Erysiphe, Sphaerotheca, Podosphaera, Microsphaera, Uncinula, Phyllactinia* etc. The ascocarps of these fungi lack an ostiole in peridium, but the asci are arranged in a hymenium without paraphyses. The peridium is provided with mycelioid or specioid or specialized appendages, which help in identification. The centrum type is described as phyllactinia type.

7.3.3.2. Order Meliolales: It comprises a single family meliolaceae. The members are called black mildews. They are ectoparasites on higher plants producing black fungal mass on the infected surface. Hyphae well developed, septate, brown with two types of special structures called hyphopodia viz.capitate hyphopodia and Mucronate hyphopodia, that helps in attaching fungus to the host surface. They do not produce any conidial stage. Ascocarps are phyllactinia type and produce dark brown ascospores.

7.3.3.3. Order Coronophorales: The order comprises a single famly coronophoraceae. These are wood inhabiting fungi. Mycelium is scanty. Ascocarps develop within the substratum without a

peridium. True ostioles absent, but openings develop at the apex by disintegration of the cells. Asci are unitunicate.

7.3.3.4. Order Sphaeriales: Majority of perithecial fungi are placed in this order. Ascocarps are spherical or flask shaped. They are mostly ostiolate, and have a bright, fleshy or dark carbonaceous peridium. Asci are unitunicate, spherical, clavate or cylindrical and are arranged in a hymenium. Paraphyses in are present among asci and ostiole is lined by periphyses. Three types of ascocarp centrum are recognized in sphaeriales viz. Xylaria-type centrum, Diaporthe-type centrum and Nectria type centrum (Fig. 7.11).

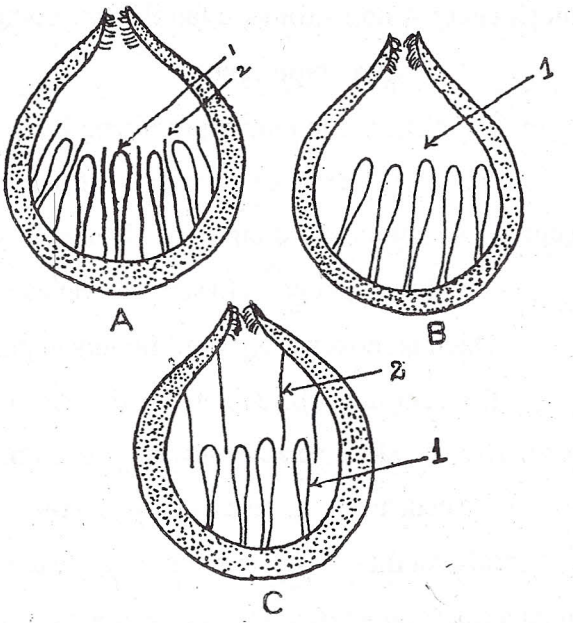

Fig. 7.11 Centrumtypes A. Xylariatype B. Diaporthe C. Nectriatype

The Xylaria type centrum is characterized typically persistent asci and paraphyses. In Diaporthe type centrum, initially asci and paraphyses are formed, but at maturity the paraphyses become evanescent, and asci may also evanescent releasing the ascopores into the ascocarp cavity or the stalks of asci become evanescent. In Nectria type centrum, apical paraphyses arise from the perithecial apex and grow down wards. As asci develop the apical paraphyses disintegrate.

15 Families are recognized in the order.

1. Ophiostomataceae: The ascocarps are provided with a very long neck and asci are distributed at various heights and do not form a hymenium.

2. Xylariaceae: Typical peritheium is formed in stroma. Centrum type is Xylaria type.

3. Diatrypaceae: Form typical peritheia in stroma, fungi are mostly saprophytic on bark. The centrum type is Xylaria type.

4. Clavicipitaceae: These fungi are plant pathogens causing disease referred to as ergot. Typical ascocarps are formed in stroma and ascospores are filiform. Centrum type is Xylaria type.

5. Polystigmataceae: The fungi cause plant diseases referred to as tar spots. Typical perithecia with Xylaria type centrumformed in the host tissue.

6. Amphisphaeriaceae: A non stromatic family producing typical perithecia with Xylaria type centrum..

7. Sphaeriaceae: A non stromatic family producing bright coloured ascocarps with typical Xylaria type perithecial structure.

8. Sordariaceae: These are mostly dung fungi. Hymenium is formed with asci and paraphyses but paraphyses are evanescent by maturity Neurospora, which is most widely used fungus in genetics, belongs to this family. The centrum is often described as Sordaria type.

9. Diaporthacae: They produce perithecia with a centrum type described as 'diaporthe type centrum'. In the ascocarps, paraphyses are evanescent by maturity and asci may be evanescent or persistent.

10. Melanosporaceae: Characterized by dark coloured ascospores

11. Halosphaeriaceae: Include marine perithecial fungi.

12. Hypocreaceae: In the ascocarps, pseudoparaphyses are prominent and centrum is described as 'Nectria type'.

13. Hypomycetaceae: They are parasitic on fruit bodies of basidiomycetes and produce ascocarps with nectria type centrum.

14. Coryneliaceae: The ascocarps are ascostroma i.e. no true peridium is formed but the asci are unitunicate.

15. Verrucariaceae: Lichenized perithecial fungi are placed in this family.

7.3.4. CLASS DISCOMYCETES:

The discomycetes are ascomycetous fungi that produce ascocarp with an exposed hymenium, called apothecia. The apothecia are macroscopic and can be seen with naked hymenium, some are relatively large like basidiocarps of agarics. Cup fungi, earthtongues, morels, truffles etc. belong to this group and some of them are collected for their delicacies species of *Morchella* are highly prized edible fungi. Most of the discomycetes are freeliving, saprophytic and occur on various substrata. Most of them are epigean i.e. occurring above the ground, but a few are hypogean i.e. occurring below the ground level. *Tuber melanosporum* and others among hypogean fungi are highly prized fungi in European countries.

7.3.4.1. General Characters: The mycelium of discomycetes is typically septate like in other ascomycetous fungi. It always occurs within the substratum and produce fruit bodies on the substratata.

The fruit body of discomycetes is apothecium. It consists of three parts: hymenium, hypothecium and excipulum. (Fig.7.12.).

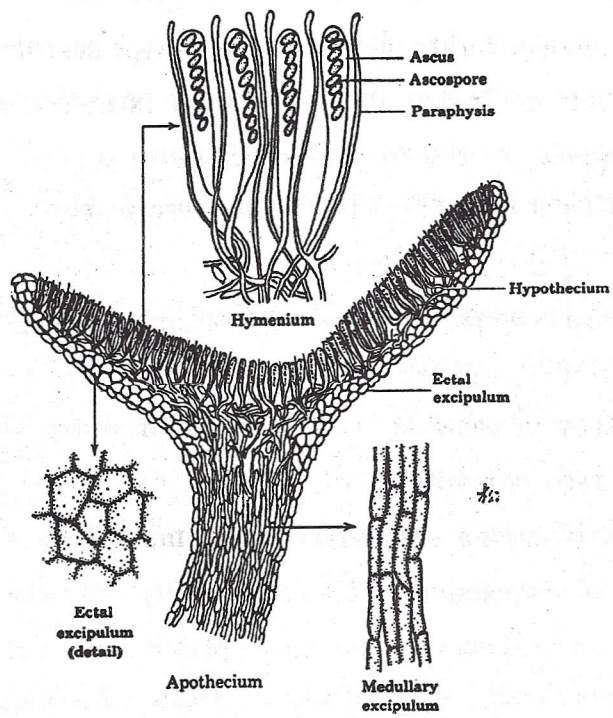

Figure 7.12. Apothecium and its various parts

Hymenium is the layer of asci that lines the surface or hollow part of the disc, cup, saddle or other variously shaped structures. The asci are unitunicate, cylindrical, and are interspersed with paraphyses, the sterile thread like structures. The paraphyses are longer than asci, they form a layer on the hymenium and it is called epithecium. The hypothecium is a thin layer of interwoven hyphae immediately below the hymenium. The fleshy part of the fruit body is called excipulum; the outer layer of apothecium is called ectal excipulum and inner portion medullary excipulum.

The asci are either operculate or inoperculate. They type of spore release is of taxonomic significance. Operculum is a shield shaped structure in the apical region of the ascus and at the time of spore release, it is thrown out and all the spores in the ascus are released either through a pore at the apex or by dissolution of the ascal apex.

The ascospores are unicellular in four orders viz. Medeolariales, Cyttariales, Tuberales and Pezizales; and transversely multiseptate in three orders viz. Oostropales, Phacidiates, and Helotiales.

7.3.4.2. Classification: Seven orders are recognized in the class.

1. Medeolariales: A single family Medeolariaceae and a single genus *Medeolaria* are recognized. The species of *Medeolaria* are pathogenic on plant *Medeola* producing rosette symptoms.

2. Cyttarials: A single-family cyttariaceae and a single genus *cyttaria* are recognized. All species of *Cyttaria* are pathogenic on *Nothofagus* producing galls on branches. The ascus opens by a broad pore.

3. Phacidiales: The fungi included in this order are all plant pathogens producing tarspots on leaves of infected plants. Ascocarps are formed in the host tissue and are covered by a black membranous structure. Three families are recognized in the order. They are 1. Phacidiacae 2. Rhytismataceae and 3. Cryptomycetaceae.

4. Tuberales: These are hypogean discomycetes and fruit bodies are fleshy and completely closed. Four families are recognized. 1. Tuberaceae 2. Elaphomycetaceae 3. Geneaceae and 4. Terfeziaceae.

5. Pezizales: These are more widely distributed discomycetes. Ascocarps are epigean, fleshy and asci are operculate. 7 families are recognized in the order. 1. Pezizaceae 2. Morchellaceae 3. Helvellaceae 4. Pyronemataceae 5. Ascobolaceae 6. Sarcoscyphaceae and 7. Sarcosomataceae.

6. Ostropales: They are wood inhabiting fungi and resemble Claviceps in ascospore structure. A single-family stictidaceae is recognized.

7. Helotiales: This is a large order of discomycetes that produce relatively small apothecia. They occur mainly on delaying plant parts. 8 families are recognized in the order. 1. Ascocorticeaceae 2. Dermataceae 3. Geoglossaceae 4. Hemiphaidiaceae 5. Hyaloscyphaceae 6. Leotiaceae 7. Orbiliaceae and 8. Sclerotiniaceae.

7.3.5. CLASS: LOCULOASCOMYCETES:

Two most characteristic features of the fungi included in this class are 1. bitunicate asci and 2. ascocarp an ascostroma with asci individually and irregularly distributed in stromatal tissue or grouped in locules.

The ascus wall is two layered outer exotunica and inner endotunica. In this class, two layers of ascus behave differently. The outer exotunica is rigid while inner endotunica is elastic. At maturity, the exotunica breaks at the apex and inner endotunica elongates through the opening made by rupture of outer wall layer. The ascogonia are always formed within a stoma, asci develop in the locules formed in the stroma either by dissolution of cells around developing asci or by division of cells and formation of pseudoparaphyses. In either case no peridium is formed around asci. Hence the ascogonium is described as a pseudothecium. Eventually a canal like opening develops over a group of asci and it may be lined by periphyses. The whole structure at maturity appears like a perithecium, and the ascocarps are also called pseudoperithecia.

Three types of centrum were recognized in this group.

1. **Elsinoe type:** Asci develop individually in ascostroma, and a locule is formed around each locule. The uniascal locules may be formed randomly in the stroma or may be formed in special rows or patterns.

2. Dothidea type: Asci develop in groups and locules are formed around each group of asci. No sterile filaments are formed among asci.

3. Pleospora type: The locule of ascostroma is formed by development or pseudoparaphyses.

The class loculoascomycetes divided into 5 orders.

1. Order Myriangiales: The fungi with uniascal locules are placed in the order. These are distributed mainly in tropics and subtropics and occur as parasites or hyper parasites. ***Piedraia***

hortai, a keratinophilic fungus colonizing human hair, causing 'black piedra', belongs to this group. 4 families are recognized in this order 1. Atichiaceae 2. Myriangiaeae 3. Saccardiaceae and 4. Saccardinulaceae.

2. Order Dothideales: These fungi mainly occurs on dead stems and leaves as saprobes, and conidial stages often cause serous diseases as various parts of the plant. The ascocarp is polyascal with dothidea type centrum. The order comprises 8 families viz 1. Dothideacae 2. Dothioraceae 3. Capnodiaceae 4. Pseudosphaeriauae 5. Englerulaceae 6. Parodiopsidaceae 7. Chaetothyriaceae 8. Trichothyriaceae.

3. Order Pleosporales: This is a very large group of fung whose conidial stages are wide spread occurring as saprophytes or plant parasites. The ascocarp shows Pleospora type centrum. 8 families are recognized in the order 1. Pleosporaceae 2. Sporormiaceae 3. Venturiaceae 4. Botryosphaeriaceae 5. Dimeriaceae 6. Mesneriaceae 7. Mycoporaceae 8. Lophiostromataceae.

4. Order Hysteriales: A small group of fungi producing ascocarp described as hysterothecium are placed in this order. Ascocarp is a boat shaped peudothecium opening by a longitudinal slit and becoming apothecioid when moistened. Asci are long cylindrical, among persistent peudoparaphyses as in Pleospoa type centrum.

The order comprises 6 families viz. 1. Hysteriaceae 2. Arthoniaceae 3. Opegraphaceae 4. phillipsiellaceae 5. Patellariaceae 6. Lecanactidiaceae.

5. Order Hemisphaeriales: They occur primarily on living leaves, young stems etc as commensal and produce flattened ascocarp with pleospora type centrum. Upper part of the ascocarp is well developed with a central pore but lower part poorly organized. This is a large group with 11 families which include *Asterinaceae*, *paronuclaceae* etc. apparently growing an plant exudates.

7.3.6. CLASS LABOULBENIOMYCETES: The organisms now classified in this group were first noticed by French entomologists Alex Laboulbene and Augusti Rouget in 1840. Since then a large number of species were recognized.

In 1965 Australian mycologists have recognized some fungi resembling laboulbeniomycetes on red algae members.

It is well defined natural assemblage, predominantly parasitic on insects. They are obligately pathogenic and highly host specific mainly confined to adult sages of hosts. They do not produce any conidial stage. The characters of Laboulbenia are shown in the figure 7.13.

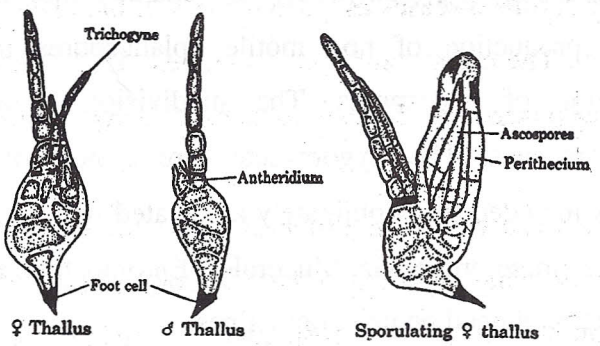

Figure 7.13. Thallus and reproductive structures of *Laboulbenia formicarum*

In this group mycelium is scanty, and thallus consists of a foot cell and limited multicellular part. The life cycle starts when a two celled ascospore is deposited on a host surface. The larger basal cell enlarges and develops the foot from which haustoria grows through the host integument and eventually reaches the living cells below. In some species, a branched rhizomycelium may be formed. Then the basal cell divides several times and forms the thallus.

No asexual reproduction structures are formed.

The sex organs are ascogonium and spermagonia. The ascogonium comprises of basal cell and terminal trichogyne. Spermatia are produced in spermagonia. Both the sex organs may be formed on the same thallus or on different thalli. The spermatia released from spermagonium reach trichogyne and transfer male nucleus to ascogonium. Asci develop without ascogenous hyphae. Ascocarp develops in place of ascogonium. Ascocarp is a perithecium. Usually a single ascus with 4 ascospores develops in each ascocarp. The ascospores are two celled and unequal.

The class Laboulbeniomycetes comprises two orders viz. Laboulbeniales and Spathulosporales.

The order laboulbeniales comprise fungi parasitic on insects and placed in 3 families. 1. Laboulbeniaceae 2. Ceratomycetaceae and 3. Peyrithiellaceae.

The order spathulosporales include the fungi parasitic on red algae. Only a single genus *Spathulospora* is recognized in this group and it is placed in the family spathulosporaceae.

7.4 SUMMARY:

The fungi of subdivision of zygomycotina are characterized by coenocytic mycelium, asexual reproduction by production of non motile aplanospores in sporangia and sexual reproduction by production of zygospores. The subdivision comprises two classes viz. Zygomycetes and Trichomycetes. The Zygomycetes are typical of the group and widely distributed. Trichomycetes included fungi obligately associated with living arthropods. The class Zygomycetes is divided into three orders viz. Mucorales, Entomophthorales and Zoopagales. The characters of these fungi are explained on taxonomic lines.

The subdivision ascomycotina comprises fungi that posses septate mycelium and produce ascospores in asci. The asci may be formed in special fruit bodies called ascocarps or farmed individually with out ascocarp formation. Basing on the strucure, the ascocarps are mainly four types viz. Cleistothecium, Perihecium, Apothecium and Pseudothecium. The ascomycetes that do not produce ascocarps are placed in the class Hemiascomycetes. The fungi that produce cleistothecia are placed in the class plectomycetes. The perithecial fungi are treated as class pyrenomycetes. Apothecial fungi are placed in Discomycetes while fungi that produce pseuothecia are placed in class Loculoascomycetes. Perithecial fungi that are parasitic on insects are placed in the class Laboulbeniomycetes. The characters of these fungi are described on taxonomic lines.

7.5. MODEL QUESTIONS:

Essay Type questions

1. Discuss the characters and classification of Zygomycotina.
2. Describe the asexual reproduction in Zygomycetes and discuss the importance of sporangial types in classification.
3. Give an account of different types of ascocarps and their importance in classification.
4. Discuss the general characters of ascomycotina fungi

Short answer type questions

5. Mucorales.
6. Trichomycetes
7. Entomophthorales
8. Hemiascomycetes
9. Types of ascocarps
10. Discomycetes

7.6. REFERENCE BOKS:

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LESSON – 8: GENERAL ACCOUNT OF BASIDIOMYCOTINA AND DEUTEROMYCOTINA.

OBJECTIVE: To explain the general characters, reproduction and classification of fungi belonging to the subdivisions Basidiomycotina and deuteromycotina.

CONTENTS

8.1 Introduction
8.2 Basidiomycotina
8.3 Hymenomycetes
8.4 Gasteromycetes
8.5 Teliomycetes
8.6 Deuteromycotina
8.7 Blastomycetes
8.8 Hyphomycetes
8.9 Coelomycetes
8.10 Summary
8.11 Model questions
8.12 Reference books

8.1. INTRODUCTION

The fungi included in the subdivision basidomycotina are called higher fungi and most of them produce characteristic fruit bodies called basidiocarps in which basidiospores are produced on a single celled structure called basidium. The basidia are formed in a fertile layer hymenium. The hymenium of most fungi are exposed from the beginning while in some fruit bodies they are not exposed because the fruit bodies are completely surrounded by peridium even at maturity. In fungi with open hymenium the basidiospores are released actively, while in closed basidiocarps they are passively released. There are some fungi within the group, which do not form basidiocarps and basidia do not occur in hymenium. The fungi which form basidiocarsps with open hymenium are classified into a class called hymenomycetes. The fungi in which basidiocarps are completely closed and hymenium may or may not form are placed in the class Gasteromycetes. The fungi which do not produce baidiocarps are placed in the class teliomycetes.

A large number of fungi do not show sexual reproductions, and perpetuate through production of asexual spores called conidia. Such fungi are placed in the sub division Deuteromycotina. This group also comprises three classes viz. Blastomycetes, Hyphomycetes and Coelomycetes.

The general characters and classification of fungi included in the subdi ons basidiomycotina and deuteromycotina are explained in this lesson.

8.2. SUBDIVISION: BASIDIOMYCOTINA

Basidiomycotina include such well known macrofungi like mushrooms, polypores, puff balls, earth stars etc. These also include important plant pathogens causing rust and smut diseases on higher plants. This group comprises about 1100 genera and 16,000 species. The most important character of all these fungi is the production of spores called basidiospores on the out side of a specialized spore producing structure, the basidium.

8.2.1 Somatic structures: The mycelium of basidiomycotina fungi consists of well-developed, septate hyphae that penetrate the substratum and absorbs nourishment.

Mycelium of basidiocarp producing fungi passes through three distinct stages of development viz. primary, secondary and tertiary stages.

Homokaryotic mycelium that develops on germination of basidiospores is called primary mycelium. Only limited amount of primary mycelium is formed and hyphae are septate with simple cross walls and cells are usually uninucleate.

The primary mycelium soon becomes dikaryotic either by somatogamy between two compatible primary mycelia or by spermatization. When two compatible homokaryotic mycelia come in contact, break down of the walls separating them occur to achieve cytoplasmic continuity. Nuclear migration fallows but there is no karyogamy but two compatible nuclei come together to form dikaryotic stage. It is the beginning of secondary mycelium. The hyphae of secondary mycelium are septate and septa are described as dolipore septa. It is characteristically barrel shaped with a central pore. The pore is surrounded by a dome shaped membranous structure called septal pore cap or parenthosane (Fig. 8.1)

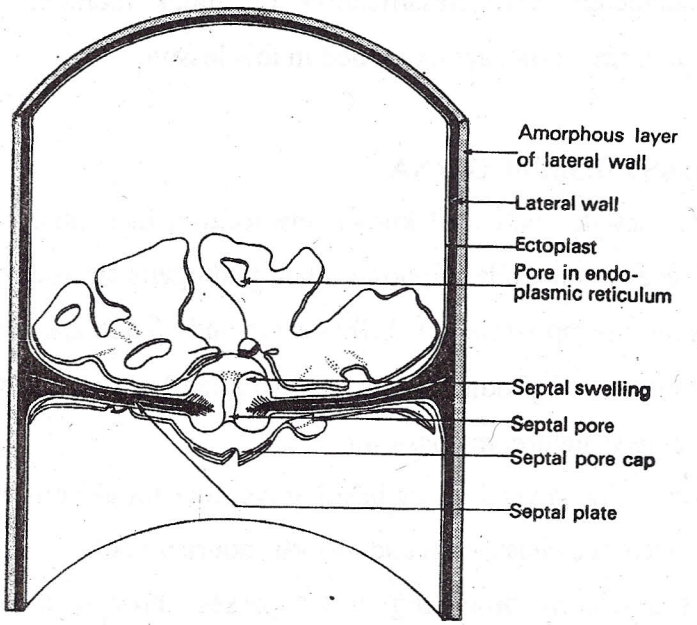


Figure 8.1 Dolipore septum of basidiomycete hyphae

The two nuclei in each of a on usually divide simultaneously and at the newly forming septum, a clamp connection is formed and it is believed to help to transfer two compatible nuclei into the daughter cells (Figure 8.2)

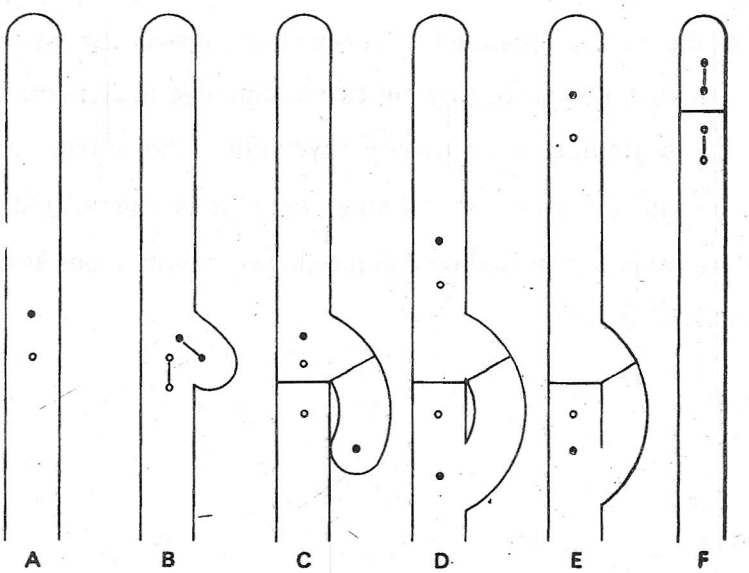


Figure 8.2. Diagrammatic representation of clamp formation.

Secondary mycelium is perennial and occurs through out the life of the fungus. The mycelium that forms the fruit bodies is called tertiary mycelium. The mycelium in the fruit bodies of mushrooms, which are soft, is almost of same type and any part of the mycelium can bear a basidium, and hence it is called generative hyphae. In the fruit bodies which are cork or woody like those in aphyllophorales thee types of hyphae have been recognized.

1. Generative hyphae - Smooth, unthickened capable of producing basidia.
2. Binding hyphae - Highly branched, thick walled, function to weave other hyphae together
3. Skeletal hyphae - Usually not branched, very thick, function to strengthen basidiocarp

When all the types of hyphae are present, mycelium is described as trim tic. When only two types (usually generative and binding) are present it is described as dimitic. If only generative hyphae are present it is monomitic. The diagrammatic representation of the three hyphal types is shown in the figure 8.3.

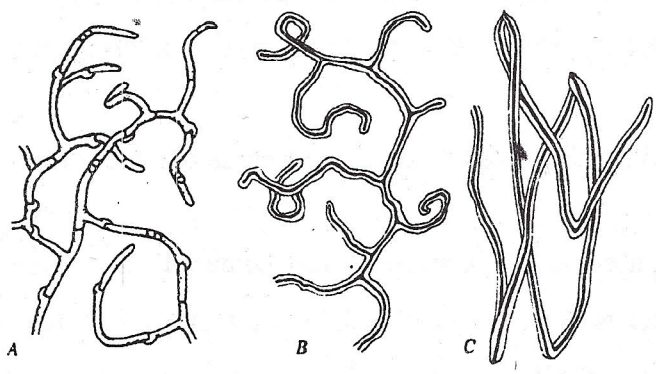


Figure 8.3. Hyphal types A. Generative hyphae B. Binding hyphae and C. Skeletal hyphae

8.2.2. Basidiocarp: The basidiocarps are the fruit bodies of the macrofungi. They occur in a variety of forms. They may be crust like, gelatinous, jelly, cartilaginous, thin and papery, corky, spongy, hard and woody or of almost any texture. They vary in size from a few millimeters to a size of more than one meter. The basidiocarps may be open from the beginning, (gymnocarpic), open at a later stage (hemiangiocarpic) or typically angiocarpic and remain permanently closed.

system loss of limbs. Pharmaceutical companies are now preparing drugs with low quantities of these alkaloids for use during childbirth to prevent excess loss of blood and contraction of uterus.

Illegal drug manufacturers are using hallucinogenic properties to produce a drug LSD (lysergic acid diethyl amide) and many drug addicts use it.

9.2.1.2.4. Alcohol production: Alcohol is a very important chemical solvent and used in bulk quantities in industry and research. Large scale production of alcohol is carried out using sugarcane molasses as raw material and *Saccharomyces cerevisiae* is the fungus used for fermentation.

9.2.1.2.5. Production of organic acids: Various organic acids are extensively used in industry and commercial production of these acids is carried out by fermentation methods using fungal species. Citric acid is one of the most important organic acid mainly used in soft drinks industry, in pharmaceutical industry etc. It is commercially produced using *A. niger.*

Itaconic acid used in manufacture of plastics is commercially produced using *Aspergillus terreus*. Lactic acid is produced using *Rhizopus oryzae*; fumaric acid using *Rhizopus arrhizus*; gallic acid by using *A.niger* and malic acid by using *Schizophyllum commune.*

9.2.1.2.6. Enzymes from fungi: A number of enzymes are used in industry and they are obtained from cultures of fungi.

Amylases used in bread making, production of beer etc is obtained from *Aspergillus niger, A.oryzae* and *A. awamori.*

The enzyme invertase used in preparation of soft centered chocolates is obtained from *Saccharomyces cerevisiae*. The enzyme β-galactosidase used in manufacture of ice creams is obtained from *Saccharomyces fragilis, Candida pseudotropicalis* or *Candida utilis.*

Pectolytic enzymes used in preparation of fruit juices and their clarification are obtained from species of *Botrytis, Mucor, Penicillium* and *Aspergillus.*

Cellulases used in paper industry are obtained from *Trichoderma viridae*, *Aspergillus niger* etc.

Enzymes used in food industry for meat tenderizing are obtained from *Aspergillus oryzae*, *A. niger*, *Mucor pusillus* and *Rhizopus sp.* etc.

9.2.1.2.7. Vitamins from fungi: One of the best sources of vitamin b complex is the yeasts. Riboflavin is now manufactured using *Ashbya gossypii and Eremothecium ashbyi.* Ergosterol, the

precursor of vitamin-D, was synthesized by a number of filamentous fungi as well as yeasts by fermentation.

9.2.1.2.8. Steroid transformations: Steroids are organic compounds with a 4 ring structure and they have many regulatory functions in the body physiology of mammals. Steroids are used as anti inflammatory agents, anaesthetics, antifertility agents, treatment for sterility etc.

For commercial use, the steroids are chemically synthesized. But, chemical synthesis is a tedious process and includes a large number of steps. However, some fungi can bring about transformations of steroids in a single step, making easy the synthesis of required steroids.

Rhizopus arrhizus and *R. stolonifer* can produce II-hydroxy progesterone by hydroxylation of steroid nucleus in a single step. A number of fungi like *Aspergillus ochraeceus*, *Mucor griseocyanus*, *Stachylidium theobromae* etc. also bring about transformations in steroids.

9.2.1.3. Fungi as Food

The edibility of mushrooms is known to man from prehistoric times. The unicellular fungi like yeasts and some filamentous fungi are now mass cultured for use as food and feed.

9.2.1.3.1. Edible fungi: More than 4000 species of agarics produce soft, fleshy fruit bodies, and at least half of them are edible. However, only about 100 species are collected in wild for edible purpose. Of these some are cultivated. The important edible fungi are

Agaricus brunnesecens – Button mushroom

Volvariella volvacea - Paddy straw mushroom

Lentinus edodes - Shiitake mushroom

Pleurotus species - Oyster mushroom

Some species of Ascomycetes also form fruit bodies that are edible. Among edible ascomycetes morels and truffles are highly prized for their delicy. Morels are species of *Morchella* and produce fruit bodies resembling *Agaricus* fruit bodies and are described as sponge mushrooms. Truffles belong to the genus *Tuber* and they are hypogean. Both morels and truffles belong to discomycetes group of Ascomycotina.

9.2.1.3.2. S.C.P (Single cell protein): Yeasts are unicellular fungi that are very rich in proteins and vitamins. They grow easily on various substrata available cheaply and in large amounts. Hence, yeasts are mass cultured and used as animal feed. Since the cells are unicellular and rich in

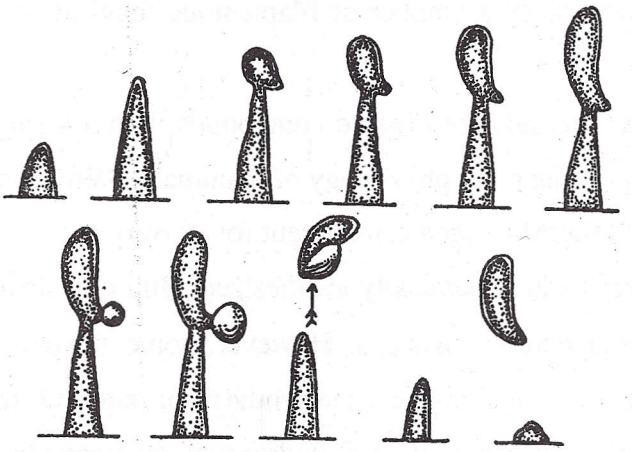


Figure 8.7 Basidiospores formation and discharge.

8.2.6. Classification:

The subdivision basidiomycotina is broadly divided into 3 Classes viz. Hymenomycetes, Gasteromycetes and Teliomycetes.

8.3. Class HYMENOMYCETES:

The class hymenomycetes include fungi, which produce basidiocarps, with open hymenium at maturity and basidiospores are actively discharged.

The class hymenomycetes is divided into two sub classes viz. 1. Holobasidiomycetidae, which include fungi in which basidium is a single called holobasidium and 2. Phragmobasidiomycetidae, which include fungi with septate metabasidium.

8.3.1. SUB CLASS HOLOBASIDIOMYCETIDAE: this is the large group of hymenomycetes, which include well-known mushrooms and polypores. This sub class includes two very large orders viz. Agaricales and aphyllophorales, and four small orders viz. Exobasidiales Brachybasidiales, Dacrymycetales and Tulasnellales. The characters are explained on taxonomic lines.

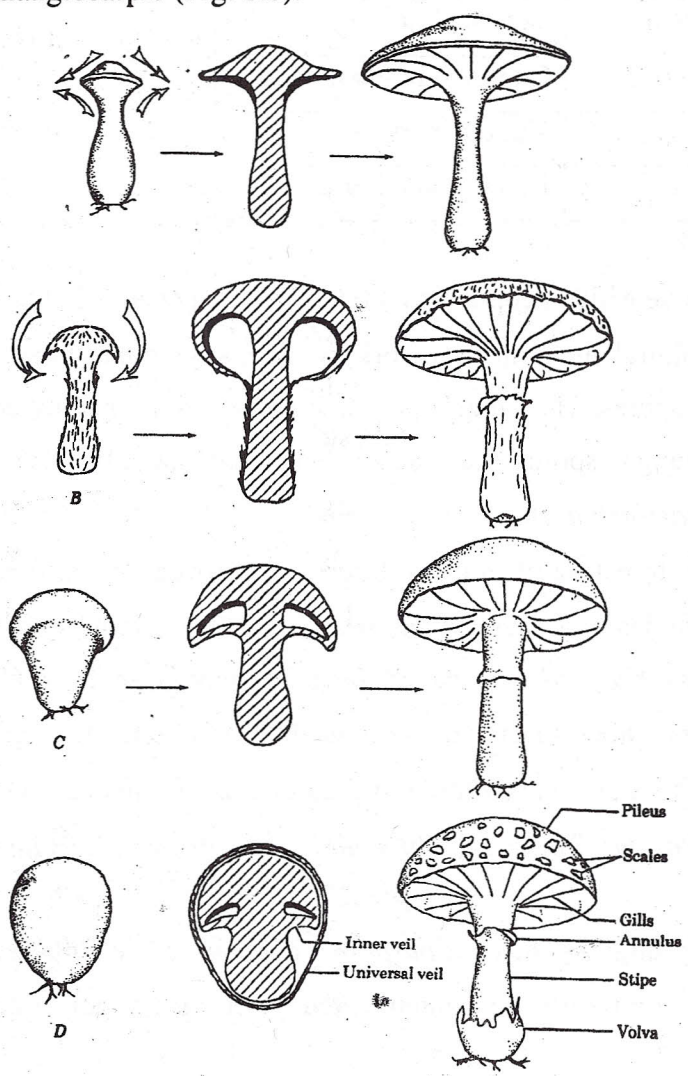
8.3.1.1. Order: Agaricales:

The members of order agaricales are commonly called 'gill fungi' and contain 270 genera and about 4000 species. The fruit bodies are called mushrooms. They are soft, flshy and evanescent. They appear mainly during rainy season. They are mainly terrestrial or lignicolous growing saprophytically. Some mushrooms are edible and people prefer them for their delicacy

and nutritious value. Some edible mushrooms are cultivated, and mushroom cultivation is a multimillion industry. Some mushrooms like species of *Amanita* such as *A. phalloides* and *A. verna* are highly poisonous.

The mycelium of agaricales is typically basidiomycetous. The dikaryotic mycelium is the major vegetative stage, which occurs in the substratum. In grasslands and open spaces, mycelium forms circular colonies and continues to grow year after year, and produce a crop of basidiocarps at the periphery of the colony during rainy season. As fruit bodies are formed in a circular ring like fashion common people call them fairy rings in the belief that they represent path of dancing fairies. *Marasmius oreades* is a common fairy ring fungus.

The fruit bodies of Agaricales are well developed with a stipe (stalk) and pileus (cap). The tissues comprising a mushroom consist of closely packed dikaryotic hyphae. Three types of basidiocarp development are recognized viz. gymnocarpic, pseudoangiocarpic and hemiangiocarpic (Fig. 8.8).



8.8. Types of basidiocarp development in Agaricales.

9.2.2.2. Fungi and disease in humans: Fungi, though not important pathogens of humans, species cause skin and respiratory tract disease. The fungal diseases of humans are chronic and difficult to cure. Some of the important fungal pathogens of humans and diseases they cause are given in table 9.2.

Table 9.2. Fungal diseases of humans

Pathogen	Disease	Infected parts
Trichophyton	Ring worm	Skin, hair, nails
Microsporum	Ring worm	Skin and hair
Epidermophyton	Ring worm	Skin and nails
Histoplasma capsulatum	Histoplasmosis	Lungs
Blastomyces dermatidis	Blastomycosis	Lungs, skin
Cryptococcus neoformans	Cryptococcosis	Lungs, skin, meninges
Candida albicans	Candidiasis	Various parts
Aspergillus fumigatus	Aspergillosis	Lungs and other parts.

9.2.2.3. Fungi as allergens: Some hyper sensitive people develop asthma as a result of allergy to fungal spores in air or house dust. Agricultural and industrial workers develop severe asthma when exposed to repeated inhalation of fungal spores. The fungal spore that cause allergy mainly below to the genera or fungal species. The fungal spores that cause allergy include *Cladosporium*, *Penicillium*, *Fusarium*, *Trichoderma*, *Chaetomium* etc.

9.2.2.4. Mycetism: Death or disease due to eating of fungi is described as mycetism. Accidental eating of poisonous mushroom may cause death or cause severe health problems. Some species of *Amanita* are highly poisonous. *Amanita phalloides* is called death cup because it is highly poisonous causing death within 24 hours. Other species of *Amanita* like *A. virosa*, *A. verna*, *A. pantherina* and *A. muscaria* are also highly poisonous. Eating of fruit bodies of other mushrooms belonging to the genera like *Galerina*, *Inocybe*, *Psilocybe*, *Clitocybe*, *Panaeolus* and *Coprinus* etc cause serious health problems.

9.2.2.5. Ergotism: The sclerotia of Ergot fungus, *Claviceps purpurea*, contain toxic chemicals like ergotoxins, ergotamines etc. and when ergot sclerotia contaminated cereal grains are used for

preparation of flour used for bread making it cause serious health problems. Ergotoxins constrict blood vessels resulting in loss of limbs and effect central nervous system causing hallucinations. In the middle ages ergotism was very serious in European countries, and it is described as St. Anthony's fire.

9.2.2.6. Fungi and food poisoning: When fungi grow in food stuffs they release different types of toxins and food becomes poisonous. Food poisoning is mainly due to the presence of Mycotoxins like aflatoxins, ochratoxins, trichothecins, zearalenone etc.

Aflatoxins: Aflatoxins were discovered in 1960 when 1,00,000 or more ducklings died after consuming groundnut cake imported from Brazil. It was found that the groundnut produced the toxin responsible for death of ducklings. The toxin as named after the fungus which produce it as Aflatoxin. Apart from *A. flavus*, other fungi like *A.parasiticus*, *Penicillium islandicum* etc. also produce aflatoxin. About 17 types of aflatoxins are recognized and among them B_1 B_2 G_1 G_2 are important and almost potent. Aflatoxins effect liver and cause cancer.

Ochratoxins: Species of *Aspergillus* and *Penicillium* produce mycotoxins referred to as ochratoxins *Aspergillus ochraceous* and *Penicillium veridicataeus* is the most important species. The ochratoxins are a group of 9 components ochratoxin-A I is the most commonly produced toxin.

Zearalenone: *Fusarium graminearum*, *F. moniliforame* and other *Fusarium* species growing on maize cobs produce mycotoxins referred to as zearalenone. The same toxin was produced by *Fusarium* species when growing on other food materials also.

9.2.2.7. Fungi spoilage and deterioration:

Fungi are saprophytic in nutrition and produce enzymes that degrade the substratum on which they are growing. It results in spoilage or rotting of the substrate. Fungi can grow on a wide variety of substrata, and the substrates spoiled and degraded by fungi are numerous. Some of the important substrates spoiled or degraded by fungi are given below.

9.2.2.7.1. Food spoilage: Most familiar example of spoilage of food by fungi is that of spoilages bread by *Rhizopus stolonifer*, the 'bread mould'. Quite a good number of other fungi also grow on bread which include *Aspergillus niger*, *Penicillium* species, member of mucorales etc.

Pickles are spoiled by pink yeasts, *Rhodotorula* species cured meat is spoiled by species of *Aspergillus, Penicillium* and *Rhizopus*.

Amanitacae: Produce basidiocarps typically with free gills and bear annulus, volva and scales. *Amanita phalloides* is the most deadly poisonous fungus and is called death cup. *Amanita muscaria* is called fly agaric as it has ant insecticidal properties and it is also hallucinogenic.

Boletacae: Fruit bodies of the family are mushroom like but bear pores instead of gills on the lower side of pileus.

Bolbitaceae: Produce clay brown spores and are called ochrosporae.

Coprinanae: Include genus *Coprinus*, which commonly occurs ondung and other places.

The pileus at matures dissolve and produce black fluid hence these are called inky cap mushrooms.

Cortinariacea: Presence cortina is a characteristic feature.

Cantherellaceae: Include fungi that produce leathery fruit bodies with gill like structures. They are often placed in aphyllophorales also.

Entolomataceae: The basidiocarps with attached gills and red spore print are characteristic features.

Gomphidiacae: Produce fruit bodies that produce olive brown bidio spores .

Hygrophoraceae: Produce large basidiocarps with waxy gills spore print is white.

Lepiotaceae: Produced large fruit bodies with scales on the pileus and annulus on stipe. The annulus can moves on the stipe like a ring.

Paxillaceae: They resemble Boletus in many characters but bear gills and produce coffee brown species.

Pluteaceae: Include basidiocarps that produce red spore print the commonly cultivated mushroom of India, paddy straw mushroom *Volvariella volvaceae* belongs to this family.

Russulaceae: Include two genera *Russula* and *Laccarius*. The characteristic feature is oozing of latex when gills are cut.

Strophariaceae: Produce basidio carps with attached gills and dark spores. The family includes *Psilocybe mexicana*, called sacred mushroom, which is a hallucinogenic fungus.

Tricholomataeae: A large family including such common genera *Armillaria* (Honey mushroom), Pleurotus (oyster mushrooms).

8.3.1.2. Order Aphyllophorales:

This is a very large order comprising about 400 genera and 1200 species placed in 23 families. All of them produce open hymenium on various structures but not on gills. The hymenium may line pores, coral like branches, teeth like projections etc. The basidiocarps are corky, leathery woody and perennial, but not soft and evanescent a in agaricales. Some of the members of the group are wood inhabiting causing wood rotting, some fungi form fruit bodies on forest trees, which partially encircles the stems, and are called shelf fungi (fig. 8.10).

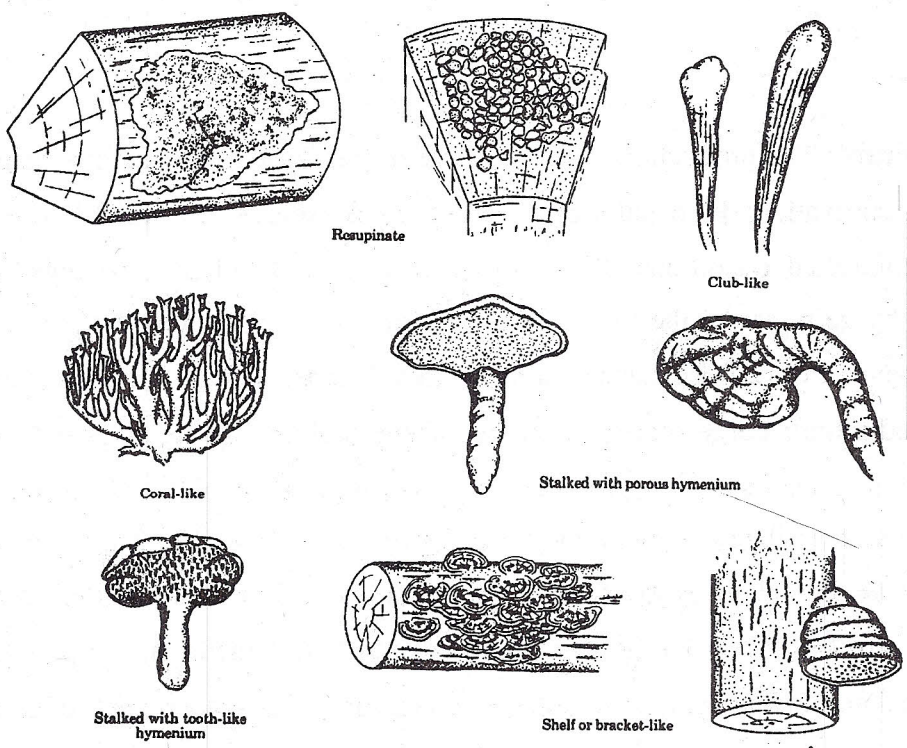


Figure 8.10. Diagramatic sketches of some common aphyllophorales

Traditionally, the order (previously called polyporals) is divided only into 4 families viz. polyporaceae, thelephoraceae, clavariaceae and hydnaceae, based on gross morphology of basidiocarp and orientation of hymenium. At present, basing on more stable microscopic characteristics, the order is divided into 23 families. For the sake of convenience they can be categorized into 5 groups viz. 1. Poriod families, 2. Hydnaceous families, 3. Clavarioid families, 4. Thelephoroid families and 5. Lamellate families.

production of mushrooms. Among the other cultivated mushrooms, *Volvariella volvacea* (paddy straw mushroom), *Pleurotus* species (oyster mushroom) and *Lentinus edodes* (shiitake mushroom) are important. Paddy straw mushroom and oyster mushrooms are mainly cultivated in India, China and South-East Asia. Shitake mushroom is cultivated mainly in China and Japan.

9.3.1. Steps involved in mushroom cultivation: The important steps in mushroom cultivation are

1. Isolation of starter culture or mother culture
2. Spawn production
3. Substrate preparation or bed preparation
4. Spawn running
5. Cropping
6. Harvesting

9.3.1.1. Mother culture: The production of pure culture of the fungus to be cultivated is the first step in mushroom cultivation. Pure culture of mycelium is isolated on special media in the laboratory under controlled conditions. It is usually done by trained professionals. The pure culture is obtained by germinating the basidiospores. It can also be produced by culturing small pieces of tissue removed from a fresh basidiocarp. It is called starter culture or mother culture.

9.3.1.2. Spawn production: Large scale preparation of pure culture for the purpose of using for mushroom cultivation is called spawn production. Spawn means inoculum. Usually for spawn production, crushed and sterilized seeds of sorghum, rye or other cereals are used as medium. To the crushed seeds water and chalk powder are added and it is filled in 1 litre bottles or polythene bags and then they are sterilized. To the sterilized containers with medium, the pure culture is inoculated in inoculation chambers under sterile conditions, The inoculated containers are incubated at room temperature for 15 to 20 days. During this period, the mycelium grows throughout medium and appears as white growth. This is called spawn. The spawn is prepared on large scale by trained people and made available commercially to the mushroom cultivation units or individuals.

9.3.1.3. Substrate preparation: The substrates used for mushroom cultivation vary with the kind of mushroom to be cultivated. Compost is the substrate for button mushroom cultivation. Paddy straw made into bundles and stalked is used for cultivation of paddy straw mushroom. Oyster

mushrooms grow on many organic wastes. Substrate is prepared with cheaply available materials. Substrate or bed preparation in each type of cultivated mushroom is described separately.

9.3.1.4. Spawn running: Inoculating the substrate with spawn is called spawning. The inoculated substrates are kept in culture rooms for mushroom production and growth or spawn in the substrate is called spawn running.

9.3.1.5. Cropping: The mushrooms formed on substrate is called crop. A number of crops may develop.

9.3.1.6. Harvesting: Removal of the mushrooms from the substrate is called harvesting. A number of crops may be harvested form each substrate.

9.3.2. CULTIVATION OF BUTTON MUSHROOM:

Agaricus brunnescens (=*A.biosporus*) is called button mushroom and it is extensively cultivated in western countries. The spawn is commercially available.

9.3.2.1. Substratum: The preferred substratum for button mushroom cultivation is compost. The compost is prepared from different materials in different countries and areas depending on the availability of raw material. Hence, different formulas for compost are available. The most common formula of ingredients for compost is as follows

Wheat straw	-	300 Kg.
Wheat braun	-	25 Kg.
Ammonium sulphate	-	9 Kg.
Super phosphate	-	3 Kg.
Urea	-	3 Kg
Murate of potash	-	3.5 Kg.
Gypsum	-	30 Kg.
Sawdust	-	30 Kg.
Zinc sulphate	-	10 Kg.
BHC powder	-	1 Kg.
Malathion	-	200 c.c.

9.3.2.2. Compost preparation:

The ground on which compost is prepared should be of cement concrete and clean, provided with a roof so as to get rid of rain and temperature due to sunlight. The wheat straw is first wetted for about 48 hours. Wheat braun, ammonium sulphate, super phosphate, urea and murate of potash are mixed in sawdust. This mixture is called fertilizer heap. It is watered to the

It is a small group with a single family Auriculariaceae. They occur on decaying plant arts. The basidiocarps of *Auricularia polytricha* are edible.

8.3.2.3 Order Septobasidiales: This is a small group of insect parasites. Mycelium is limited and produce thick walled spores, which function as probasidia and give rise to a transversely septate, 4 celled metabasidium. From each cell of metabasidium a basidiospore is formed on a small sterigma as in rust fungi.

In this order, a single family septobasidiaceae is recognized.

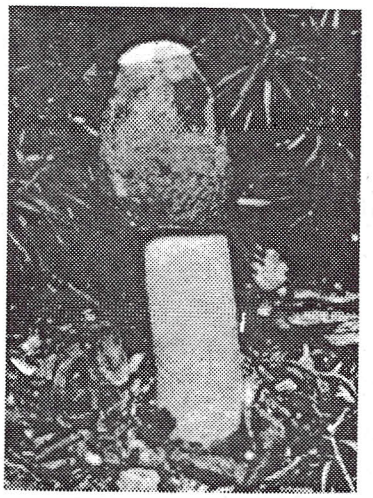
8.4. CLASS: GASTEROMYCETES

The class gasteromycetes includes such commonly known fungi like puff balls, earth starts, stink horns, birds nest fungi etc. (Fig. 8.11 see page 16) Some of the fruit bodies are very large. They are found in a variety of habitats, on soil, below soil (hypogean), on decaying plant parts, on dung etc, and some are mycorrhizal

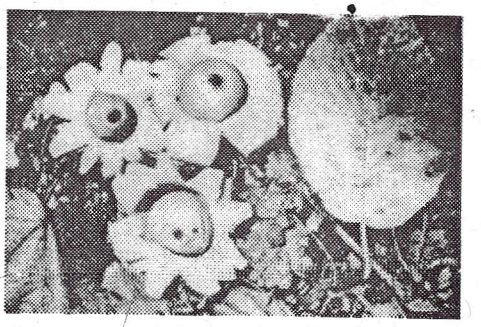
The class is an unnatural assemblage of basidiomycetes that share a common negative character that their basidiospores are not discharged actively as in Hymenomycetes. The basidiospores are described as statismospores, and they are symmetrically poised on sterigmata or sessile. The fruit bodies usually remain closed or open only after the spores are mature. Clamp connection may or may not be present. Basidium is a holobasidium, the fruit bodies have a distinct outer wall called peridium. Within the peridium, the spore forming portion is called gleba.

During basidiocarp formation, hymenium may be formed at least in the early stages in many fungi, while others completely lack hymenium. Presence of absence of hymenium is taken as a fundamental character in classification. 9 orders are recognized in this class. 5 orders viz. Podaxales, Phallales, Hymenogastrales, Lycoperdales and Gautieriales possess hymenium, and in 4 orders viz. Tulostomatales, Sclerodermatales, Melanogastrales and Nidulariales do not possess hymenium

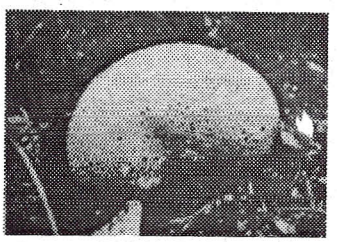
Podaxis



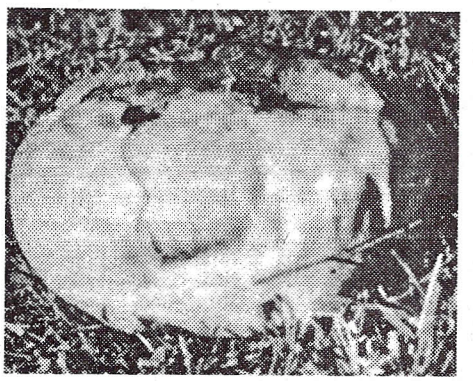
Phallus (Stink horn)



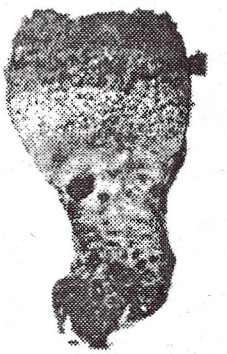
Geaster (Earth Star)



Scleroderma
(Stony Puff ball)

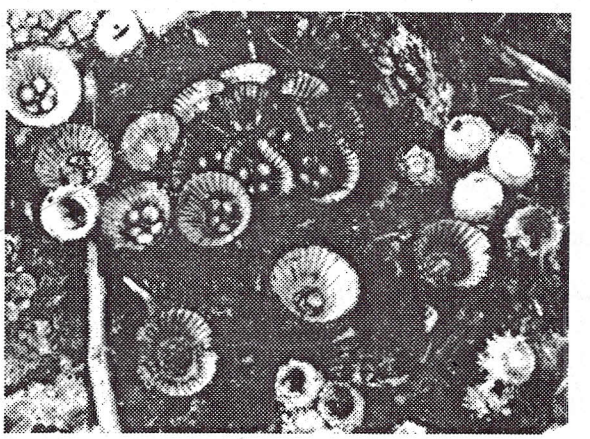


Clavatia (Giant puff-ball)



Pisolithus

Rhizopogan



Cyathus (Bird's nest fungus)

Figure 8.11. Fruit bodies of some common genera of gasteromycetes

9.3.3.3. After care of beds:

Water is sprayed 2-3 times in hot day and 1-2 times in rainy season. If necessary, 0.1% Malathion 0.2% Dithane Z-78 is sprayed to control insects, pests and other problems.

9.3.3.4. Harvesting and marketing:

Cropping starts 10-12 days after spawning and continues up to 15-20 days. Mushroom is harvested at button stage or just before rupturing of the cap by twisting so that broken pieces are not left in the beds. After harvesting, the mushrooms should be used within 8 hours or kept at 10-15°C for 24 hrs. Otherwise these get spoiled. They can be kept in a refrigerator for one week.

Fresh mushrooms are dried in sun or in an oven at 55°-60°C for 8 hrs. After drying they are packed and sealed otherwise they absorb moisture spoils then.

9.3.4. CULTIVATION OF OYSTER MUSHROOM:

Pleurotus species are known as oyster mushrooms. Different species of *Pleurotus* are cultivated in different regions. Some of the cultivated species of *Pleurotus* are

Pleurotus ostreatus : White oyster
P. caejar-kaja : Ash coloured oyster
P. citrino-piletus : Golden oyster
P. flabellatus : Rose coloured oyster
P. sapindus : Black oyster

Important steps involved in cultivation of oyster mushrooms are choice of substratum, bed preparation, cropping, after care of beds, harvesting and marketing.

9.3.4.1. Substratum: It grows on many organic wastes. Crushed maize cobs, wheat straw, paddy straw, dried and pulverized grown, compost, wooden logs etc. Chopped paddy straw is the most commonly used substratum for oyster mushroom cultivation.

9.3.4.2. Bed reparation and cropping: Chopped paddy straw is soaked in water tank for 8-12 hours; washed again with fresh water, and allowed to dry. Then it is filled in wooden trays measuring about 1x ½ x ¼ meter. The entire trays is filled with substratum and transferred to cultivation room.

9.3.4.3. Spawning: The entire surface of substratum in tray is sprinkled with spawn, and after spawning the trays are covered with polythene sheet.

9.3.4.4. After care of beds: Water is sprayed as and when necessary, temperature in the range of 25±5°C and relative humidity in the range of 85-90% are maintained in the production room. To over come pest problems 0.1% malathion and 0.2% dithane Z-78 are sprayed in the room.

9.3.4.5. Cropping: Mushrooms begin to appear after 10-15 days. Then the polythene sheet is removed. Production of mushrooms continues up to 1 to 1½ months (30-45 days) after the appearance of the first flush.

9.3.4.6. Harvesting and Marketing: Mushroom is harvested when the pileus reaches about 8-10cms in diameter. The harvesting is done by twisting so that broken pieces are not left in the tray. Harvested mushrooms are dried in sun or in an oven at 55-60°C for 8 hours. After drying they are packed in polythene bags, sealed and marketed.

9.4. SUMMARY:

Fungi are heterotrophic organisms showing saprophytic, parasitic and symbiotic types of nutrition. They are ubiquitous and occur every where on substrates with some moisture and little organic matter. The activities of fungi are both beneficial and harmful to man and hence are of great economic value.

The beneficial activities of fungi in agriculture include soil formation, soil aggregation, humus formation and maintaining soil fertility, forming mycorrhizal association with crop plants which help is phosphate accumulation and takes up of other nutrients. Some fungi like *Trichoderma* species which are parasitic on plant pathogenic soil fungi are used as biopesticides for control of plant diseases.

The industrial uses of fungi include production of alcoholic beverages such as wine, beer etc. Antibiotics like penicillin and cephalosporins are commercially produced using fungi. Production of alcohol, organic acids, enzymes vitamins etc. are other industrial uses of fungi. Fungi also bring about steroid transformations very effectively.

Mushrooms are used directly as food and unicellular fungi like yeasts are mass cultured for production of SCP, used as food and feed. Fungi are also used in preparation of some foods like tempeh, miso, soysuceae, sufu, Lao-chao, cassava and cheeses.

The harmful activities of fungi include causation of diseases in plants, animals and humans. Mycetism is death or disease due to consumption of poisonous mushrooms. Ergotism is

teliospores or teleutospores. During germination, karyogamy occurs in the teliospores, hence it is equivalent to probasidium. The diploid nucleus enters the germ tube where it undergoes meiosis and become septate. Then it is called promycelium and it gives rise to 4 basidiospores, one from each cell. Hence, the promycelium is equivalent to metabasidium. In rust fungi, the basidiospores are produced on small sterigmata while in smut fungi the basidiospores are budded off directly from the cells without sterigmata.

The class teliomycetes comprise two orders 1. Uredinales – the rust fungi and 2. Ustilaginales – the smut fungi.

Order Uredinales: There are about 100 genera and 4000 species all causing rust disease on higher plants, gymnosperms and pteridophytes. They are ecologically obligate.

A typical rust fungus produces 5 spore types in specialized structures. The 5 stages are referred as O, I II, III, and IV.

Stage O – Spermatia or pycniospores pro ed in spermagonia or pycnia.

Stage I – Aeciospores produced in aecia.

Stage II – Urediniospores produced in uredinia

Stage III – Teliospores produced in telia

Stage IV – Basidiospores produced on promycelium.

When all the 5 spore types are present in the life cycle, it is called macrocyclic rust. If one stage, usually uredinial stage, is lacking it is called demicyclic rust. When only three stages viz. O, III and IV are present, it is called microcyclic rust.

When all the stages are formed on a single host, it is called autoecious rust. When two hosts are required for completing the life cycle, it is called heteroecious rust. In heteroecious rusts, stages O and I are formed on one host, and stages II and III are formed on another host. The host on which stage III (telia) formed is called primary host, because telial stage is the perfect stage, when karyogamy occurs. The other host is called alternate host. There may be no connection between primary and alternate host. For example, for *Puccinia graminis tritici*, which cause wheat rust, wheat plant is primary host and barberry (*Berberis*) is the alternate host.

Stage-O (pycnial): Though this stage was first recorded by persoon in 1800, its function was discovered only in 1927 by Cragie, a Canadian mycologist. By this time the sequence of formation

of other spore stages was well established and they are referred as I, II, III and IV stages. Hence, this stage is called 'O' stage.

The spermagonia or pycnia are flask shaped structures usually formed on the upper surface of the leaves. They produce large number of spermatia on short stalks at the base. The spermagonia also posess long receptive hyphae projecting from the ostiole and function like trichogyne to receive spermatia of compatible strain. The spermatia produced in the same spermagonium do not fuse with receptive hyphae of the same spermagonium when a compatible spermatium attaches to receptive hyphae, the nucleus from it passes into the receptive hyphae and dikaryotic stage develop.

Stage – I (Aecia): The dikaryotic mycelium develop from the dikaryotic receptive hyphae, on the lower surface of the same leaf and this dikaryotic mycelium produce spores in chains in a cup shaped fruit body called aecium. The spore mass (sorus) is surrounded peridium which is perfectly cup shaped. In some rusts, the peridium of aecial cup protrudes beyond the leaf and curl backwards. Such an aerial cup is called rostelium. When the peridium covers entire spore mass it is called peridermium. When there is no peridium, the sorus is called caeoma (fig.8.12).

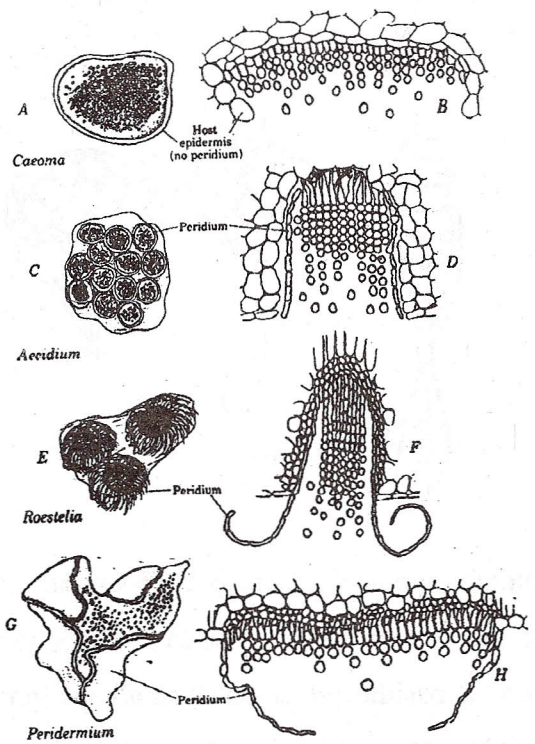


Figure 8.12.Different types of aecia found in uredinales

Stage – II (Uredinia): This is the repeating spore stage of the rusts. Urediniospores are always single celled, thick walled, dikaryotic and are produced on the tips of thin, delicate, evanescent stalks. As the spore mass increases, epidermis ruptures and the spore mass is exposed. The urediniospores are aerially dispersed and when infect the host, it once again develop uredinial stage. Hence, the uredinial stage is repeating stage in the life cycle of rusts and urediniospores are often described as conidia of rust fungi.

Stage – III (Telia): The dikaryotic mycelium, towards the end of the crop season, produce thick walled, dikaryotic resting spores called teliospores or teleotospores. The teliospores differ greatly in their morphology. They may be single celled or multicellular, produced singly or in chains, possess persistent stalks or sessile. Different genera of rust fungi are recognized basing on the type of teleutospores formed. Some common types of teleutospores found in rust fungi are shown in the figure 8.13.

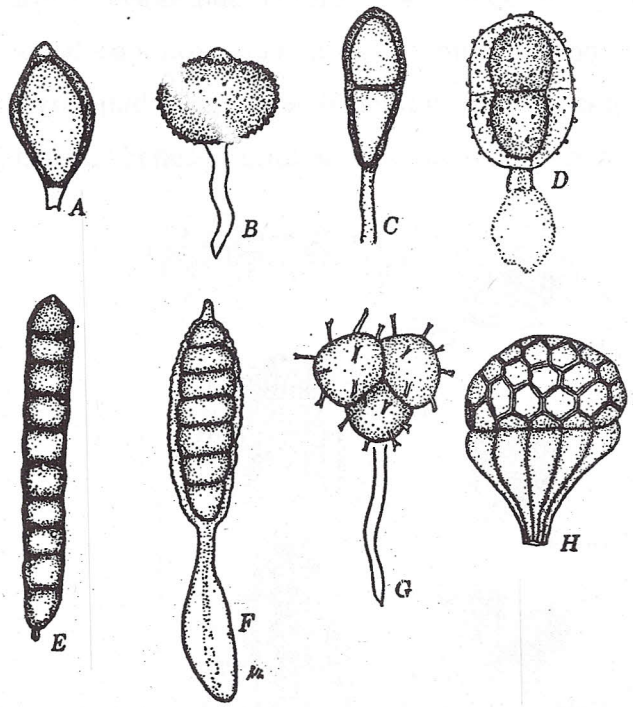


Figure 8.13. Some common types of teleutospores found in rust fungi

Stage – IV (Basidiospores): The teliospores give rise to promycelium which become transversely septate and each cell produce a basidiospores on a small sterigma. Karyogamy occur in the teliospores and meiosis in the germ tube, which become septate after wards.

Classification: Tradionally, rust fungi are divided into two families viz. 1. Pucciniaceae with stalked teleutospores and 2. Melampsoraceae with sessile teleutospores. However presently 4 families are recognized.

1. Pucciniaceae - teleutospores are stalked Eg. *Puccinia*, *Uromyces*.
2. Melampsoraceae - Teleutospores are sessile and formed in a single layer Eg. *Melampsora*.
3. Cronartiaceae - Sessile teleutospores are formed in chains.
4. Coleosporiaceae - Sessile teleutospores are formed in one or two layers. During germination, teleutospores become septate and each cell gives rise to basidiospores directly. No promycelium is formed.

Uredinales imperfecti: The rust fungi in which telial stage is not found or absent are called Uredinales imperfecti. In such rusts, only uredinia may be found, then they are generally placed in the genus 'Uredo'. In heteroecious rusts, on altrnate hosts aecial cups are usually prominent. If the main host for such rusts was not identified, it is not possible to identify. Hence such rusts with only Aecial stage are placed in 4 genera viz. Aecidium, Roestelium, Peridermium and Caeoma, basing on the nature of peridermi of the sorus.

Order Ustilaginales: The members of this order are generally known as smut fungi. About 50 genera and 950 species are recognized and they attack host plants spread over 75 families of angiosperms. They produce smut sori mainly in place of grains, but other parts are also sometimes infected.

In smut fungi, the lifecycle is simple. The basidiospores, often described as sporidia are uninucleates. Dikaryotization takes place by fusion of any two compatible cells (basidiospores), mycelial fragments or germ tubes. The dikaryotic mycelium infect the host and when a mass of mycelium is formed, the mycelium fragments into individual cells and each cell becomes thick walled round spore called smut spores or chlamydospores or teliospores. During germination of the teliospore, karyogamy occurs in the spore and diploid nucleus moves into germ tube and under goes meiosis. The germ tube then become septate to form 3 or 4 celled promycelium. Basidiospores are formed from sides of the cells of promycelium by budding without sterigmata.

In 3 – celled promycelium, the tip cell bears two spores one at the tip and another laterally. The life cycle pattern of Ustilago, the most common genus in the group is shown in the figure 8.14.

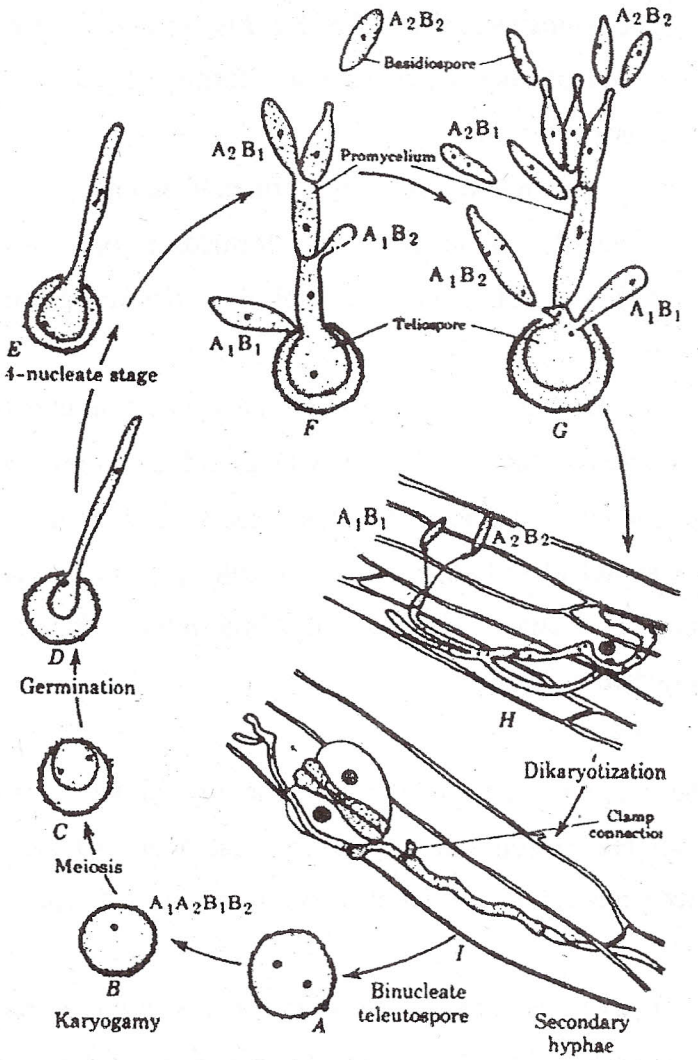


Figure 8.14. Life cycle of *Ustilago*

In some smuts, generally called bunts, the promycelium do not become septate but bear 8 elongate basidiospores at the tip; and two compatible basidiospores fuse while still attached to germ tube. Then, from one of the fusion cells binucleate spores, described as conidia or sporidia are formed on stalks.

Many smuts are systemic in nature. The infection occurs during seed germination and symptoms are produced when inflorescence emerges. In the smuts attacking vegetative parts infection is localized.

Unlike rusts, smuts are culturable and in culture many fungi show yeast like growth.

Classification: The order ustilaginales is divided into 2 families.

Ustilaginaceae: Smut spores on germination produce septate promycelium and basidiospores are formed on cells of promycelium by budding.

Tilletiaceae : On spore germination, aseptate promycelium is formed, which bear 8 uninucleate elongate spores at the tip. These spores unite in pairs, while still attached to promycelium.

In general, the members of ustilaginales are called smuts while the members of tilletiaceae are called bunts.

8.6. SUB DIVISION: DEUTEROMYCOTINA

A great many fungi are known that have septate hyphae and reproduce only asexually by producing conidia. These are placed in the subdivision Deuteromycotina Hawksworth *et al.* (1983) recognized 1680 genera and more than 17,000 species in this group. Since most of these fungi apparently lack a sexual phase (perfect stage), they are commonly called 'imperfect fungi' or 'Fungi Imperfecti'. The conidial stages of most of these fungi are very similar to conidial stags of some well known ascomyceties. Intensive search for perfect stages revealed that some deuteromycotina fungi produce perfect stages that are described as ascomycetes genera. However, the different species in a deuteromycotina genus may have perfect stages in different genera of ascomycetes, and vice – versa may also be true as seen in imperfect – perfect stage relations in he table 8.1.

Name of the fungus of conidial stage	Name of the fungus of perfect stage
Aspergillus glaucus	*Eurotium glaucum*
A. fumigatus	*Sartorya fumigate*
A. nidulans	*Emericella nidulans.*
Septoria rubi	*Mycosphaerella rubi*

Cercocspora arachidicola	*Mycosphaerella arachidis*
Ramularia fragariae	*Mycosphaerella fragariae*
Dreschlera sativa	*Cochliobolus sativa*
Dreschlera teres	*Pyrenophora teres*
Monilia fruictigena	*Sclerotina fructigena*
Monlia sitophila	*Neurospora sitophila*

Some imperfect fungi have perfect stages in basidiomyceties also. For example, for *Cryptococcus neoformans*, an important human pathogen, the perfect stage is *Filobasidiella neoformers* of basidiomycetes. Similarly for *Rhizoctonia solani*, a soil borne fungus attacking a large number of host plants, the perfect sage is *Thanatephorus cucumeris* of basidiomyces. Hence, we may consider deutermycotina fungi as conidial stages of ascomycetes or more rarely basidiomycetes, whose sexual stages have not been discovered or do not exist.

General characters

Somatic structures: Except in a small group of imperfect yeasts, the fungi of the group produce well developed, septate, branched hyphae. The cells are usually multinucleate. The septa possess of a simple pore as in mycelium of ascomycetes.

Asexual Reproduction: Most of the fungi reproduce asexually by producing conidia on conidiogenous cells borne directly on hyphae or on specialized stalks called conidiophores. The conidia and conidiophores are the only structures, which are very distinctive and used in classifying the fungi. Another character, the method of production of conidia on conidiophore, i.e. conidiogenesis is now a days used, especially to distinguish closely related fungi.

Conidia: A conidium is a non-motile, asexual spore formed at the tip or side of a conidiogenous cell. There is tremendous variety of morphologically different types of conidia. They may be spherical, ovoid, elongated, cylindrical, thread like, spirally curved, star shaped and soon. Conidia may be one celled to many celled with either transverse septa or both transverse and vertical septa.

In addition, conidia may be hyaline or coloured. Various types of conidia found in deuteromycotina fungi are shown in figure 8.15.

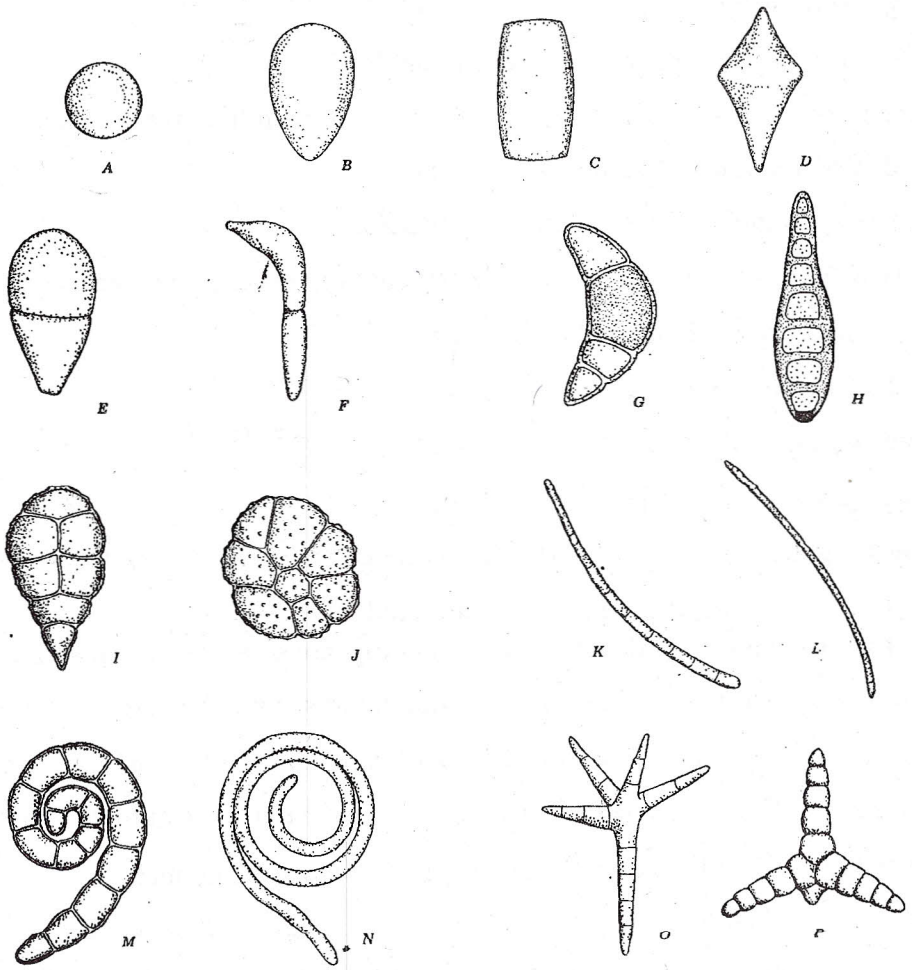


Figure.8.15. Various types of conidia.

Saccardo (1899) in his monograph 'Sylloge fungorum' grouped conidial fungi into 7 sections based on various characters. Though these sections have no taxonomic validity, they are very much useful for descriptive purpose.

Section I. Amerosporae - Single celled conidia

Subsection: 1. Allantosporae – Slightly curved (allontoid) conidia, hyaline to pale coloured

2. Hyalosporae – Hyaline conidia

3. Phaeosporae – colored conidia

Section II. Didymosporae – Two celled conidia, ovoid to oblong in shape.

Subsection 1. Hyalodidymae – Hyaline two celled conidia

2. Phaedidymae – Coloured two celled conidia

Section III. Phragmosporae – Conidia are 3 to many celled, with transverse septa.

Subsections: 1. Hyalophragmiae - hyaline phragmospores

2. phaeophragmiae – coloured phragmospors.

Section IV. Dictyosporae – Multicellular conidia with both transverse and vertical septa.

Subsections: 1. Hyalodictyae – hyaline dictyospores

2. Phaeodictyae – coloured dictyospores.

Section V. Scolecosporae – Conidia long, thread like

Section VI. Helicosporae – Helically coiled conidia, hyaline or colored.

Section VII. Staurosporae – Star shaped or radically lobed conidia; continuous or septate; Hyaline or coloured.

Conidiophore: The conidia are produced from a conidiogenous cell borne on somatic hyphae or distinct branches called conidiophores. The conidiophores may be free or organized into specialized structures. Depending on their nature, conidiophores are variously described.

1. Micronematous – Conidiophores are similar to vegetative hyphae and not distinct.
2. Macronematous – Conidiophores are distinct from vegetative hyphae.
3. Mononematous – Conidiophores are free, may occur singly or in fascicles.
4. Synnemata – A synnema consists of a group of conidiophores often united at the base and part way up the top. Conidia nay be formed along the synnema or only at its apex. The conidiophores comprising a synnema are often branched at the top.
5. Sporodochium: In a sporodochium, the conidiophores arise from a central cushion shaped stroma. The conidiophores are packed tightly together and are generally shorter than those composing a synnema.
6. Pycnidium – A pycnidium is a globose or flask shaped body that is lined on the inside with conidiophores. Pycnidia may be completely closed or may have an opening (ostiole). They vary greatly in size, shape, colour and may be superficial or sunken in the substratum.

7. Acervulus – An acervulus is typically a flat or saucer shaped bed of generally short conidiophores growing side by side and arising from stromatic mass of hyphae. In nature they are typically produced on plant tissue subepidermally and eventually become erumpent.

The major types of asexual fructifications are shown in the figure 8.16.

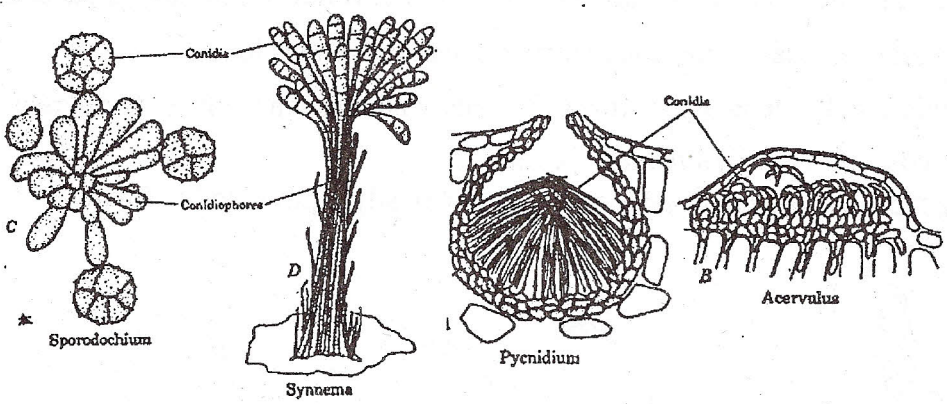


Figure 8.16. Asexual fruit bodies found in deutermycotina.

Conidiogenesis: The pattern of conidial development is called conidiogenesis. The conidia may be formed singly or in groups. Conidia produced in chains are described as catenulate. If the oldest conidium is at the tip and the youngest at the base, then the conidia are said to form in basipetal fashion. If the oldest conidium is at the base and youngest at the tip, it is described as acropetal.

The process of development of conidium from the conidigenous cell is recognized as two main types viz. 1. Thallic and 2. Blastic.

Thallic mode of conidiogenesis: In this method, an entire pre-existing hyphal cell is transformed into a conidium. The cell may be either terminal or intercalary. The thallic conidia are described as arthrospores.

Thallic mode is two types viz. holothallic and enterothallic.

Holothallic: If all the layers of the wall of conidigenous cell are involved in the formation of the conidium, the conidia are described as holothallic.

Enterothallic: If the outer layer of the wall of conidiogenous cell does not become part of the conidium wall, the conidia are described as enterothallic.

Holothallic conidia are more common than enterothallic conidia.

Blastic mode of conidiogenesis: In this mode, there is marked enlargement of the recognized conidial initial before it is delimited from the conidiogenous cell by a septum. It is also mainly two types viz. holoblastic and enteroblastic.

Holoblastic: Both outer and inner walls of blastic conidiogenus cell contribute towards the formation of conidium. Two subcategories are recognized in this type viz. monoblastic and polyblastic. If conidiogenous cell blows out at several points it is described as polyblastic type.

Enteroblastic: In this method only inner wall layer of the conidiogenous cell or neither wall layers contributes to the formation of conidium. Three sub categories are recognized in enteroblastic type of conidiogenesis.

Phialide: A flask shaped or other types of conidigenous cell with fixed conidiogenous locus is called a phialide. The conidia are produced from phialides. Best examples are *Aspergillus* and *Penicillium*.

Annelide: In this type, conidia are produced in a basipetal succession and conidigenous cell elongates with the production of each conidium. As the first formed conidium is pushed up in the chain, it leaves a ring like scar on the outer surface of conidiogenous locus.

Tretic: When conidium develops by protrusion of inner wall through a channel or pore in the outer wall, it is described as tretic. Eg. *Helminthosporium*.

Different types of conidiogenesis are diagrammatically represented in figure 8.17

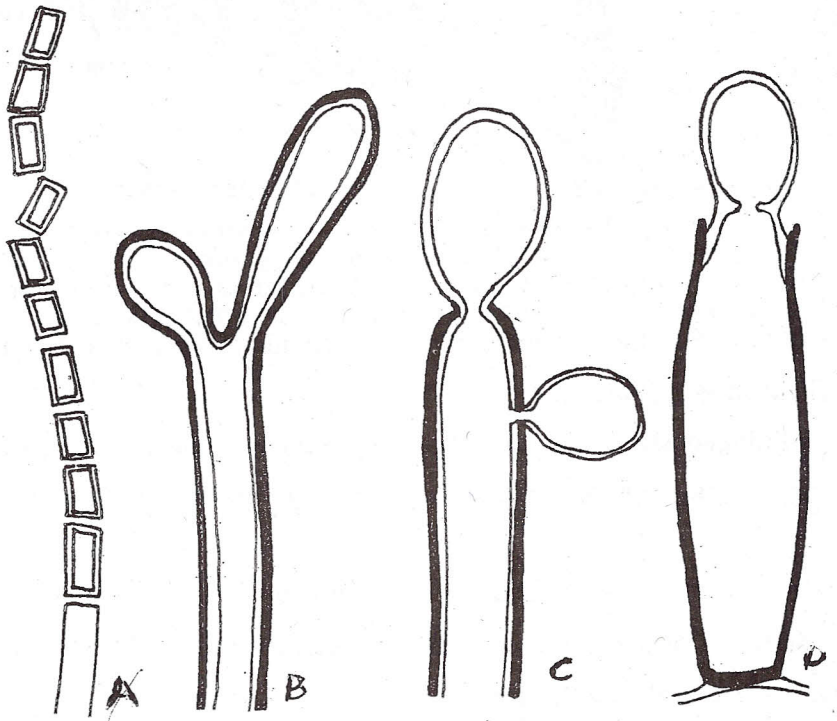


Figure 8.17. Methods f conidiogenesis A: holothallic type. B: holoblastic. C,D: Enteroblastic types

Classification: The subdivision deuteromycotina comprises three classes. 1. Blastomycetes 2. Hyphomycetes 3. Coelomycetes.

8.7. CLASS BLASTOMYCETES:

The conidial fungi which do not form true mycelium but occur as yeast like organisms are placed in this class. They are often described as imperfect yeasts, which reproduce only by budding.

In this class two orders are recognized. 1. Sporobolomycetales and 2. Cryptococcales.

In the order Sporobolomycetales, the spores produced by budding i.e. blastospores, are forcibly discharged like basidiospores, and hence they are called ballistospores. This order comprises only 4 genera, all placed in a single family sporobolomycetaceae and are considered as imperfect stages of basidiomycetes. Important genera are *Sporobolomyces* and *Bullera*.

In the order Cryptococcales, the blastospores are not discharged actively as in sporobolomycetaceae. It is a relatively large group with more than 15 genera and all are placed in a single family cryptococcaceae. The important genera are conidia and *Cryptococcus*. Some species of the genera are important human pathogens *Cryptococcus neoformans*.

8.8. CLASS HYPHOMYCETES:

This is one of the largest groups of fungi with more than 1000 genera and 9000 species. Fungi that produce free or aggregated conidiophores are placed in this. Some fungi included in this class do not produce any spores and perpetuate only in mycelial form, often producing sclerotial bodies.

Two orders are recognized in the class viz. Moniliales and Agonomycetales.

Order Moniales: This is the large order comprising mycelial fungi that reproduce asexually by producing conidia on specialized conidiophores. Four families are recognized.

1. Moniliaceae: Mycelium and conidiophores are hyaline; conidiophores are free. Eg. *Aspergillus* and *Penicillium*. The colonies of the fungi may appear in various colours but the colour is imparted by the conidia while mycelium and conidiophores are hyaline.

2. Dematiaceae: Mycelium and conidiophores are dark coloured, conidiophores unorganized either free or occur in fascicles. Eg. *Alternaria*, *Curvularia*, *Cercospora* etc. Conidia of some of the fungi may be hyaline. Eg. *Cercospora*.

3. Stilbellaceae: Conidiphores are organized into synnemata. These are called synnematial fungi. E.g: *Isariopsis*, *phaeoisariopsis*, *Graphium* etc.

4. Tuberculariaceae: Conidiophores organized to form sporodochium. Eg: *Fusarium*. In cultures, sporodichium may or may not be clearly formed.

Order Agonomycetales: The fungi included in this order are generally described as Mycelia sterilia; since they do not produce any spores. Reproduction is by random fragmentation of the hyphae. Some of the fungi may produce sclerotia under unfavourable conditions. There are about 40 genera in this group, and best known examples are *Rhizoctonia* and *Sclerotium*. All the fungi are placed in a single family Agonomycetaceae.

8.9. CLASS COELOMYCETES:

The fungi that produce conidia in a pycnidium or in acervulus are placed in this class. Two orders are recognized 1. Sphaeropsidales and 2. Melanconiales.

Order sphaeropsidales: The fungi that produce conidia in a pycnidium are placed in this order. They are commonly called pycnidial fungi. In this order 4 families are recognized.

1. Sphaeropsidaceae: Produce typical ostiolate pycnidia which are hard, leathery and dark coloured. E.g: *Septoria*, *Phoma*, *Phyllosticta* etc.

2. Nectrioidaceae: Produce typical ostiolate pycnidia which are light coloured, soft or waxy in texture. Eg: *Aschersonia, Zythia* etc.

3. Leptostromataceae: Pycnidia are elongate or flattened with well developed upper part, but lower part poorly developed. Eg: *Leptostroma* etc.

4. Excipulaceae: Pycnidia open out early to form a cup or saucer shaped structure. Eg: *Excipula, Sporonema* etc.

Order Melanconiales: The fungi included in this order produce conidia in acervulus. A single family melanconiaceae is recognized. The most important genus in this group is *Colletotrichum*

8.10. SUMMARY

The subdivision Basidiomycotina includes highly developed fungi that produce basidiospores. Mycelium of the fungi is well developed, much branched and possess a septum called dolipore septum in the hyphae between the cells. Clamp connections are also seen in the mycelium. The subdivision comprises three classes viz. Hymenomycetes, Gasteromycetes and teliomycetes. In hymenomycetes, the fruit body posses open hymenium, in Gasteromycetes, the peridium remains closed up to maturity while in teliomycetes, no fruit bodies are formed. The general characters of basidiomycotina are explained on taxonomic lines.

The subdivision Deuteromycotina comprise fungi which do not posses any sexual stage, and perpetuate only in asexual stage producing conidia, and in some fungi, even asexual spores are not formed. In classification of these fungi the structure of conidia conidia and conidiophores and the mode of conidiogenesis are considered. The subdivision comprises three classes viz. Blastomycetes, Hyphomycetes and Coelomycetes. The general characters and classification were explained.

8.11 MODEL QUESTIONS

Essay type questions

1. Give an account of basidiomycotina and its classification
2. Explain the fruit bodies of basidiomycotina and their significance
3. Discuss the general characters and classification of Deuteromycotina
4. Discuss the variation in conidia and conidiophores in deuteromycotina

Short answer type questions

1. Agaricales
2. Basidiocarps
3. Gasteromycetes
4. Deuteromycotina
5. Hyphomycetes
6. Conidiogenesis

8.12 REFERENCE BOOKS

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2. Webster, J. 1980. Introduction to Fungi 2nd Edn Cambridge University Press, London.
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LESSON 9: ECONOMIC IMPORTANCE OF FUNGI AND MUSHROOM CULTIVATION

OBJECTIVE: To know the economic importance of fungi which include both beneficial and harmful activities and also to explain the methods of cultivation of common edible mushrooms.

CONTENTS:

9.1. Introduction
9.2. Economic importance of fungi.
9.2.1. Beneficial activities of fungi
9.2.2. Harmful activities of fungi.
9.3. Mushroom cultivation
9.4. Summary
9.5. Model Questions.
9.6. Reference books.

9.1 INTRODUCTION

Fungi are ancient and ubiquitous organisms occurring in all hospitable areas with some organic matter and moisture. Most of them are saprophytic, free living organisms getting nourishment from the dead organic remains. Together with bacteria, these are termed as 'universal scavengers' which cleanup the dead organic debris that accumulates in the environment and make the earth hospitable for future generations. Because of their universal occurrence and wide variety of activities fungi effects man in various ways. Some of the activities of fungi are beneficial and some are harmful. These activities of the fungi are explained under the economic importance of fungi.

Fungi are both macroscopic and microscopic. Some of the macrofungi which produce soft fleshy fruit bodies, belonging to the group Agaricales are edible. People prefer the edible mushrooms for their delicacy and nutritive value. Because of immense consumption of mushrooms; some of them are cultivated on commercial scale. The methods of cultivation of some important edible fungi are also explained in this lesson.

9.2. ECONOMIC IMPORTANCE OF FUNGI:

Fungi are heterotrophic organisms showing absorptive type of nutrition. They produce a variety of enzymes to degrade the organic matter and take up soluble nutrients. Fungi utilize a

great variety of substrates, and nutritionally they show saprophytic, parasitic and symbiotic modes. During the normal metabolic activities they degrade the substrates or parasitise other living organisms including plants animals and man. Some of the activities of the fungi are beneficial to man and some are harmful. For example, during their growth when they degrade the dead organic debris of plants and animals, they release the bound nutrients which increase soil fertility and when they grow on food stuffs and other substrates useful for man, they spoil the substrate and it is harmful to the man. The parasitic organisms when attack plants, animals or man, they cause harm or economic loss. The metabolic pathways and products are exploited by human to produce useful products commercially. Hence, the activities of fungi can be divided into beneficial activities and harmful activities. Both beneficial and harmful activities of fungi are of considerable economic importance to humans.

9.2.1. BENEFICIAL ACTIVITIES OF FUNGI:

The natural activities of fungi in soil are of considerable importance in agriculture, and ability of fungi to produce various metabolic products is utilized by humans in industrial production of pharmaceutics, organic solvents, enzymes vitamins etc. In addition to agricultural and industrial uses of fungi, some macrofungi especially edible mushrooms can be directly used as food, and microfungi like yeasts can be mass cultured to produce SCP (single cell protein) which can be used as food and feed. Some food and dairy products are prepared by using fungi. Hence the beneficial activities of fungi can be conveniently categorized as 1. Agricultural importance 2. Industrial uses and 3.as food and preparation of fermented foods and 4. dairy products.

9.2.1.1. Agricultural importance:

Fungi are useful in agriculture in various ways like soil formation, maintaining soil fertility, soil conservation, phosphate solubilization, mycorrhizal association and biopesticides.

9.2.1.1. Soil formation: Some fungi, in symbiotic association with algae, form lichens which are pioneers in ecological succession. They cause withering of rocks, thus contributing to soil formation.

9.2.1.1.2. Maintaining soil fertility: Fungi, along with other heterotrophic microbes, play an important role in decomposition of organic matter, thus releasing the nutrients locked up in dead bodies in nature. In organic mater degradation fungi are the primary colonizers attacking carbohydrates, release Co_2 into atmosphere, and release nutrients board to organic carbon into soil.

The products of microbial activity in soil constitute the nutrients for higher plants. The organic fraction of soil, often termed as humus, is a product of synthetic and decomposing activities of fungi and other soil microorganisms. The amount of humus content in soil is an indicator of fertility level of soil.

9.2.1.1.3. Soil Conservation: Soil fungi with their extensive mycelial growth bind the soil particles resulting in aggregation and soil conservation. Some fungi produce mucilage that also helps in soil aggregation.

9.2.1.1.4. Solubilization of phosphates and other inorganic nutrients: Many soil fungi, pecially species *Aspergillus Penicillium* and others produce organic acids during their growth and these acids solubilize insoluble phosphates and other inorganic nutrients into soluble form, thus increasing their availability to plants. Among phosphate solubilizing microorganisms *Aspergillus awamori*, *A niger*, *Penicillium digitatum* etc. are used as bioinoculants.

9.2.1.1.5. Mycorhizal formation and phosphate nutrition of plants: More than 90% of the land plants have mycorrhizal association, the mbiotic association of fungi with roots of higher plants. Majority of tree species have ectomy .izae in which mycobiont, normally a member of basidiomycetes, forms a thick mantle or sheath on the surface of the root. More than 85% of land plants form vesicular arbuscular mychorrhizae, in which the fungal symbiont is a mucoralean fungus belonging to *Endogone*. The fungus forms arbuscules and vesicles in the root and extend into the surrounding soil extensively. By increasing the area of absorption around the roots, the mycorrhizal fungus accumulates phosphorus and other nutrients and makes them available to the host plants. Mycorrhizal association is especially useful in phosphate nutrition and these associations mainly form in phosphate deficient soil.

Ectomycorrhizal fungi are used as bioinoculants for seedlings used for social forestry, afforestation programmes and plants used for reclamation of denuded soils. VAM fungi are mainly useful for cultivated plants.

9.2.1.1.6. Fungi as biopesticides: Fungi parasitic on insects and other pathogenic fungi are now used to develop biopesticides to control insects and some important soil borne plant pathogens. *Beauvaria bassiana* is used as an insectide. Species of *Trichoderma* viz. *T. harzianum* and *T. viridae* are parasitic on mycelium of *Rhozoctonia solani* and *Sclerotium rolfs*, which are wide spread soil pathogens causing damping off and root rot of young seedlings. Species of

Trichoderma are now used as bioinoculants to control seedling diseases caused by *R. solani* and *S. rolfsii.*

9.2.1.2. Industrial uses of fungi:

Fungi are exploited industrially to produce a number of chemicals. Among industrially useful fungi, yeasts, especially *Saccharomyces cerevisiae*, are most important. Other industrially useful fungi include species of *Penicillium, Aspergillus, Rhizopus, Mucor, Claviceps* etc.

9.2.1.2.1. Alcoholic beverages: Alcoholic drink are used allover the world and companies producing them are called Breweries. It is a multibillion industry. The basis of beverage production is fermentation of carbohydrates to alcohol by yeasts.

Beer is an alcoholic beverage produced from barley malt and alcohol in this beverage is derived from malt sugar through fermenting activity of *Saccharomyces cerevisiae* or *S. carelsbergensis*. The alcoholic content is usually 3-8 %.

Wine is produced from grape juice or other fruit juices by fermentation activity of *S. cerevisiae*. The alcoholic content is generally up to 14%.

The alcoholic content of various beverages is increased either by aging (long periods of incubation) or by distillation.

9.2.1.2.2. Antibiotics from fungi: Penicillin is the first antibiotic discovered by Alexander Fleming and it is produced by *Penicillium notatum* and *P. chrysogenum*. It is a wonder drug useful mainly against infections caused by Gram-positive bacteria. Various second-generation penicillins are produced by partial chemical modification of the original penicillin and they have extended spectrum of activity.

Penicillium griseofulvum produces an antifungal antibiotic 'griseofulvin' which is very effective against skin infections of human caused by fungi such as *Trichophyton*, *Macrosporum* and *Epidermophyton.*

Cephalosporium group of antibiotics are produced by a fungus *Cephalosporium acremonium* and they have similar activity as penicillin.

9.2.1.2.3. Ergot alkaloids: The sclerotia of ergot fungus *Claviceps purpurea* contain a large number of alkaloids, which when consumed in large quantities seriously affect central nervous

8.2.3. Basidium: A basidium may be defined as a structure bearing on its surface a definite number of basidiospores (usually four) that are typically formed as a result of karyogamy and meiosis. In the fruit bodies, the basidia usually form in a layer and it is called hymenium. In addition to basidia, hymenium in basidiocarps also comprises sterile structures called cystidia and immature basidia called basidioles. (Fig 8.4)

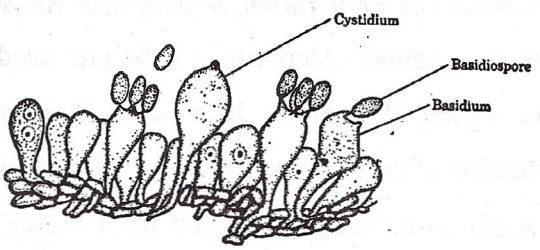


Figure 8.4. Hymenium of a basdiomycetes.

In the hymenium, basidium usually develops at the tip of a generative dikaryotic hypha. The two nuclei fuse to form diploid nucleus. Then immediately it undergoes meiosis forming four nuclei. As meiosis occurs, the cell enlarges to appear like a distinct globose cell called basidium. On the developing basidium, four small sterigma develop at the apex and eventually a single basidiospore develop on each sterigma (Figure 8.5)

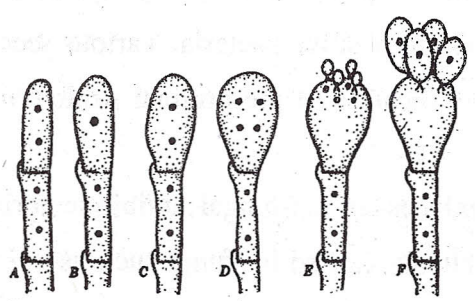


Figure 8.5. Different stages in the development of a basidium

Depending upon the stage of development and structure, basidia are variously described as probasidium, metabasidium, holobasidium and phragmobasidium.

Probasidium – the portion of basidium in which nuclear fuion takes place

Metabasidium – the part of basidium where meiosis occurs.

Holobasidium – One celled basidium

Phragmobasidium – Basidium divided into 4 cells either transversely or longitudinally.

In majority of basidiomycetous fungi, pro and meta basidia only refer to same structure at different stages of development. However, in some fungi, they are morphologically distinct.

Another term heterobasidium is used by many to describe any type of basidium, other than the single celled club shaped holobasidium. Different types of basidia are shown in the figure 8.6.

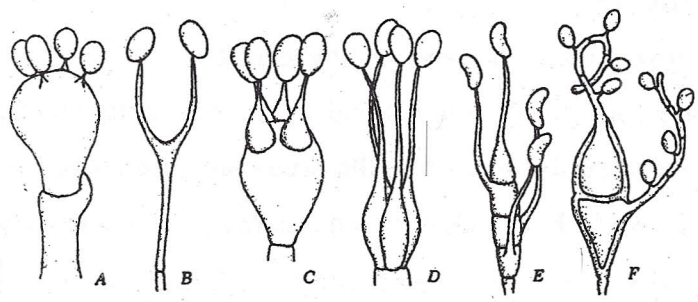


Figure 8.6 Different types of basidia. A, B: Holobasidium types,
C, D&E: Phragmobasidium types.

8.2.4. Basidiospore: The basidiospore is typically unicellular, maybe globose, oval elongated or variously shaped. They may be colourless or pigmented. In many cases the pigments are very dilute and can be detected when spore mass is observed. The spore prints maybe green, yellow, orange, ochre, pink, brown, violet or black.

8.2.5. Basidiospore release: A basidiospore rests on the tip of a sterigma in an oblique fashion and, in forms with exposed basidia, is forcibly discharged from the sterigma. Very close to the point of its attachment to the sterigma, the spore has a minute projection referred to as hilar appendix. A small bubble or drop form at the hilar appendix as the spore mature is thought to be responsible further discharged of basidiospores. Buller first described such a drop formation and hence it is described as Buller's drop. Various others theories such as electrostatic repulsion, jet propulsian, surface tension, rounding off of turgid cell etc have also been proposed to explain the forcible release of basidiospores. The basidiospore formation and discharged is shown in the figure 8.7.

protein, it is called single cell protein (SCP). The species of yeasts used for SCP are *Candida utilis*, *C. lipolytica*, *Saccharomyces* species, *Klyuveromyces fragilis*, *Debaryomyces klocckeri* etc.

9.2.1.4. Fungi in preparation of fermented foods: Bread and various other food items are prepared using fungi.

9.2.1.4.1. Bread: For preparation of bread, the flour of wheat or other cereals is made into paste, called dough, and it is mixed with yeast cultures and incubated for a few hours at room temperature. By fermentative action of yeast the carbohydrates splits into alcohol and carbondioxide. The gaseous product makes the dough soft and levened. From it bread is prepared by baking. The yeast strains used in bread making belongs to *Saccharomyces cerevisiae*. This yeast, hence, is also called Baker's yeast.

9.2.1.4.2. Fermented products of soybean: The seeds of soybean (*Glycine max*) are very rich in proteins (up to 50%) but they are conjugated to lipids and trysin inhibitors, and hence are not easily digested. To make them more easily digestible, the seeds are inoculated with fungi and resulting fermented products are consumed Tempeh in Indonesia, Miso in Japan, and sufu in china are popular fermented products of soybean.

For preparation of Tempeh, the soybeans are soaked in water for 2 hrs, cooked, cooled, inoculated with *Rhizopus oligosporus* and incubated for 24 hrs. The fungus grows extensively and binds the cooked beans and held them together like cakes. The slices of the cake are fried in oil and eaten.

For Miso production, the fermentation of soybeans is brought about by *Aspergillus oryzae* and *Saccharomyces rouxii*.

Sufu is prepared using *Actinomucor elegans* or *Mucor hiemalis* or *Mucor subtilissimus*.

9.2.1.4.3. Lao-Chao: Lao-Chao is a fermented product of rice used in china. Cooked, waxy rice is inoculated with two fungi viz. *Rhizopus chinensis* and *Endomycopsis* and incubated for 2-3 days. The final product is soft, juicy, sweet and mildly alcoholic.

9.2.1.4.4. Cassava: The roots of *Mannihot esculentus*, commonly called cassava, are tuberous and edible. They are extensively used as food in West African countries and in rural India. The rots of cassava contain a toxic substance called cyanogenic glucoside. Hence, roots should not be eaten raw. But they can be fermented to remove the toxic substance. Fermentation is carried out in two

stages. In the first stage, *Corynebacterium manihot* and in second stage species of *Geotrichum* are used. The first one is a bacterium and the second a fungus.

9.2.1.4.5. Cheeses: Cheeses are important diary products and different microorganisms are used in ripening of cheeses to impart distinctive flavour. *Penicillium roqueforti* and P. camemberti are used in prepafation of Roquefort cheese and Camembert cheese, respectively.

9.2.2. HARMFUL ACTIVITIES OF FUNGI:

The harmful activities of fungi include causing diseases in plants, animals and humans, causing food poisoning and mycetism, spoilage of various substrates etc.

9.2.2.1. Fungi and disease in plants: Fungi are the most important group of plant pathogens causing severe epidemic diseases such as rusts, smuts, powdery mildews, downy mildews, leaf spot diseases of crop plants, Yield losses running into thousands of crores of rupees occur every year. Complete devastation of food crops by fungal pathogens resulted in wide spread famines also. Among these, the great Irish famine of 1845-46 is very important one and it is due to the failure of potato crop in Ireland due to late blight disease caused by *Phytophthora infestans*. During this famine an estimated 1.5 million people died. In 1942, a wide spread famine occurred in Bengal region of India. The Bengal famine is due to failure of rice crop due to brown leaf, spot disease caused *Helminthosporium oryzae*. Some very important fungal disease of crop plants is given in the table 9.1.

Table 9.1. Important fungal diseases of crop plants

Crop	Disease	Pathogenic fungus
Wheat	Rust Smut Powdery mildew	*Puccinia graminis tritici* *Ustilago tritici* *Erysiphe graminis*
Rice	Blast Brown leaf spot	*Pyricularia oryzae* *Helmithosporium oryzae*
Sorghum	Smuts	*Sphacelotheca sorghi,* and Others
Bajra	Green ear	*Sclerospora graminicola*
Ground nut	Tikka leaf spot	*Cercospora arachidicola* *Cercospodium personatum*
Cotton	Wilt	*Fusarium oxysporum*
Sugarcane	Red rot Whip smut	*Colletotrichum falcatum* *Ustilago scitaminea*
Chillie	Die back and fruit rot	*Colletotrichum capsici*
Cabbage and cauliflower	Club root	*Plasmodiophora brassicae*
Grapes	Downey mildew	*Plasmopora viticola*

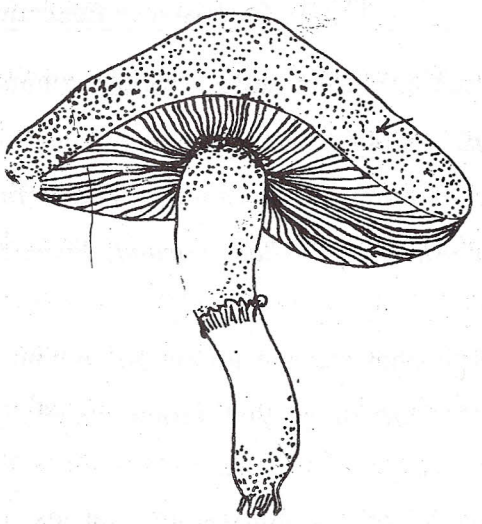
In gymnocarpic type of development, hymenium's remains always naked. In pseudoangiocarpic development, hymenium's becomes enclosed by the incurving margin of the pileus and at maturity it breaks exposing hymenium. In hemiangiocarpic development, hymenium is closed by the tissues of the basidiocarp. Typically the margin of the pileus is connected to stipe by a membrane called inner veil. As the fruit body matures the veil is severed from the margin of the pileus and remains attached to the stipe in the form of a ring or annulus. In some species, veil tears in much a manner that the broke membrane hangs down from the margin of the pileus like a cobweb curtain, and it is described as *cortina*. In some fungi like species of *Amanita* species, baidiocarp peridium is also covered by a membranous structure called universal veil. When the fruit body expands, the universal veil tears and leaves a cup shaped body called volva at the base, and the part that covers pileus can be seen in the form of scales on the top of the cap. These vestigial structures, annulus, cortina, volva, and scales, resulting from hemiangiocarpic mode of development are important in the classification of many agarics.

In Agaricales, hymenium is formed lining the lamellae or gills formed beneath the pileus. Gills are generally them strips of tissue radiating from the margin of the pileus towards the stalk. The gills may be free from the stripe, and in such fungi stalk can be easily removed from the pileus without disturbing he gills. In some fungi, gills may directly attach to the stalk and such gills are described as adnate gills. If the gills run down the stalk for a distance, the gills are described as decurrent. The nature of gill attachment to the stalk is an important taxonomic character.

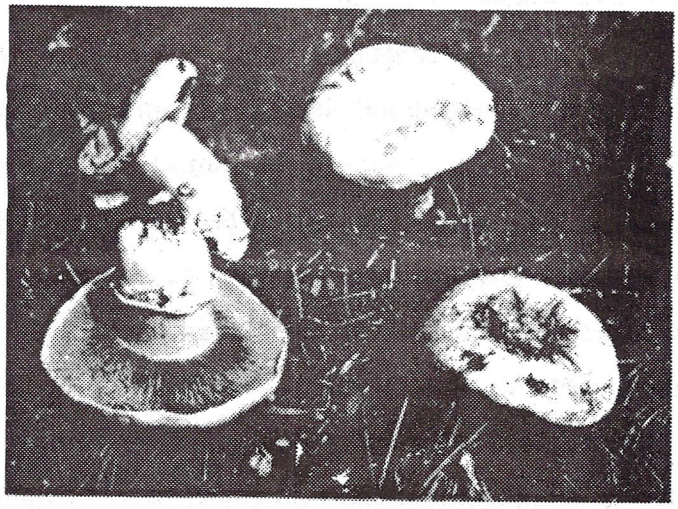
The inner tissue of the gills is called trama. Basically a trama consists of plectenchymatous tissue made up of elongate hyphal cells. Some specialized cells like sphaerocysts (round cells) are present in the genera *Russula* and *Laccaria*. The hymenium is formed on either side of the gills and each basidium bears 4 basidiospores, which are actively discharged.

The order agaricales is divided into 16 families.

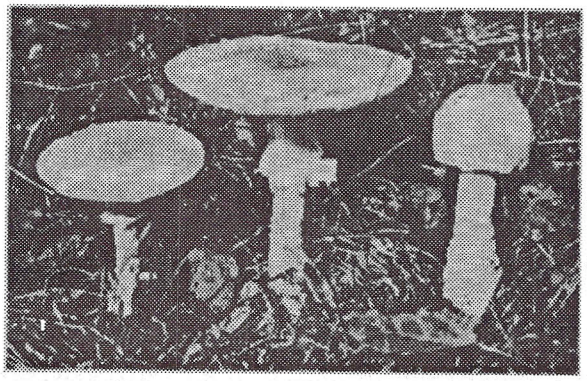
Agaricaceae: Includes well known genus *Agaricus* called field mush room. The basidiocarps have white or brown smooth pileus, stipe bears annulus but no volva, gills are free, and appear rose coloured at the beginning but becomes dark brown as the spores mature. *Agaricus bisporus* (*A.brunnescens*) is the cultivated mushroom. Some common agaric fruit bodies are shown in the figure 8.9.



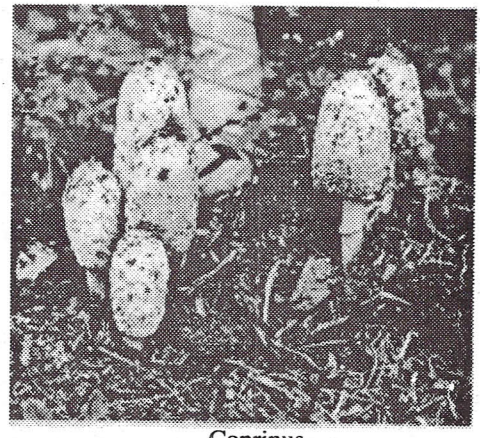
Agaricus campestris



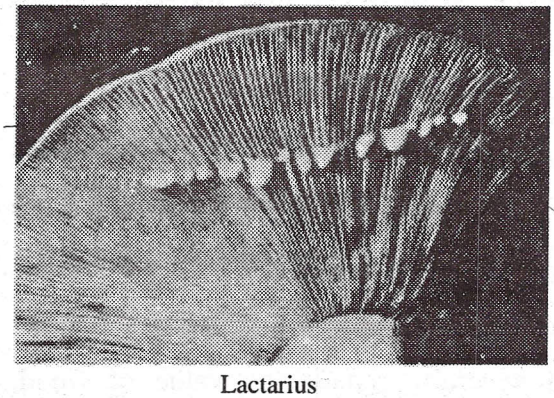
Agaricus rodmani



Amanita

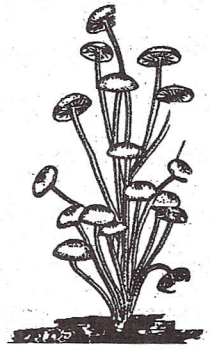


Coprinus

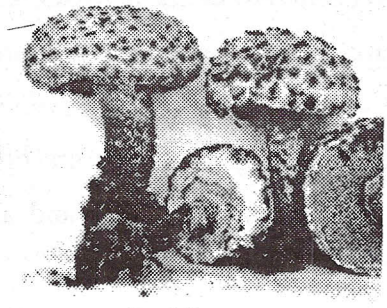


Lactarius

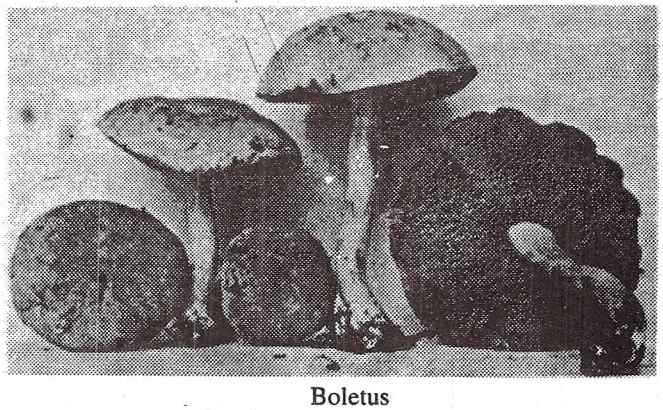
Pleurotus



Marasmius



Strobilomyces



Boletus

Figure 8.9. Some common agaric fruit bodies

Fruits and vegetables also suffer from fungus infestation during storage. Some common examples are *Penicillium digitatum* on citrus fruits, *Rhizopus stolonifer* on sweet potatoes etc.

Grains of cereal crops are infested with a number of fungi during storage. The storage fungi include species of *Aspergillus Penicillium, Phoma, Alternaria, Cladisporium, Fusarium Curvularia, Drechslera, Mucor* etc.

9.2.2.7.2. Spoilage of cellulosic products: Paper, textiles, leather etc. are important commonly used materials mainly composed or cellulosic substance. The fungi that produce cellulose degrading enzymes grow on them and spoil them.

Wood pulp the most important raw material of paper industry is spoiled by fungi. Species of *Alternaria, Aspergillus, Cladosporium, Trichoderma, Mucor, Polyporus* etc. are commonly encountered in bulk storage vats. Yellow discoloraton and spoiling (called mildewing) of paper is caused by species or *Aspergillus, Penicillium* and *Chaetomium.*

In spoilage of textiles, the important fungi are species of *Aspergillus Penicillium, Cladosporium herbarum, Chaetomium globosense* etc.

9.2.2.7.3. Deterioration of wood: Wood is the secondary xylem of the tree secies and extensively used in construction activities. A number of fungi, especially those belonging to Aphyllophorales, such as species of *Armillariella, Collybia, Polyporus, Ganoderma, Coniophora* etc. attack wood and cause decay. Two basic types of wood decay are recognized viz. brown rot and white rot. In brown rot, the cellulose component of the wood is decomposed by the fungus while the brown lignin component is left unaltered. Hence it looks brown. In white rot, both cellulose and lignin are enzymatically attacked, resulting in a white appearance of the wood.

In addition to decay, fungi also cause another type of wood deterioration called staining of wood. The colouring or staining is due to the presence of fungus in the wood fungi causing staining in stored wood belong to the genera such as *Alternaria, Aspergillus, Penicillium, Mucor, Cladosporium, Aureobasidium* etc. Staining of wood generally reduce the value of wood, however, wood stained by *Chlorociboria aeruginascens* is green and preferred in making of certain wooden ornaments, the wood of *Aguilaria agallocha* infested with *Epicoccum granulatum* gives perfume odour.

9.2.2.7.4. Deterioration of fuels: Hydrocarbon fuels such as kerosene and lubricants are often attacked by fungi. The fungal growth forms matted mycelium, which clog fuel lines and also

changes the properties of fuels. The fungus *Cladosporium resinae* (the perfect stage is *Amorphotheca resianae*) degrade kerosene and other hydrocarbons and is commonly called 'kerosene fungus'.

9.2.2.7.5. Deterioration of optical equipment: Although glass as such is not utilized by fungi as a nutrient source, fungal growth on lenses of microscopes, binoculars, cameras, viewfinders etc cause spoilage of the equipment. Damage is due to the growth of fungi, living on accumulations of dirt on the lens surface. This growth either obscures vision by its presence or more seriously by etching the lens surface by its corrosive secretions. Species of *Aspergillus*, *Penicillium* and *Scopulariopsis* are the common moulds associated with the deterioration of optical equipment.

9.2.2.7.6. Deterioration of other substrates: Fungi frequently cause deterioration of paintings, films etc. under humid conditions the fungi involved are mostly the species of *Cladosporium*, *Phoma* and *Pullaria*.

The clear, smooth surface of many plastics is spoiled by even slight growth of fungi. Plastic surface itself do not supports growth of fungi but debris accumulated may support the growth. The most common fungi seen on plastic materials in refrigerators are species or *Aspergillus*, *Penicilluim*, *Mucor*, *Rhizopus* etc.

The insulation material or electrical equipment also gets spoiled because of mould growth.

9.3. MUSHROOM CULTIVATION:

Mushrooms are the fruit bodies of fungi belonging to the order Agaricales of Basidiomycotina. They are soft, fleshy, short lived and generally occur during rainy season. More than 4000 species of mushrooms are known and about nearly two thousand species are known to be edible. However, only about 100 species are mainly collected as edible fungi and of them only a few are cultivated on commercial scale. Most people who eat mushrooms do so for their flavour, and they are also a good source of vitamins. The fibre content of the fruit bodies is very high and it cleanses the digestive system. Mushrooms are good for diabetics also because starch is absent completely. Hence, mushroom consumption is on increase now a days, and mushroom cultivation is being run commercially in many countries. The most important cultivated mushroom is *Agaricus brunnescens* (=*A.bisporus*) which is commonly called 'button mushroom'. It is extensively cultivated in Western countries and accounts for more than 50% of commercial

1. Poroid families: The basidiocarps are pileate, either stalked or sessile. When stalk is present it may be central or lateral. The basidiocarps are very hard and woody. The most important character is formation of closely packed tubular structures lined by hymenium. The central part of these tubes appears as pores on the lower surface of pileus.

Five families possess period hymeneal structure 1.polyporaceae 2.Ganodermataceae 3. Fistulinaceae 4.bondarzewiaceae and 5.podoscyphaceae.

2. Hydnaceous families: In this group the basidiocarps are mushroom like, with teeth like projections on the lower surface of pileus. The fruit bodies are relatively smooth and monomitic with only generative hyphae. Such structures are formed in 5 families viz. 6 Hydnaeae, 7. Auriscalpiaceae 8. Bankereaceae 9. Echinodontiaceae and 10. Hericiaceae.

3. Clavarioid families: The fruit bodies erect, clavate or corolloid branches and hymenium form on the external surface of the branches

Such an organization is found in 4 families viz Clavariaceae, Clavulinaceae, Lacnocladiaceae and 'Sparassidaceae'

4. Thelephoroid families: The basidiocarps are simple, sessile, resupinate (cnust like or flat) on the substratum. They are membranous, leathery or hard with monomitic construction. Hymenium is formed in different ways. 7 families are having the thephloeerioid basdiocarps 1. Thelephoraceae, 2. Corticiaceae 3. Coniophoraceae 4. Streaceae, 5.Punctulariace, 6. Hymenochaetaceae and 7. Gomphaceae.

5. Lamellate families: The hymenium is formed on the gill like structures. But the fruit body is leathery and perennial but not fleshy and evanescent.

Two families viz. Cantherellaceae and Schizophyllaceae are recognized. In Chantherella, basidiocarps are half funnel shaped, on the outer surface thin folds resembling gills are formed, and hymenium is formed on the gills. In *Schizophyllum*, fruit bodies are leathery and gill like structures on the lower surface of the pileus are typically split in the middle. Hence the gills of *Schizophyllum* are called split gills.

8.3.1.3. Order Exobasidiales: This is a small order fungi parasitic on flowering plants producing galls and blisters. *Exobasidium vixans*, which cause blister blight of tea plants, is the best known species. Mycelium is internal and form hymenium on leaf surface, and no basidiocarp is formed. The number of basidiospores produced per basidium varies from 2 to 8. A single family Exobasidiaceae is recognized in this order.

8.3.1.4 Order Brachybasidiales: This is a very small order comprising a single family Brachybasidiaceae. The fungi are parasitic on higher plants producing leaf spots, and no basidiocarps are formed. Basidium is bisterigmate producing only two basidiospores.

8.3.1.5 Order Dacrymycetales: The members of this order are characterized by forked basidium producing two basidiospores. The members are saprophytic on decaying wood producing gelatinous or waxy fruit bodies often coloured yellow or orange. A single family Dacrymycetaceae is recognized.

8.3.1.6 Order Tulasnellales: The order tulasnellales includes saprobic as well as plant parasitic species. They produce effused basidiocarps that are often waxy in appearance. The most important character of this group is formation of greatly inflated broad sterigmata on basidium.

In this order, two families viz. Tulasnellaceae and Ceratobasidiaceae are recognized.

Rhizoctonia solani, on of the very common soil borne plant pathogen, some times produce a perfect stage in cultures. The perfect stage is recognized as *Thanetophorus cucumeris* of Ceratobasidiaceae.

8.3.2 SUBCLASS PHRAGMOBASIDIOMYCETIDAE:

The subclass phragmobasidiomycetidae comprises of fungi whose metabasidium is typically divided into 4 cells by transverse or longitudinal septa. It is a very small group with 3 orders 1. Tremellales 2. Auriculariales and 3. Septobasidiales.

8.3.2.1 Order Tremellales: These are commonly known as jelly fungi because of the gelatinous jelly like nature of basidiocarps. They are free living, and on substrata form a thin layer of gelatinous hyphae that produce basidia. In some fungi, basidiocarps are cushion shaped. The metabasidium in which meiosis occurs divide into 4 cells by longitudinal septa. Each cell of the metabasidium bears an elongate sterigma at the tip of which one basidiospore is formed.

The order comprises 3 families 1. Tremellaceae 2. Sirobasidiaceae and 3. Hyaloriaceae.

8.3.2.2 Order Auriculariales: Members of this group are characterized by the production of metabasidium that is divided by transverse septa into 4 cells. Each of the 4 cells bears a long sterigma with a single basidiospore at the tip.

water adequately for about a day. Next day the mixture is mixed to the watered wheat straw and stacked into a neat heap of 1½ x 1 x 1 meter size. It is well watered but the water should not seep from below. If possible, this heap is covered with polythene sheet. Temperature of the heap rises steeply (up to 78°C) on second or third day.

9.3.2.3. Turning of compost:

The first turning is done on 6th day. About one foot deep compost is scrapped from out side and top and is stacked at one side. Again same process is repeated till one finds a new stack. Meanwhile a little amount water is added so that no dry patch remains left. The second turning is similar to the first one and is done on10th day. The third turning is done on 13th and 10 Kgs of gypsum is added during this turning. The fourth and fifth turnings are done on 16th and 19th day respectively and 10 kgs of gypsum added in each of two turnings. The 6th turning is done on 22nd day. The remaining ingredients 200 c.c. of malathion and one litre / kg of lintaff, are added to prevent insect infestation.

9.3.2.4. Bed p : paration and cropping:

Compost is now filled in wooden tray, each measuring 1½ x ¼ metre, pressed firmly and slightly watered. The trays are arranged are above the other in tiers in pasteurization room. Temperature of pasteurization room is raised up to 60^0c and maintained for a period of two days after which the trays are transferred to production hall, provided with 24^0C temperature.

Small pieces of commercially produced spawn are inoculated on to the compost bed. After spawnning the bed is covered with neat, clean and covered with wet newspaper. It is important that news papers should be always wet. A good white cottony growth is observed on the compost surface below the newspaper after 10– 15 days. The newspaper is removed after 20 days and now the trays are subjected to 'casing'.

Casing denotes the covering of beds with soil. Casing soil can be prepared with three parts ordinary soil and one port sand. It is sterilized before casing. The casing soil is laid over the beds up to 2-2.5cm height. Cased trays are watered by spraying. Casing of beds is essential as it induces the formation of fruiting bodies.

It takes about a month for the fruiting bodies to appear on beds. Temperature less than 15^0C favour fruiting body production, so the temperature in the production hall should be maintained blow 15^0C.after the fruiting bodies start appearing on beds.

9.3.2.5. After care of the beds:

Humidity is maintained about 70-80% Watering twice a day with a very fine sprayer is required. Insecticides (lintaff) and fungicides (dithane Z-78) may be sprayed after 10 days so as to avoid insect and other pathogens.

9.3.2.6. Harvesting and Marketing:

Mushrooms are harvested at button stage (just before opening) by twisting them so that no part remains in compost Plucked mushrooms are dried, packed and finally marketed. One can get a yield of about 3 - 4 kg trays.

9.3.3. CULTIVATION OF PADDY STRAW MUSHROOMS:

Volvariella volvacea is the paddy straw mushroom, widely cultivated in India and other South East Asian Countries. It is a tropical fungus and can be grown in summer also. Important steps involved in cultivation are choice of substratum, bed preparation, cropping, after care of beds, harvesting and marketing.

9.3.3.1. Choice of substratum: Paddy straw is considered to be the best substratum, but straw of other cereal crops can also be used.

9.3.3.2. Bed preparation and cropping:

Hand harvested, 3-4 ft long, well dried and disease free, paddy straw is collected and it is made into bundles of 1 to 1½ kg. in weight. About 35 bundles of straw are required for one bed. Before preparing the bed, the bundles are soaked in water for 8 to 16 hrs, taken out of water, washed with fresh water and allowed to drain off excess water.

Now bed is prepared by putting 4 layers of paddy straw bundles one over the other, each layer contains 8 bundles. The spawn bottle opened and raked with a glass rod. The spawn is now sprinkled by hand all over on the margin of each bed about 10 cm away from the edge and central portion is left for spawning in the first layer. The sprinkled spawn is covered with gram powder. The second, third and fourth layers are also prepared in the same way as in the 1st layer. On the fourth and top layer spawn is sprinkled on the entire surface instead of at periphery only. This layer should also be covered with gram powder. After that a thin layer of straw up to 8 cm thickness is placed on the top.

Order podaxales: A single-family podaxaceae and a single genus *Podaxis* are recognized in this order. The fruit bodies of *Podaxis* are quite common in grasslands. They maybe up to 25 cm tall and 5cm wide. It appears like unopened *Coprinus*. Hymenium is pileate and spores are dark brown. The peridium completely covers the basidiocarp and may rupture at maturity.

Order Phallales: The members of the order phallales are commonly called stinkhorns because of the fetid odour that accompanies the exposure of gleba. The fruit bodies are simple, hollow column like receptacle which bears gleba on its outer surface near the top. Some are hypogeal and others mainly the members of phallaceae and clathraceae are epigeal.

The young fruit body is whitish, eggs shaped, and of hen's egg size. The development of basidia and basidiospores occurs within the eggshell. At maturity the egg hatch. The pressure caused by enlargement of internal structures break the peridium and a long spongy receptacle emerges carrying the gleba on the surface at the tip. The egg shell (peridium) remains as a volva. After exposure gleba undergoes autodigestion and spore mass enmeshed in a foul smelling gelatinous greenish matrix. Flies are attracted to fruit body by the smell. Spores cling to the body and mouthparts and are disseminated.

There are 6 families in the order. The members of two families viz. phallaceae and clathraceae are epigeal and produce conspicuous fruit bodies. The members of other four families viz. protophallaceae, clastulaceae, hysterangiaceae, and gelopellidaceae are hypogeal and inconspicuous.

Order Hymenogastrales: The members are intermediate in structure between hymenomycetes and Gasteromycetes. Most of them are hypogeal and are often called " false truffles". Some are mycorrhizal. They are often described as 'secotioid fungi' – the agarics in which cap failed to expand. Three families are recognized – 1. Hymenogastreaceae 2. Gastrosporiaceae and 3. Protogastraceae.

Order Lycoperdales: Puffballs and earthstars belong to this group. The giant puff ball (*Clavatia gigantea*) may be up to 70 – 80 cm in diameter. The puffballs are sessile and liberate spores from an apical pore when pressure is applied.

The peridium of the fruit bodies consists of two layers, the outer exoperidium and inner endoperidium. In puffballs a pore is present at the top, and spores are released in a puff like smoky cloud, and hence they are called puffballs. In fungi called earthstars, exoperidium splits radially

exposing intact smooth endoperidium. The entire structure appears like a star on earth, and hence the name earth star. In this also, spores are released like puff.

The order comprises 4 families. The common puffballs are placed in the family Lycoperdaceae, and earthstars in the family Geastaceae. The other two families are Arachniaceae and Mesophelliaceae. These families mainly include hypogeal genera.

Order Gauteriales: This is a small group of hypogeal, mycorrhizal fungi and only single family Gautieriaceae is recognized.

Orders without hymenium

Order Melanogastrales: These are hypogeal fungi, resemble hymenogastrales but hymenium is not formed at any stage. Two families viz. Melanogastraceae and Torrendiaceae are recognized in the order.

Order Tulostomatales: The fruit bodies of these fungi appear like puff balls, but unlike puff balls which are sessile, these fungi produce fruit bodies on prominent stalks. Hence these are called stalked puffballs.

Two families are recognized in the order 1. Tulostomataceae and 2. Calostomataceae.

Order Sclerodermatales: They resemble puffballs, but the peridium is very hard. Hence, these are called stony puffballs. In this order, two families are recognized 1. Sclerodermataceae and 2. Astraceae.

In this group, *Pisolithus tinctorius* is a very important ectomycorrihizal fungus extensively used in afforestation programme to inoculate young seedlings.

Order Nidulariales: The members of this order are called bird's nest fungi. The fruit bodies are inverted bell shaped and contain peridioles which are dark globose structures that contain spore mass. The hollow fruit body with its peridioles appears like a miniature bird's nest with eggs. Hence the name.

Two families 1. Nidulariaceae and 2. Sphaerobolaceae are recognized.

8.5. CLASS TELIOMYCETES:

This class includes the plant pathogenic fungi commonly known as rusts and smuts. They do not form basidiocarps. The septal pore of mycelium is simple and clamp connections are absent. They are characterized by production of thick walled, binucleate, resting spores called

due to consumption of ergot sclerotia. Inhalation of airborne fungal spores cause allergy in sensitive people. Mycotoxins are toxins produced by fungi in food materials and it is also a health hazard. Fungi growing on different substrates cause spoilage and deteriorations. Spoilage of food and food products, spoilage of cellulosic products like textiles, paper etc. rotting of wood, staining of wood, deterioration of fuels, optical instruments etc. are all harmful activities of fungi.

Mushrooms are fruit bodies belonging to the order Agaricales of basidiomycotina. Most of them are edible. Mushrooms are preferred for their delicacy and nutritive value and mushroom consumption is on the increase. To meet the growing demand of edible, mushrooms some are cultivated on commercial scale. The most important cultivated mushroom are button mushroom (*Agaricus brunnescens*), paddy straw mushroom (*Volvariella volvacea*), Oyster mushroom (*Pleurotus species*) etc. For cultivation of mushroom the important steps are: obtaining pure culture or mother culture of the mushroom; spawn production; substrate selection, substrate preparation, spawning, spawn running, cropping and harvesting. The steps are explained for cultivation of button mushroom, paddy st v mushroom and Oyster mushroom.

9.5. MODEL QUESTIONS

Essay type questions

1. Discuss the economic importance of fungi
2. Give an account of beneficial and harmful activities of fungi.
3. Write an essay on mushroom cultivation.
4. Discuss the methods of cultivation of button mushroom and Paddy straw mushroom.

Short answer type questions

5. Industrial uses of fungi.
6. Agricultural importance of fungi.
7. Fungi as food and use of fungi in fermented foods.
8. Harmful activities of fungi.
9. Cultivation of paddy straw mushroom
10. Cultivation of oyster mushroom

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Unit-III

Lesson 10

PLANT DISEASES

10.0 OBJECTIVE:

The concept of plant disease, symptoms produced by plant pathogenic microorganisms and classification of plant diseases are discussed.

10.1 CONCEPT OF DISEASE IN PLANTS

10.2 DEFINITION OF PLANT DISEASE

10.3 SYMPTOMS CAUSED BY PLANT PATHOGENIC FUNGI, BACTERIA AND VIRUSES

10.4 CLASSIFICATION OF PLANT DISEASES

10.5 SUMMARY

10.6 MODEL QUESTIONS

10.7 REFERENCE BOOKS

10.1 CONCEPT OF DISEASE IN PLANTS

Since plants are so important to man's survival and enjoyment of life, anything which is detrimental to plants directly affects man. One of the most widespread destroyers of plants are the plant diseases. Man has been aware of plant diseases for many centuries. The Bible contains many references to mildews and blights and the damages caused by these diseases.

An example of the tremendous impact that a plant disease can have on the course of human history is given by late blight of potatoes, caused by the fungus *Phytophthora infestans*. Due to this potato famine, one million Irish died of starvation and 1½ million emigrated to North America. Thus, a single plant disease played a major role in shaping the destiny of nations in several different ways. The beginnings of modern plant pathology were also a direct outcome of this disease.

To outline the basic concepts and principles of plant pathology, the meaning of certain terms should be discussed. The word pathology is derived from two Greek words – **Pathos** meaning "suffering" and **logos** meaning "speech". Thus plant pathology is concerned with the study of the suffering of plants.

The plant pathologist is concerned both with the **Science** of plant pathology, such as understanding of the nature of a plant disease, and also the **art** of plant pathology, such as diagnosis, treatment or control of plant diseases. In many instances, it is difficult to separate the science and the art of plant pathology, since development of control methods is often closely tied to an understanding of disease processes.

10.2 DEFINITION OF PLANT DISEASE

Various definitions have been proposed for a diseased or suffering plant. J.C. Walker states that "diseased plants are distinguished by changes in their structure or physiological processes which are brought about by unfavourable environment or by one or several parasitic agencies. There is no clearly defined line of distinction between normal or healthy plants and abnormal or diseased plants. Plant disease, therefore, is like many biological phenomena, difficult to define. We may, however, think of diseased plants as those which have become altered in their physiological and their morphological development to such a degree that signs of such effects are obvious."

E.C. Stakman and J.G. Harrar have provided a more inclusive definition: "The term plant disease is properly applied to any deviation from normal growth or structure of plants that is sufficiently pronounced and permanent to produce visible symptoms or to impair quality and economic value".

Disease, therefore, involves a malfunctioning of various plant processes and may occur while the plant is growing or while it is in a dormant condition. The term **pathogen** may be defined as "anything or entity that causes or incites a plant disease". Several other terms are of importance in understanding the concept of plant disease. **Pathogenesis** is the complete process of

causing a disease, a series of events from infection of the plant to development of the pathogen within the plant and the subsequent production of disease symptoms.

Pathogenicity is the ability of a pathogen to generate or cause a disease. **Symptoms** refer to the visible effect that the pathogen may have on the plant, such as yellowing of leaves. All these terms are summarized in Fig. 10.1. Symptoms may indicate the existence of a disease, but they are not the disease themselves. Plant pathologists use them as a guide in diagnosing the nature or cause of a particular disease.

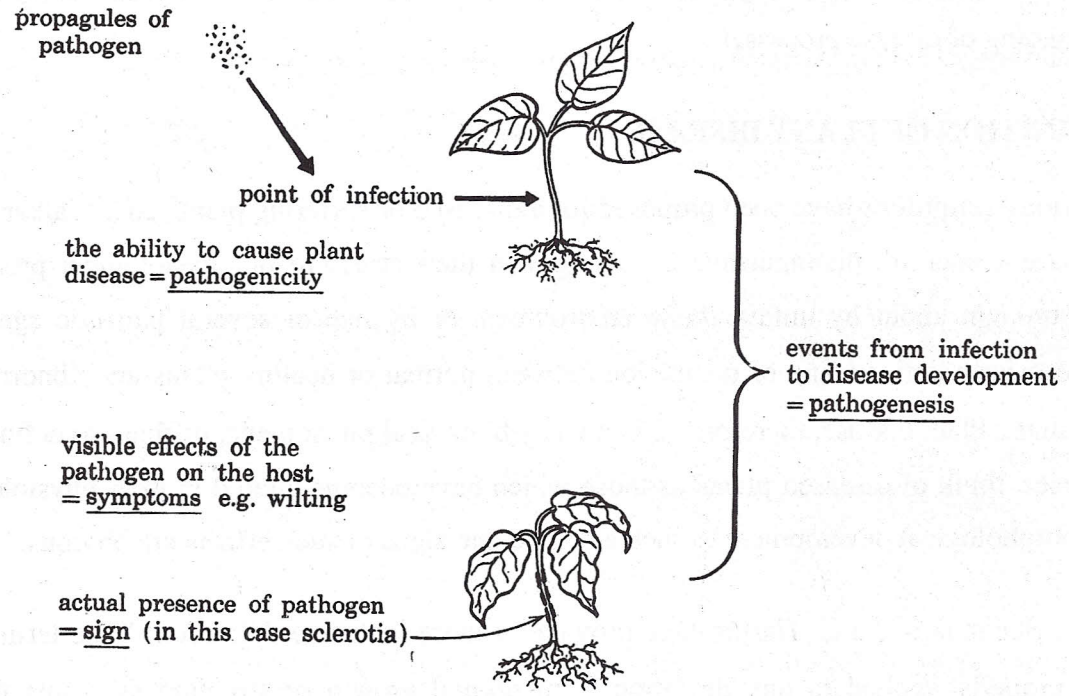


Fig. 10.1 The Development of a plant disease

Whether in any one given situation, a plant disease will develop is dependent up on three things. First of all a susceptible host is required. Next, a virulent pathogen is required. Finally, a favourable environment of sufficient duration must be present to allow infection by the pathogen and subsequent development of the disease.

The causes of plant diseases can be divided into two main groups: those that are non-infectious in nature, and those that are biological or infectious in nature. The two broad categories of the non-infectious pathogens include the chemical agents and the physical agents. Chemical agents causing plant diseases include excess or deficiencies of plant nutrients, toxic or misused pesticides etc.

Physical agents causing plant diseases include such varied things as unfavourable temperatures for proper growth of plants, excess or deficient moisture conditions and harmful aspects of radiation from the sun such as excessive ultraviolet light.

The infectious pathogens are also of varied nature and include the viruses, bacteria, fungi, algae, higher plants such as mistletoes, and animals ranging from the nematodes to insects, on up to higher animals including man himself.

Many organisms are associated with diseased plants. Therefore, one of the tasks the plant pathologist faces is to determine which of the associated organisms is the actual cause of the disease. To do this, plant pathologists have borrowed a procedure from scientists studying animal diseases. Robert Koch first showed that anthrax bacillus actually caused the anthrax disease in animals by using a procedure since called **Koch's Postulates**:

1. Constant association of the organism with the diseased host.
2. Isolation of the suspected pathogen from the diseased host and establishment of the pathogen in pure culture.
3. Inoculation of a disease-free host with the pure culture of the suspected pathogen with the resulting reproduction of the disease.
4. Reisolation in pure culture of the pathogen from the inoculated and diseased host.

Although not all infectious pathogens of plants will grow in culture, many microorganisms have been shown to be the causal agents of plant disease by use of Koch's postulates.

10.3 SYMPTOMS CAUSED BY PLANT PATHOGENIC FUNGI, BACTERIA AND VIRUSES

The two visible aspects of disease diagnosis are **symptoms** and **signs**. Symptoms are the outward expressions of a plant that indicate that it is suffering. Signs on the other hand, are the actual presence of a pathogen such as mycelium, sclerotia or bacterial ooze. Symptoms of plant diseased take many forms. These enormous variety of symptoms basically belong to any of the three main categories: (1) Necrosis, (2) Hypertrophy and Hyperplasia, and (3) Hypoplasia.

Necrosis: One of the major categories of plant disease symptoms is necrosis or cell death. Tissue that is composed of dead cells is said to be necrotic. Necrosis can involve one or a few cells or the entire plant. When necrosis of cells is confined to a fairly small area or to a distinct area, it is called localized necrosis. Symptoms of this type can be found on any part of the plant.

Of localized necrotic areas become numerous enough, they eventually coalesce, and entire organs or plants become necrotic. Again, all organs and tissues of a plant can be affected, and such symptoms can be produced by a variety of pathogens. Necrosis is caused by fungi, bacteria and viruses. The symptoms which are of necrotic nature are as follows:

Leaf spots: The cells are killed in a limited area to form lesion or spot. The spots are of various sizes and shapes (Fig. 10.2). On leaves, necrotic areas or spots that develop as a result of bacterial infection often first appear as small water-soaked areas. These are often confined by vascular tissue, thus giving the spot an angular appearance. Later the cells die, resulting in a dark brown or black lesion or spot.

Localized spots on leaves are also a characteristic symptom of many virus diseases, particularly those of stone fruit trees caused by the various ring spot viruses. These necrotic areas may eventually drop out of the leaf, thus giving it a "shot-hole" appearance. Fungi produce similar symptoms on leaves ranging from small necrotic spots to broad oval necrotic areas. Tar spots are characteristic type of leaf spots which appear as raised, black coated fungus bodies giving the appearance of a flat drop of tar on leaves as in *Phyllachora.*

Streaks and Stripes: Minute linear lesions may appear on the leaf blade, leaf, sheath, stem and other plant parts. Such streaks may be limited in number and size. But in some diseases they enlarge and spread to form stripes of variable length and breadth. Streaks and stripes may be yellow, brown or black. In advanced cases, they may turn necrotic to cause shredding of the tissues.

Blight and Blast: They refer to diseases that kill the affected organs suddenly. Due to severity of infection, the tissues are rapidly killed, resulting in the death of foliage, blossom or other above-ground plant parts. Such a symptom is called blight. When the entire leaf blade, bud or other plant parts are involved resulting in quick death of the part or plant as a whole, then it is known as blast.

Damping-off: Seeds of high germinative capacity are not able to develop into seedlings due to pre-emergence damping-off. In post-emergence damping-off, the stem is attacked near the ground level. The affected portion becomes constricted and weak and finally disintegrates causing the seedling to topple down and die. *Pythium* and *Phytophthora* often cause damping-off of nursery seedlings and young plants.

Pots: The affected tissues disintegrate leading to rot. Depending upon its characteristics, it may be classified as soft rot, dry rot, hard rot, heart rot, white rot, black rot and pink rot. Succulent fruits and vegetables are often spoiled by fungal and bacterial infections that produce soft rot. In advanced stages of certain fruit rots, the fruits dry and form a dark, wrinkled, hard mass called mummification.

Cankers: These are the necrotic lesions in the cortical tissues of stem, fruits or leaves. Corky growths often develop in the affected part. Cankers mainly cause localized death of the tissues but in severe infections they may girdle the stem and kill the twig. These are mainly due to fungal or bacterial infections, but sometimes due to virus infections also.

Anthracnose: The symptoms appear as circular to angular, sometimes irregular spots occurring along the leaf veins, petioles, stems and fruits. The affected tissues are discolored and killed, resulting in characteristic lesions.

Burn, scald or scorch: In succulent organs like fruits, limited areas die and turn brown.

Die-back: It results from the dying of twigs or branches from the tip downward, giving them a burnt appearance.

Wilting: It is a flaccid or drooping condition of the plant caused by many non-parasitic and parasitic diseases. Plant wilt often results from vascular infections of the basal stem or the root system.

Scab: This is a rough, crust – like lesion commonly found in fruits and vegetables.

Rust pustules: These are the characteristic symptoms of infection of obligate parasites. The pustules may be erumpent or submerged, large or minute, circular or linear or irregular. They occur on all parts, but more frequently on the leaf blade. Several rust pustules sometimes coalesce to cover large areas of the leaf blade and stem, sometimes deforming the affected plant parts. The color of the pustules may also vary from brown, red, yellow to dark. These are most commonly caused by basidiomycetous fungi. In addition to them, white rusts are caused by *Albugo* spp. of the oomycetous fungi, and the red rust caused by the green alga *Cephaleuros*.

Smuts: The word smut means a sooty or charcoal-like powder. The symptoms usually appear on floral organs. Smut affected parts of the plant show a black or purplish-black dusty mass.

Mildews: The pathogen is seen as white, grey, brownish or purplish growth on the host surface. In *Downy mildews* the superficial growth is a tangled cottony or downy growth while in *powdery mildews*, the host surface becomes dusty or powdery, due to the production of enormous number of spores by the pathogen.

Hypertrophy and Hyperplasia

Some pathogens induce excessive growth of host tissues, which results in abnormal increase in size of affected organs. It is brought about by one or both of the two processes called hypertrophy and hyperplasia. In hypertrophy, abnormal increase in size of individual cells of

affected tissue occurs. Hyperplasia is the abnormal increase in the size of a plant organ due to increase in number of cells of which the organ is composed. Club-like swelling of infected roots is the characteristic symptom of club-root of crucifers.

Galls or tumors: These are globose, elongated or irregular outgrowths formed on affected plant parts. The commonly observed galls on the ears of corn are caused by the smut fungus *Ustilago maydis*. Galls may also be caused by bacterial pathogens and may occur on root or stems (crown gall caused by *Agrobacterium tumefaciens*).

Curls: Increased growth on one surface of a leaf results in leaf curling as in peach leaf curl caused by *Taphrina deformans* or in curly top of sugarbeets.

Elongation of Internodes: Rice plants infected with the fungus *Fusarium moniliforme* are much taller than uninfected plants. The increased height is due to elongated internodes.

Phyllody: These are the symptoms of abnormal development expressed in floral parts. It means an abnormal growth or proliferation of tissue, usually leaf tissue, in the flower. It results from infection by several viruses or by such fungi as the smut fungus *Sphacelotheca reiliana*.

Witches' broom: This symptom is produced by some fungi, bacteria and certain viruses. The broom appears as an upright cluster of small shoots. *Taphrina cerasi* on cherry, stimulate shoot production by the host resulting in a dense cluster of twigs called a witches, broom, which persists for many years.

Hypoplasia: These symptoms reflect a reduction in size of plant parts. **Variegation**, a pattern of white patches in leaves and other plant parts results from the failure of chlorophyll development in certain cells. **Chlorosis** is due to partial failure of chlorophyll development in leaves causing more or less uniform discoloration of the tissues. Another colour variation symptom is **mosaic** which results from an uneven development of chlorophyll that produces alternate light green patches with dark green areas. Such symptoms are often associated with virus infections. **Vein-clearing**, a translucent appearance of the veins and **vein-banding** in which dark green bands bound the veins are also common in virus infected leaves.

Stunting of the plant can result from attack by various plant pathogens. The viruses are particularly effective in causing this symptom, as illustrated by the barley yellow dwarf disease. Reduction in leaf size is also a common symptom of virus infection and in some cases, the leaves may be much reduced in size giving **shoe-string** appearance. In **Little leaf** disease, the leaves become very small and their petiole size is so much reduced that they appear as sticking to the stem. This disease is caused by phytoplasma.

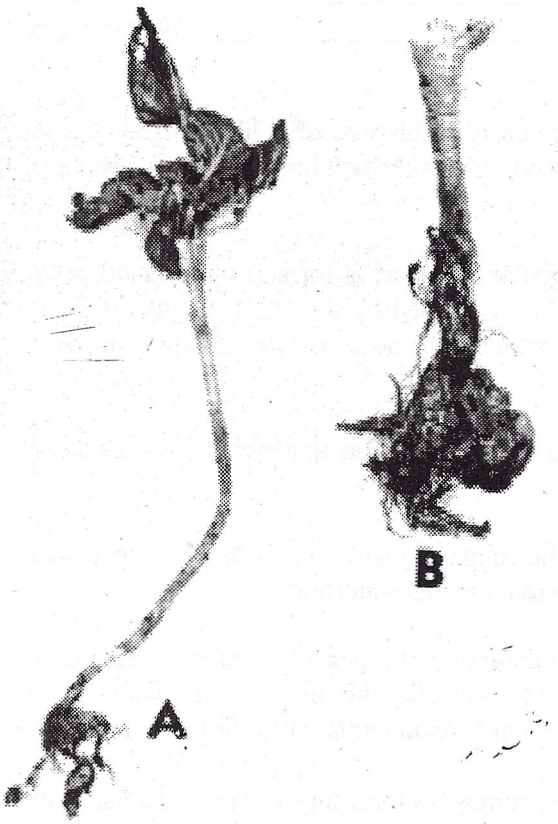


FIGURE Club-root of cabbage. (a) 'clubs' on a young transplant, (b) much malformed root of an old plant.

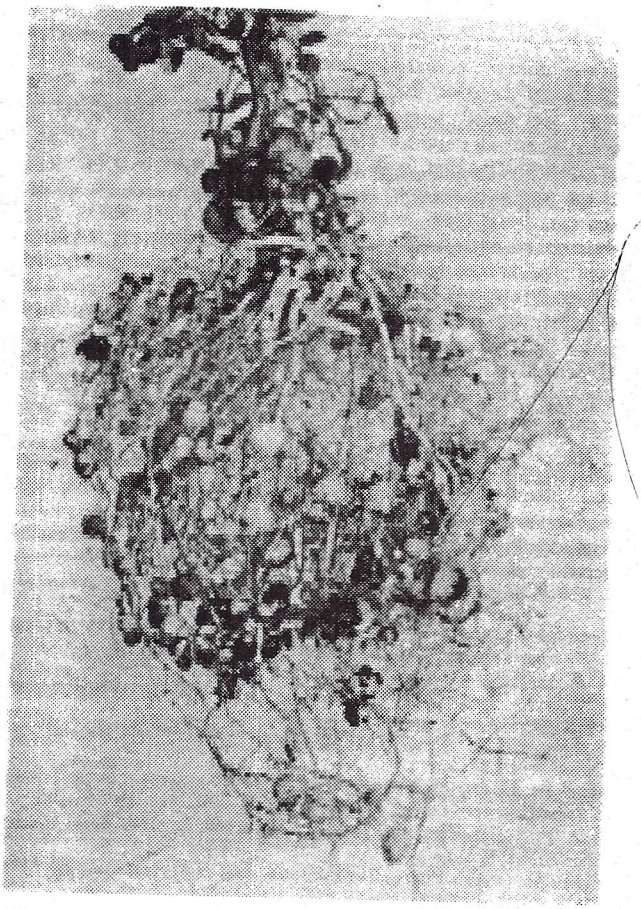


FIGURE Galls induced by wound-tumour virus on the roots of sweet clover.

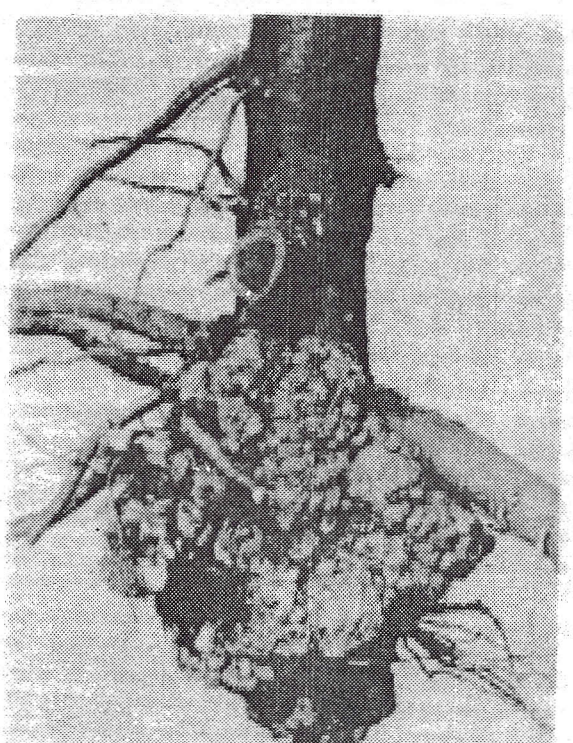


FIGURE Crown gall on an apple rootstock.

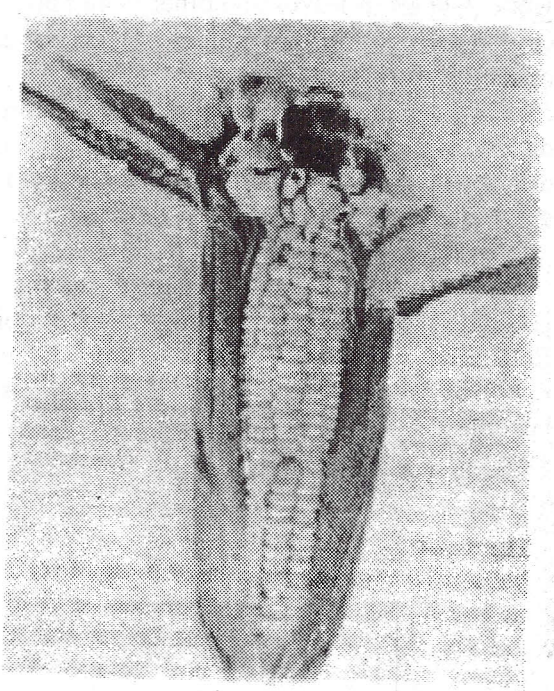


FIGURE Maize smut. Only the top grains of the cob have been infected by *Ustilago maydis*.

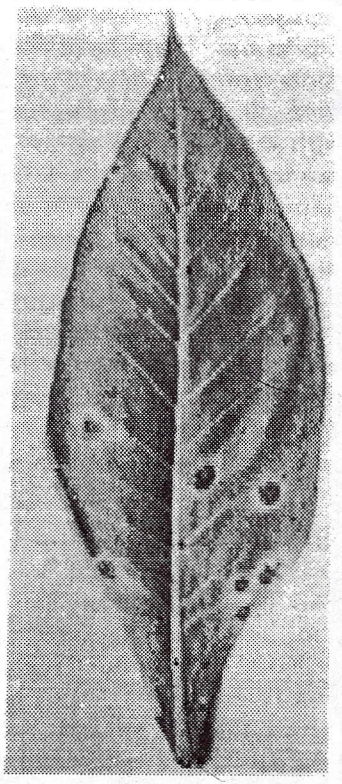
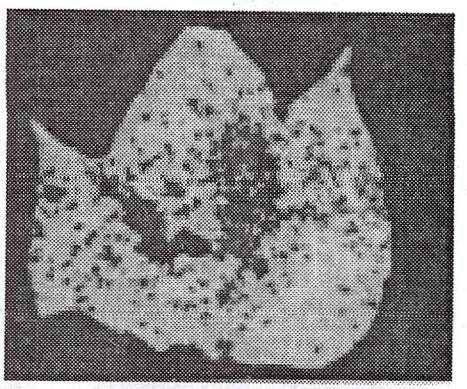


FIGURE *Alternaria* leaf spot of tobacco, showing the necrotic lesions and surrounding chlorotic zones.

FIGURE *Helminthosporium* leaf spots of maize



Angular leaf spot of cotton

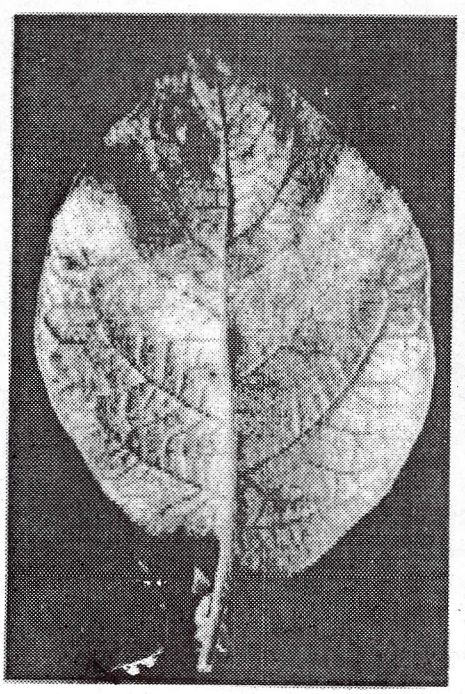
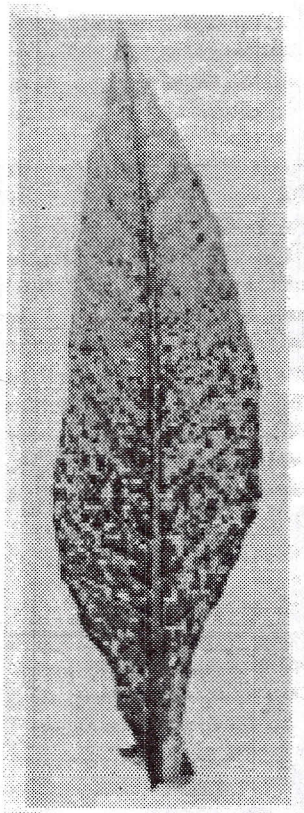
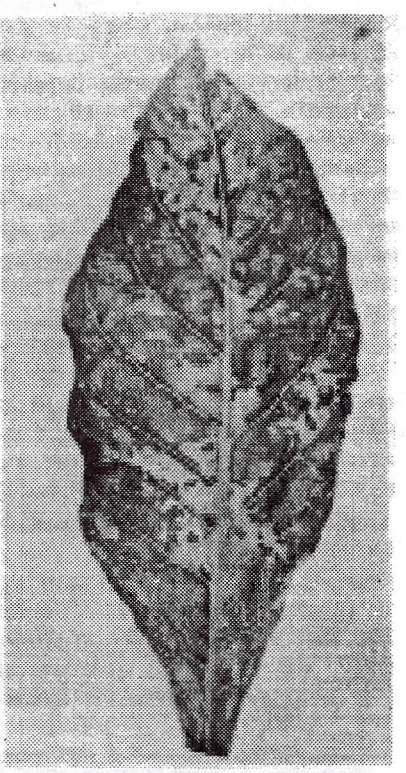


FIGURE Late blight of potatoes. The under surface of an infected leaf with *Phytophthora infestans* sporulating at the edge of the lesion.



(a)



(b)

FIGURE Tobacco mosaic.

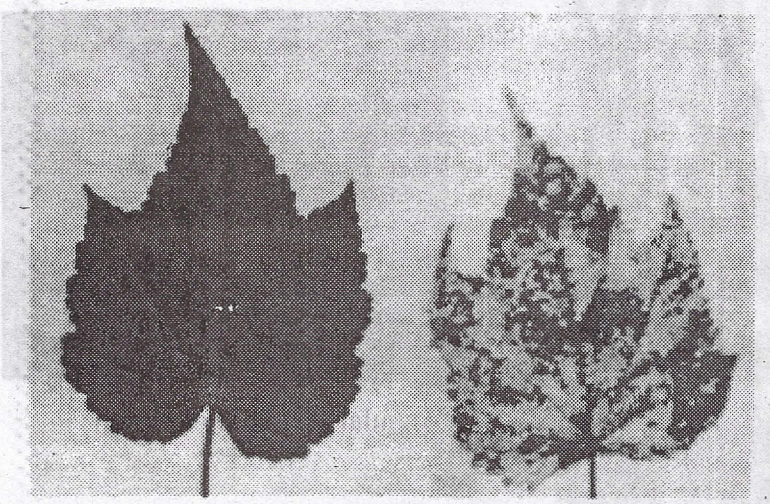


FIGURE Abutilon variegation. The leaf on the right came from a plant with the virus, the one on the left from a non-infected plant.

FIGURE Tobacco leaf curl.

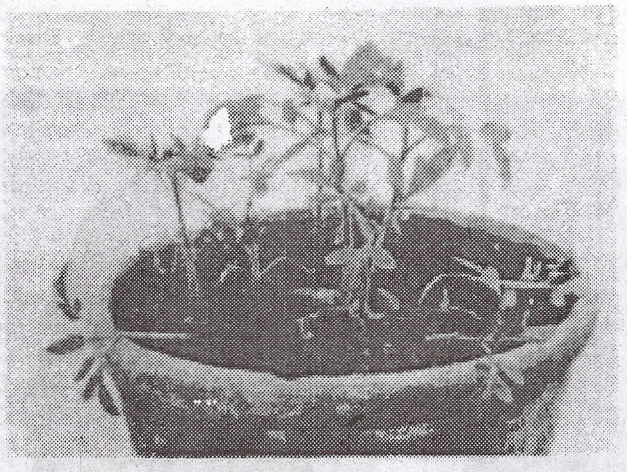
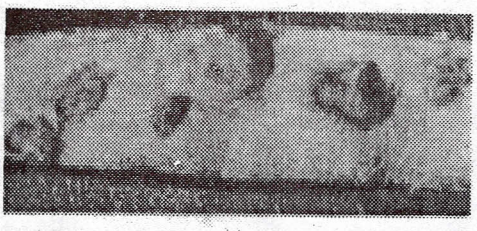


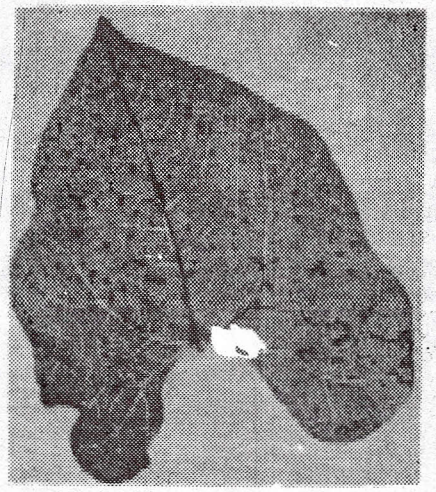
FIGURE Post-emergence damping-off of tomato seedlings.

FIGURE Downy mildew of vine. Groups of sporangia phores on the lower leaf surface.

FIGURE Powdery mildew of cucumber. The leaves on the left are completely covered with mycelium and conidiophores.

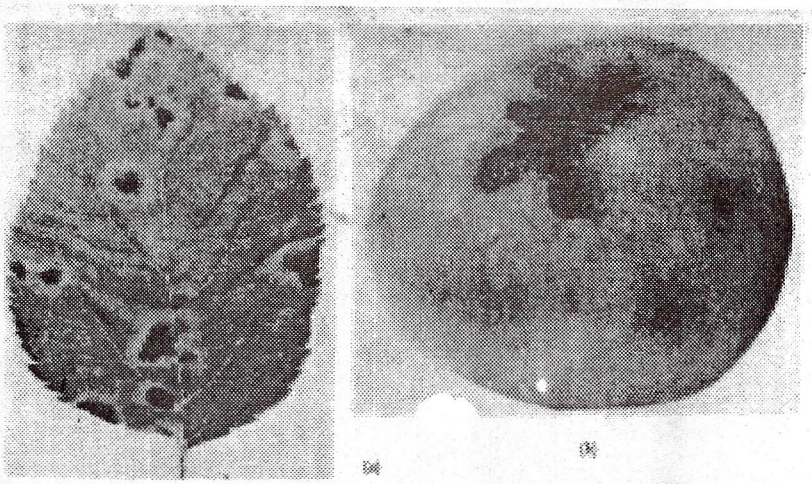


(a)



(b)

FIGURE Bean anthracnose. (a) on the pods. (b) on the lower leaf surface.



(a) (b)

FIGURE Apple scab. Lesions on the leaf (a) and fruit (b).

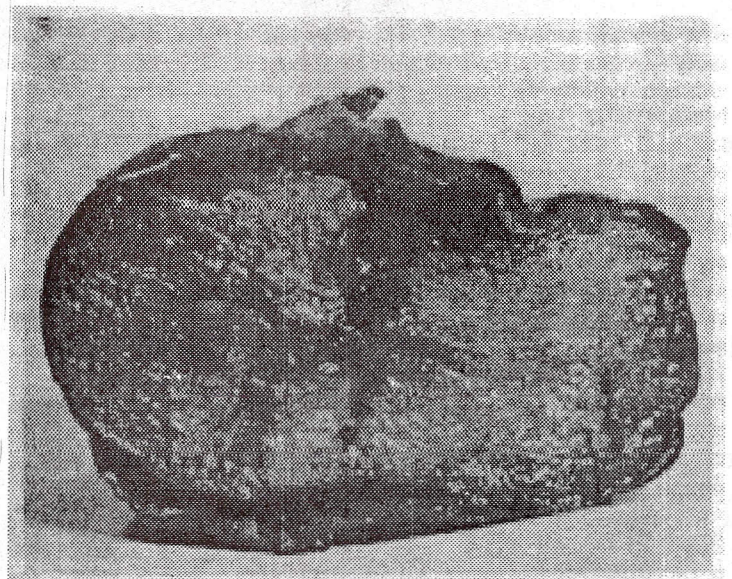


FIGURE Soft-rot of potato. The mass of cells is held together only by the skin, and this can be readily detached, as is seen at the base of this tuber.

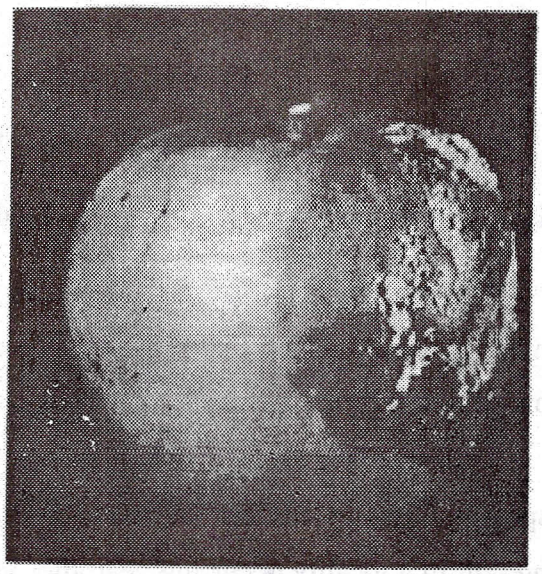


FIGURE Brown rot of apple caused by *Sclerotinia fructigena*. The conidial cushions are clearly visible on the rotted tissue.

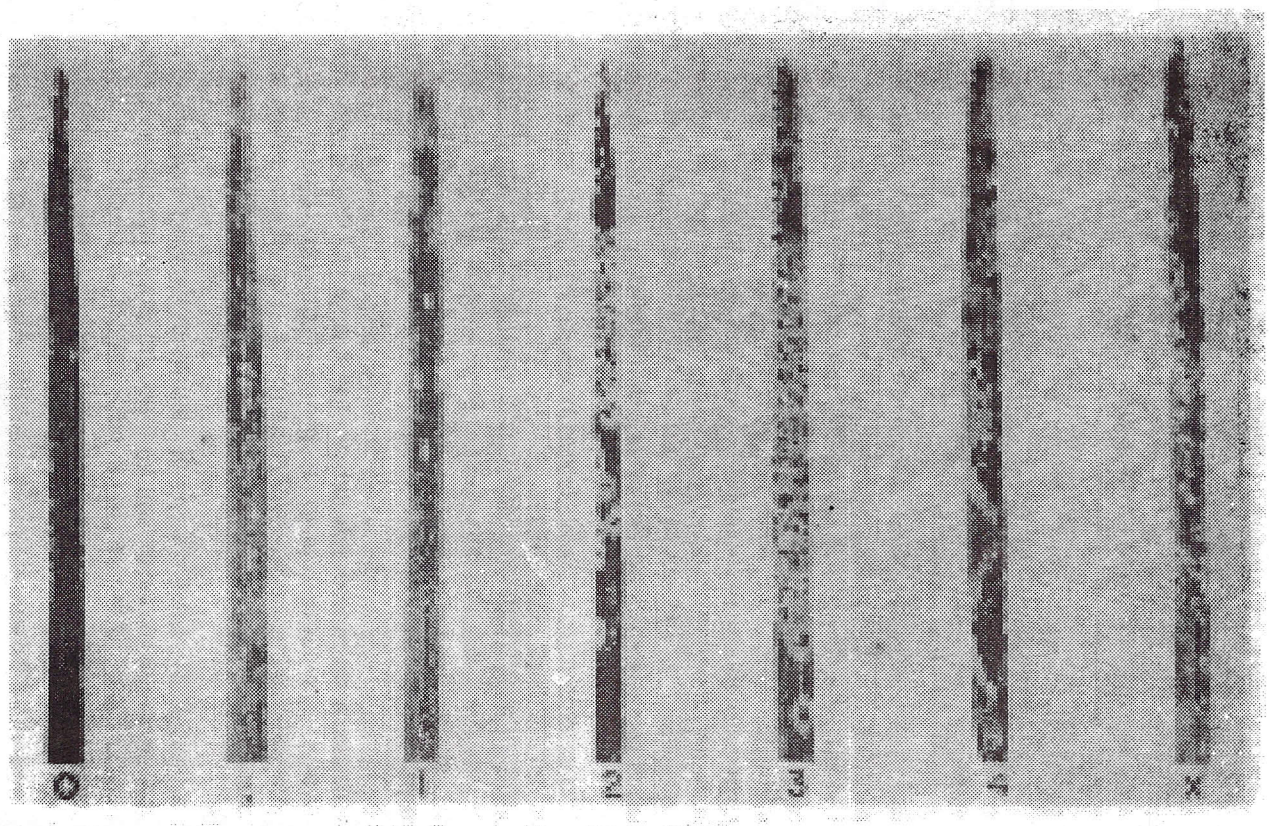


Fig. 10.2 Some of the symptoms of plant diseases

10.4 CLASSIFICATION OF PLANT DISEASES

Plant diseases may be grouped in various ways. Basing on the host plants affected, they may be classified as cereal diseases, vegetable diseases, root crop diseases forage diseases and so on.

Depending on the mode of spread, diseases may be classified into epiphytotic, endemic and sporadic diseases and also as seed-borne, soil-borne and air-borne. When a disease is constantly present in a locality occurring year after year it is called **endemic**. An **epiphytotic** disease occurs periodically in a widespread area causing devastating damage to crop plants. If the disease occurs at irregular intervals over limited areas in a tract it is said to be **sporadic**.

The diseases may also be classified based on the plant parts affected as foliar diseases, root diseases, fruit diseases, stem diseases and so on. Based on the symptoms produced on host plants, the diseases can be classified into necrotic, hypoplastic (atrophic) and hypertrophic diseases.

Basing on the causal agents, the diseases may be classified into non-parasitic diseases and parasitic diseases. Non-parasitic diseases are incited primarily by:

(a) Soil conditions such as: (i) soil moisture imbalances, (ii) unfavourable physical structures, (iii) nutritional imbalances etc.

(b) Meterologic conditions such as: (i) light deficiency, (ii) low relative humidity, (iii) unfavourable atmospheric structures etc.

(c) Defective agricultural practices.

(d) Industrial and other chemical contaminants of the atmosphere.

Parasitic diseases are incited by

(a) Members of animal kingdom: (i) insects, (ii) nematodes, (iii) mites;

(b) Phanerogamous parasites

(c) Microorganisms including slime moulds, fungi, bacteria, algae etc.

(d) Viruses and mycoplasmas.

Some diseases are caused by more than one causal agent. They are called disease complexes. Examples of such complexes include nematode-virus, nematode-fungus, fungus-virus-nematode, fungus-bacterium-nematode etc.

10.5 SUMMARY

Diseased plants are those whose physiological functions have been altered to the extent that symptoms are evident. Plant diseases develop when a virulent pathogen attacks a susceptible host within a favourable environment. The outward expressions of plant diseases are known as symptoms. They are a manifestation of the disruption of various plant functions. Plant disease

may be classified basing on – the host plants affected, the plant parts affected, the mode of spread of the pathogen, the symptoms produced by the pathogen, the causal agents etc.

10.6 MODEL QUESTIONS

1. Write in detail about the symptoms produced by plant pathogens.
2. Write short notes on:
   a) Concept of Plant disease
   b) Classification of plant diseases

10.7 REFERENCE BOOKS

1. **Introduction to Principles of Plant Pathology** – R.S. Singh, Oxford & IBH Publ. Co. Pvt. Ltd., New Delhi, 1999.

2. **Plant Pathology** – P.D. Sharma, Rastogi Publications, Meerut, 2001.

3. **Diseases of Crop Plants in India** – G. Rangaswamy and A. Mahadevan, Prentice Hall of India Pvt. Ltd., New Delhi, 1999.

4. **Plant Diseases** – R.S. Singh, Oxford & IBH Publications Pvt. Ltd., New Delhi, 1993.

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■ **Dr. M. VIJAYALAKSHMI**

Unit-III

Lesson 11

ENTRY AND ESTABLISHMENT OF PATHOGENS AND DISPERSAL OF PLANT PATHOGENS

11.0 OBJECTIVE

The various ways by which pathogens enter into the host plants are discussed. Methods of dispersal of inoculum are also explained.

11.1 INTRODUCTION
11.2 PENETRATION BY VIRUSES
11.3 PENETRATION BY BACTERIA
11.4 PENETRATION BY FUNGI
11.5 DISPERSAL OF PLANT PATHOGENS
11.5.1 Wind
11.5.2 Water
11.5.3 Insects
11.5.4 Fungi
11.5.5 Nematodes
11.5.6 Birds
11.5.7 Mammals
11.5.8 Man
11.6 SUMMARY
11.7 MODEL QUESTIONS
11.8 REFERENCE BOOKS

11.1 INTRODUCTION

Parasitic plant pathogens must gain entrance into the host plant to cause disease. Plant pathogens enter plants through wounds, natural openings, or directly through the cell wall. In the

case of many fungi, the process of gaining entry begins when the germinating spore produces an infection peg. For other pathogens such as the viruses and bacteria, the entire process of gaining entrance is usually passive. The process by which a pathogen enters a plant and establishes a parasitic relationship between it self and the host is called **infection**.

In order to gain entry into plants, the pathogens have to overcome several barriers in the plants. For leaves and young stems with an intact epidermis, a cuticular layer covers most of the outer portion of the epidermal cell. Beneath the cuticule lies the primary cell wall composed mainly of cellulose. A secondary wall composed mainly of lignin occurs inside the primary cell wall in some plants.

In woody stems and roots where the true epidermis has been sloughed off, a layer of cork cells forms the outer covering. Such cells are heavily impregnated with the fatty substances cutin and suberin. The epidermis of young roots usually lacks cuticle but does bear root hairs. The wall of the root hair is merely a lateral extension of the wall of the cell from which it originates. This wall is very thin and is composed of an inner cellulose layer covered on the outside by pectin.

It might appear that the surface of the plant is a tightly closed layer of various materials and cells. When viewed microscopically, many tiny breaks are found in the cuticular layer which may be caused by wind movements, abrasion with dust and sand particles. The other openings commonly called natural openings include the stoma, lenticels and hydathodes.

11.2 PENETRATION BY VIRUSES

A virus particle is by itself unable to move, its entry into a plant cell is completely dependent on some other agency. Viruses often enter plant cells and/or tissue only through wounds. A break in the cell wall and in the plasmalemma must usually occur to allow the virus particle to come into contact with the host cell cytoplasm. An exception may be the pollen-borne viruses. The wounds may be caused by mechanical handling or by organisms such as insects, fungi or nematodes.

Mechanical Wounding: On leaves of some plants, such as tomatoes, relatively large trichomes or leaf hairs are found. These are easily broken off during handling. If a person handling such plants has virus particles on his hands, infection can be initiated when these virus particles are brought into contact with such wounds. Usually the wounding process and exposure to the virus particle occur simultaneously. TMV is spread in tomatoes or tobacco by the mechanism described above. Other types of mechanical wounds that serve as infection sites for viruses are those made during grafting operations. In fact this is one of the main ways that the viruses of trees are spread.

Wounds caused by organisms: In nature, the most common source of wounds associated with virus infection are those caused by various organisms such as insects, nematodes or fungi. The organism causing the wound almost always is carrying the virus. In most of the cases, the insect performs the inoculation as well as making the wound. However, wounds alone do not always permit virus inoculation. There is a high degree of specialization between vector-virus-host.

Some viruses are capable of infecting isolated protoplasts (cells from which the cell walls have been removed enzymatically). Electron micrographs indicate that virus particles may enter isolated protoplasts through vesicular invaginations of the protoplast surface – a process called endocytosis. Polyornithine, an agent known to damage cells and disrupt permeability, enhances viral infection of isolated protoplasts.

11.3 PENETRATION BY BACTERIA

Plant pathogenic bacteria enter plants by two means: through wounds of various types and through natural openings. Since none of the plant pathogenic bacteria are spore formers, they must quickly find a suitable site for multiplication or death may result. The requirements for multiplication are an adequate nutrient source and usually a high relative humidity to prevent desiccation.

Penetration through wounds: Wounds through which bacteria penetrate plant tissue must usually be much larger than those needed for virus entry. The wounds needed for entry of bacteria are of three main types. 1) mechanical damage to cells and tissues, 2) insect wounds and 3) natural wounds.

The wound - invading bacteria usually are those found in the soil or its close proximity. The crown gall bacterium, *Agrobacterium tumefaciens*, requires not only a wound but a host response as well for bacterial proliferation and gall development.

Several bacterial plant pathogens are carried within and on insects. *Erwinia tracheiphila* (the cause of cucumber wilt) enters the cucumber plant when its vector, the cucumber beetle, feeds on young leaves or cotyledons. The bacterium causing olive knot (*Pseudomonas savastanoi*) often enters the host through natural wounds. Scars formed by the abscission of leaves are the most common avenues for entry into twigs.

Penetration through natural openings: Many of the bacteria that attack the aerial portions of plants can gain entrance to the plant through natural openings such as stoma, lenticels and hydathodes. When the bacteria are non-motile, they must enter the stoma in a passive manner. This can occur when the bacteria-infested water film is drawn into the stoma by capillarity or as drying occurs. In some cases where there is a continuous film of water from the leaf surface through the stoma into the substomatal cavity, motile bacteria may be able to swim through the stoma.

Lenticels are naturally occurring openings of loosely arranged cells more or less lacking in suberization. They occur as breaks in the outer covering of stems, roots and some tubers. Since these structures are composed of loosely packed ruptured cells and are continuous with the cells in the periderm layers beneath, they provide an ideal site for infection by certain microorganisms such as *Streptomyces scabies* (the cause of potato scab).

Hydathodes usually occur at the tips or edges of leaves and are at the terminus of a vascular bundle. Under conditions of high relative humidity, guttation water is exuded through the hydathode. While the guttation water is exposed, it may become contaminated with bacteria. Then, as the water is retracted into the plant as conditions become drier, the bacteria are also drawn into the plant. Cabbage black rot bacterium, *Xanthomonas campestris* often enters the plant by this way.

Bacterial plant pathogens also enter plants through various flower parts, in particular the nectaries. *Erwinia amylovora* (the cause of apple fire blight) is carried to blossoms by insects or wind blown rain. Penetration takes place through the stomatal - like openings in the nectaries at the base of the flower from which nectar is secreted.

11.4 PENETRATION BY FUNGI

Fungi are able to penetrate the barriers of host plants in a variety of ways. Many fungi require a break or wound in the surface layers of the host. Others penetrate through natural openings, such as stoma, while many have developed the ability to penetrate the host surface directly. In some cases, the pathogens can penetrate by more than one means.

Penetration through wounds: Man's activities are responsible for many of the wounds through which fungal penetration occurs. Many fruit and vegetables at the time they are in their prime for marketing quite succulent. In this condition, they are most susceptible to wounding by rough handling. *Rhizopus* rot of sweet cherries and peaches develops in wounds of this type. When tomatoes are mechanically harvested they are often dropped into the harvesting wagon with such force that they crack open, thus providing an ideal site for infection by *Rhizopus stolonifer* and other fungi.

Other organisms besides man are responsible for making wounds through which fungi gain entrance to plants. The root - invading vascular pathogen, *Fusarium oxysporum* f. sp. *vasin fectum*, gains entrance to the roots of susceptible cotton plants through the injuries caused by the attack of the root knot nematode.

Wounds are an important entry point for many fungal pathogens, providing a pathway past the barriers of cuticle, epidermal cells, bark tissue, etc. In wounded areas, nutrients released from injured cells allow the pathogen to get established in a plant before live tissue is invaded.

Penetration through natural openings: The stoma of plants serve as an ideal site for penetration of important plant pathogenic fungi such as rusts and downy mildews. These fungi can also penetrate the plant directly but the main penetration route is through stoma.

The process by which fungi enter through stoma can be illustrated by the stem rust fungus *Puccinia graminis tritici*. Uredospores of this fungus will germinate in water on the leaf surface. The germ tube elongates until it reaches a stoma. Over the stoma, the top of the germ tube enlarges to form an *appressorium*. From the appressorium, a penetration tube then extends down through the stomatal opening into the substomatal cavity where it then enlarges to form the substomatal vesicle. From the substomatal vesicle, hyphae then emerge to make contact with the surrounding parenchyma cells and thus establish the parasitic relationship with the host. Some of the rust fungi, such as *Puccinia striiformis* (the cause of wheat stripe rust), do not form an appressorium over the stoma but penetrate directly through the stomatal opening (Figs. 11.1 and 11.2).

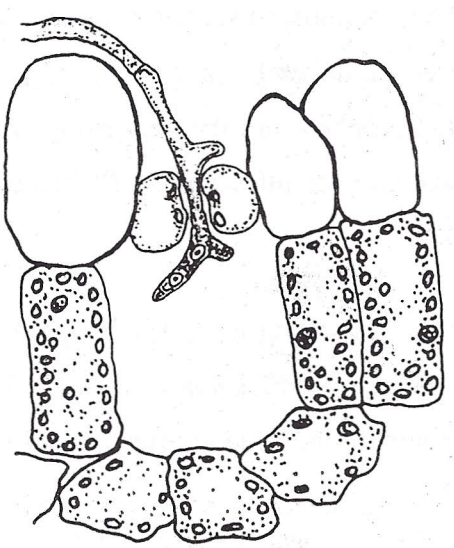

Fig. 11.1 Penetration of a stoma of wheat by *Puccinia graminis*

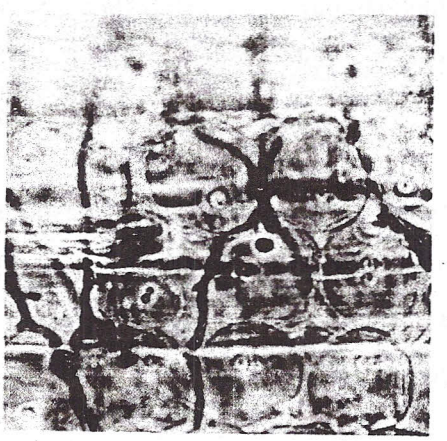

Fig. 11..2 Intercellular mycelium of *Puccinia striiformis* spreading out through a wheat leaf.

Direct Penetration: Many fungi developed the ability to penetrate directly through the uninjured host surface, both cuticle and primary cell wall. The mechanism of direct penetration has been

thought to be a strictly mechanical action. Several early workers showed that fungi such as *Botrytis cinerea* could penetrate inanimate materials such as thin gold leaf. Studies with the light microscope of the penetration process through plant tissue also revealed no evidence of a dissolving or chemical action during the penetration process.

Recently, however, evidence has been obtained that the penetration by some fungi may involve a chemical process. Several fungi such as *Penicillium* spp. produce enzymes that will break down plant cutin. Studies concerned with the penetration of onion roots by the pink root fungus *Pyrenochaeta terrestris* revealed that the onion cell wall appears to be dissolved in the area around the penetrating hyphae (Fig. 11.3). The area beneath the penetrating hypae, the host cell wall has a different staining characteristic, indicating a change in its chemical or physical structure.

P. terrestris is known to produce cellulase, so the action observed in this case may be due to the dissolving action of this enzyme on the cellulose microfibrils in the host primary cell wall.

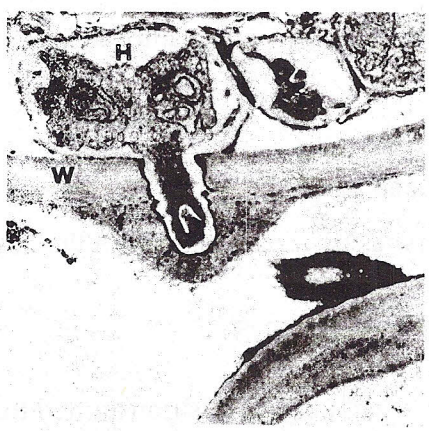

Fig. 11.3 Electron micrograph of an onion root showing penetration of the host cell wall (W) by a hypha (H) of the pink root fungus *Pyrenochaeta terrestris.*

Detailed studies of cuticular penetration have been carried out with powdery mildews (*Erysiphe* spp.) and anthracnoses (*Colletotrichum* spp.). Scanning electron micrographs of germinated spores of *Erysiphe graminis* f. sp. *hordei* on its host barley indicated the nature of penetration holes as round with smooth edges and with no signs of physical stress or tearing.

Moreover, on barley the surface wax crystals appear dissolved in areas beneath germ tubes and hyphae. These observations suggest that enzymatic activity is involved in cuticular penetration.

Transmission electron micrographs of sections through penetration pegs of *Colletotrichum graminicola* on maize showed the distinct inward depression of cuticle as the penetration peg enters the cell wall. Such inward displacement indicates that mechanical forces play some role in cuticular penetration by such pathogens. Evidence from electron microscopy also indicates that enzymatic activity plays an important role in cell-wall penetration.

Enzymatic activity during cell-wall penetration by fungi such as *Erysiphe* and *Colletotrichum* appears sharply localized. With some pathogens, enzymatic activity during cell-wall penetration appears diffuse rather than localized. Cuticular penetration by *Botrytis cinerea* is followed by rapid swelling of the epidermal cell wall. Cell walls beneath infection cushions of *Rhizoctonia solani* are also swollen and apparently partially degraded. Such diffuse effects on cell walls may be related to the ability of these two pathogens to produce large quantities of wall-degrading enzymes.

Once the pathogen has penetrated the outer barriers of the host by any of the means discussed, it usually establishes a parasitic relationship with the host.

11.5 DISPERSAL OF PLANT PATHOGENS

Inoculum is that portion of a pathogen that is transmitted to or contacts a host and is capable of infecting the host. Inoculum is the starting point for the development of plant disease. It consists of unit particles such as virus particles, bacterial cells, or fungal spores, or of more complex structures such as the nematodes or mycelial structures such as rhizomorphs. The reservoirs and sources of inoculum are many. Soil and infected plant tissue are excellent inoculum reservoirs, while air and water are usually poor reservoirs of inoculum. However, unless inoculum happens to be produced at the same place the host plant is growing, it must be transported from the point of its production to the host plant before it can infect it. The distances may be minute, for

example, a zoospore swimming through soil water to a host root, or they may be great, for example, wind carrying rust uredospores hundreds of miles.

Plant pathogens spread by an enormous variety of means. So pathogens spread in many ways - by wind, insects, water etc., while other pathogens spread in only one way, such as by a specific insect species. The development of disease control measures is based on a knowledge of how the pathogen is disseminated.

The best way to control a disease is by attacking the weakest link in the disease cycle. One such weak link may be the dispersal of the inoculum. A good example of a control based on knowledge of inoculum dispersal is that worked out for white pine blister rust. This pathogen has two hosts, the 5-needle pine and *Ribes* spp. (gooseberries and currants). The pine trees can only be infected by thin-walled basidiospores that are produced on *Ribes* leaves. These spores are wind disseminated and remain viable for only a short time, usually no longer than the time it takes them to be blown 300 meters. By using this knowledge of dispersal, white pine stands, particularly nurseries, can be protected from this pathogen by removing all *Ribes* bushes within a 300 meter radius around the stand. Some important agents of dispersal are discussed below.

11.5.1 Wind

The movement of air can carry the inoculum of many plant pathogens. The distances can range from a few centimeters up to hundreds of kilometers. Wind does not play a role in the dissemination of plant viruses except as it affects the movement of insect or mite vectors of viruses. Bacteria are also not often wind disseminated except when wind carries bacteria - laden water droplets or polysaccharidal strands of bacteria.

Fungi are the main group of plant pathogens disseminated by wind. Three aspects of wind dissemination of fungal inoculum are important in determining the success of dissemination: a) liberation of inoculum into the air from its source of production, b) the movement or dissemination of the liberated inoculum by wind, and c) the landing or contact of the inoculum with a susceptible host.

Most wind-disseminated fungal plant pathogens are in the form of spores. Liberation of spores from the parent mycelium or sexual structures can be a passive or an active process. In passive liberation, the fungus's only role is to produce the spores in a manner that allows the spores to be blown off, fall of, or knocked off by plant debris or passing insects. Many fungi produce conidia on long conidiophores that elevate the conidia to a point where they can be easily blown off. Active liberation of spores occurs in the sexual stages of many of the Ascomycetes and Basidiomycetes. Here the fungus performs a functional role in getting the spores air-borne.

Spores are so light that they are easily carried by very light winds. The distance that spores can be effectively disseminated is determined in past by the ability of the spores to remain viable under many and often extreme environmental conditions. Spores of some fungal plant pathogens have been trapped at extremely high altitudes, ranging up to 4500 meters above infected grain fields. Once spores reach an altitude of a few to several hundred meters they can then be transported horizontally, from a few meters to hundreds of kilometers. The dissemination of the rust fungi has probably been studied in greater detail in this respect than all other pathogens.

The fact that inoculum is liberated from its source of production and disseminated over various distances does not insure that it will make contact with a susceptible host. The inoculum must in some way be deposited on the host. Since spores do have a mass they are under the effect of gravity. The deposition of spores on a host surface strictly through the action of gravity is termed **sedimentation**. This method of landing probably occurs only rarely and then only in calm air.

Impaction of spores occurs when air movements forcibly drive a spore onto a surface. Large spores, such as *Helminthosporium* spp, are believed to be deposited quite frequently by this method. In **turbulent deposit** method, spores are deposited on the lower side of horizontal surfaces, particularly lower leaf surfaces where there is often an abundance of stoma. Finally, spores may reach host surfaces by being "washed out" of air with rain drops. This process of bringing the spore into contact with a host is probably the most important of the methods mentioned, particularly with large spores such as rust uredospores.

11.5.2 Water

Water is not as important in disseminating plant pathogens as is the wind. However, there are some examples where water is important in localized dissemination. Bacteria and the spores of some fungi are often spread by water, usually in the form of rain water or as dripping water that originates from rain or irrigation sprinklers. Many of the bacterial leaf spots, such as angular leaf spot of cotton (*Xanthomonas malvacearum*), are spread in this manner, particularly when the bacterial - laden water is blown about by wind.

11.5.3 Insects

Next to wind, some of the most effective disseminators of plant pathogens are the insects and related animals such as mites. A large number of economically important plant viruses are disseminated by insects, including aphids, leaf hoppers, thrips, white flies, beetles and mealy bugs. The aphids and leaf hoppers are probably the most important. The relationship of the virus to its vector may range from a strictly mechanical contamination of mouth and body parts to one of intimate biological association of the virus with the insect (Figs.11.4)

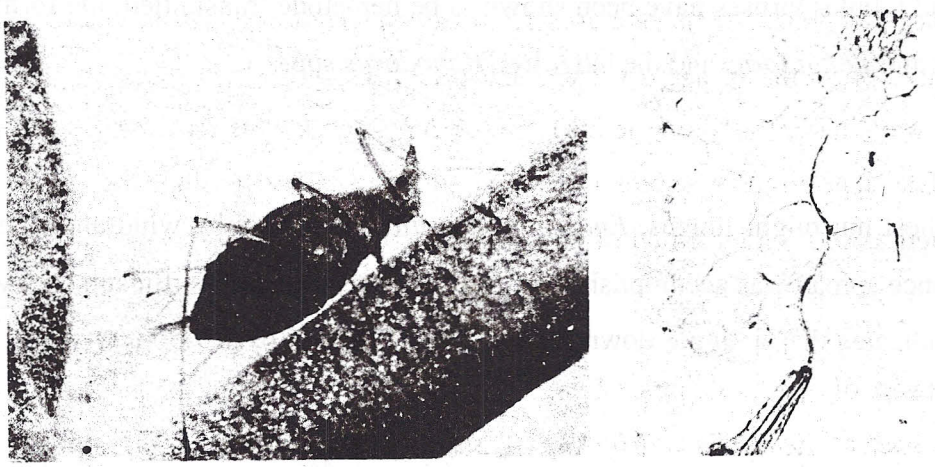

Fig. 11.4 Aphid beginning to probe a plant. Aphid extending its stylet into a leaf.

Several bacterial pathogens are disseminated by insects. The cucumber wilt pathogen, *Erwinia tracheiphila*, is completely dependent upon two species of cucumber beetles (*Acalymna*

spp.) for survival as well as dissemination. *Xanthomonas stewartii*, the cause of Stewarts wilt of sweet corn is dependent upon corn flea beetle for dissemination.

Plant pathogenic fungi also have some unique relationships with insects, varying from complete dependence on the insect for spread to a rather casual relationship. For example, members of the genus *Ceratocystis* are spread by beetles of various types.

11.5.4 Fungi

Several viruses have been shown to be transmitted by fungi. The big-vein virus of lettuce is transmitted by the zoospores of *Olpidium* and survives within the resting spores of this fungus. *Olpidium* also transmits other viruses including tobacco necrosis virus.

11.5.5 Nematodes

Like fungi and viruses, nematodes have also been shown to be disseminators of viruses. Most notable is that of the grapevine fan leaf virus and the nematode *Xiphinema index*. Both polyhedral and tubular viruses have been shown to be nematode transmitted, the former by species of *Xiphinema* and *Longidorus* and the latter by *Trichodorus* spp.

11.5.6 Birds

The chest nut blight fungus, *Endothia parasitica*, is spread by wind and rain but much of the long distance spread was accomplished by birds such as sapsuckers, flickers and wood peckers. One report indicates that a single downy wood pecker carried over 7,50,000 viable spores on its feet, head and bill.

11.5.7 Mammals

Both wild and domestic mammals have been implicated in spreading several pathogens. Squirrels have been known to eat the mycelial mat of the oak wilt fungus, *Ceratocystis fagacearum*. Conidia are produced abundantly on these mats and are able to survive the passage through the squirrel's digestive tract. Insects that visit such mats are also able to disseminate this

pathogen. Some viruses that are easily transmitted mechanically are also known to be spread by animals.

11.5.8 Man

Man serves to disseminate pathogens in two ways - on his person, or in the things that he transports from one area to another. Local dissemination of pathogens on man's person is illustrated by the angular leaf spot of cucumber caused by the bacterium *Pseudomonas lachrymans*. When cucumbers are grown for pickles, the fields are picked many times so that only small fruit will be picked. When an infected plant is contacted or picked by a person, particularly when the plants are wet with dew, the hands of the picker become contaminated. The pathogen is thus spread down the row.

The long-distance dissemination of plant pathogens by man is usually accomplished by the transport of infected plant parts such as seeds or nursery stock. Grape powdery mildew (*Uncinula necatur*) was transported to Europe from the United States on nursery stock. The white pine blister rust fungus (*Cronartium rubicola*) was also introduced into the United States from Europe.

11.6 SUMMARY

Plant pathogens enter plants through wounds, natural openings, or directly through the cell wall. Viruses penetrate only through wounds which may be caused by mechanical handling or by organisms such as insects, fungi or nematodes. Bacteria enter plants through wounds caused by rough handling of plants, through natural wounds caused by abscission of plant pants, or through insect wounds. Some bacteria enter through stoma, hydathodes or lenticels. Some fungal plant pathogens may penetrate plant tissues directly by mechanical or chemical means while others penetrate only through wounds or natural openings.

Plants pathogens spread by an enormous variety of means. Some pathogens spread in many ways; by wind, insects, water etc., while other pathogens spread in only one way. Spread can be of the short distance of one leaf to another or can be over immense distances from one continent to another.

11.7 MODEL QUESTIONS

1. Discuss the ways by which plant pathogens gain entry into the plants.
2. Describe different modes of inoculum dispersal.
3. Write short notes on:
   a) Role of insects in inoculum dispersal.
   b) Role of wind in inoculum dispersal.
   c) Penetration of plant tissues by fungi.

11.8 REFERENCE BOOKS

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Unit-III

Lesson 12

ROLE OF ENZYMES, TOXINS AND PHYTOALEXINS IN PATHOGENESIS AND PHYSIOLOGICAL CHANGES IN DISEASED PLANTS

12.0 **OBJECTIVE:**

Involvement of enzymes, toxins and phytoalexins in pathogenesis is described and physiological changes found in diseased pants are discussed.

12.1 INTRODUCTION

12.2 ENZYMES

12.3 TOXINS

12.3.1 Phytotoxins

12.3.2 Vivotoxins

12.3.3 Pathotoxins

12.4 PHYTOALEXINS

12.5 PHYSIOLOGICAL CHANGES IN DISEASED PLANTS

12.5.1 Changes in respiration

12.5.2 Effects on CO_2 fixation

12.5.3 Nucleic acids and proteins

12.5.4 Phenol metabolism

12.6 SUMMARY

12.7 MODEL QUESTIONS

12.8 REFERENCE BOOKS

12.1 INTRODUCTION

For a pathogen to infect a plant, it must be able to make its way into and through the plant, obtain nutrients from it and neutralise its defense mechanisms. The pathogens employ mechanical as well as chemical weapons in order to infect the plants. The mechanical forces include mainly

appressoria in fungi. The chemical weapons, mainly of pathogen origin include enzymes, toxins and growth regulators. In general, pathogens' enzymes disintegrate the structural components of host cells, break down food substances in cells or affect the protoplast directly and interfere with its functioning systems. Toxins seem to act directly on the protoplast and interfere with the permeability of its membranes and with its function. Growth regulators exert hormonal effects on the cells and either increase or decrease their ability to divide and enlarge.

11.2 ENZYMES

Pathogens are known to have both constitutive and inducible enzymes. Different types of enzymes that are shown to play some role in disease development are as follows:

Cutinases: Aerial plant surfaces consist primarily of cuticle and cellulose, whereas root surfaces consist mainly of cellulose. Cutin is the main component of cuticle. The upper part of the cuticle is admixed with waxes, whereas its lower part is admixed with pectin and cellulose. Cutin is an insoluble polyester of C_{16} and C_{18} hydroxy fatty acids.

Many fungi and bacteria are known to produce cutinases that degrade cutin. There is good evidence to show that cutinases are involved in the penetration of host cuticle by plant pathogenic fungi. The highest concentration of cutinase has been reported at the penetration point of germ tube and at the infection peg of appressorium forming fungi.

Enzymatic degradation of cell walls: Complete degradation of cell walls would require cellulolytic, hemicellulolytic, pectolytic and proteolytic enzymes capable of attacking each of the major polymeric components. Although enzymes of each of these four types readily attack model substrates, pectolytic enzymes appear effective in initiating the degradation of whole isolated cell walls. As pectolytic enzymes have been associated with lethal effects during pathogenesis, this group has received much attention.+

Pectolytic enzymes: These enzymes are of two major types – a) **Pectin methyl esterases** which remove methoxyl groups from pectin to yield pectic acid, and b) **Chain splitting enzymes** which cleave the α-1,4-glycosidic bonds that link the uronic acid units of pectic polymers.

Chain-splitting pectic enzymes are separated into two major categories: a) hydrolases, which cleave bonds by hydrolysis, and b) **lyases** or **trans-eliminases** which cleave bonds through trans-elimination. Members of each category are subdivided into endo- (random cleavage) and exo- (terminal cleavage) types. Further separation is based on which substrate, pectic acid or pectin, is most readily attacked. Many pathogenic fungi and bacteria especially those that cause soft rots or water-soaked lesions produce these enzymes, e.g. *Erwinia carotovora, E. chrysanthemi, Sclerotinia fructigera, Sclerotium rolfsii* etc. Due to the action of pectolytic enzymes, the wall structure is loosened and the remaining components are exposed to sequential attack by hemicellulases, cellulases and proteases of pathogen origin.

Cellulolytic enzymes: Many phytopathogenic fungi and bacteria are known to produce cellulases. They play an important role in softening and disintegration of cell-wall material, thus facilitating penetration and spread of pathogens. Break down of cellulose into glucose is brought about by **cellulase complex**. One cellulase (C_1) attacks native cellulose by cleaving cross-linkages between chains. A second cellulase (C_2) also attacks native cellulose and breaks it into shorter chains. Third group of cellulases (C_X) which attack shorter chains degrade them to cellobiose. Finally, cellobiose is degraded by β-glucosidase into glucose.

Hemicellulases: Hemicelluloses are complex mixtures of polysaccharides forming a major component of the primary cell wall and may also make up a varying proportion of the middle lamella and secondary cell wall. Many plant pathogenic fungi produce hemicellulases. Depending upon the monomer they release from polymer, Hemicellulases are designated as xylanase, glucanase, galactanase, arabinase, mannase etc.

Primary cell walls, besides cellulose, hemicellulose and pectin, also contain some structural proteins. Proteases or proteinases produced by plant pathogens degrade these proteins. Several fungi and bacteria are able to degrade lipids of plant cells by producing lipases, phospholipases and so on.

12.3 TOXINS

Toxins are injurious substances produced by organisms. The term is generally restricted to substances active at physiologically low concentrations. For the nomenclature and classification of toxins, systems based on the organism producing the toxin, on the organism affected by the toxin, on the toxin's chemical nature, on its mode of action or on its effects have been proposed, but none has gained acceptance.

The toxin hypothesis states that a toxic substance (x) is directly responsible for the symptoms of disease (y). When x is a toxin produced by a microbial pathogen, the hypothesis predicts:

a) the toxin will produce all symptoms characteristic of the disease.
b) sensitivity to the toxin will be correlated with susceptibility to the pathogen.
c) toxin production by the pathogen will be directly related to its ability to cause disease.

These requirements were clearly met for the first time by a toxin produced by *Helminthosporium victoriae*, the fungus which causes **Victoria blight of oats**.

The vast majority of toxins associated with plant diseases fail to exhibit all the properties expected from the simple relationship toxin **x** causes disease **y**. In such cases, indirect lines of evidence for a role in pathogenesis must be sought. Reproduction of particularly distinctive symptoms, demonstration of the toxin in diseased plants in quantities sufficient to cause symptoms and the nature of the toxin and its mode of action may provide such indirect evidence.

Toxins can be categorized into:

a) **Phytotoxins** which induce few or none of the symptoms usually caused by the pathogen from which they originate.

b) **Vivotoxins** which produce at least a portion of the disease syndrome and are not specific to the host on which they exert an effect, and

c) **Pathotoxins** which produce all or most of the symptoms characteristic for the pathogen from which they originate and exhibit the same host specificity as the causal organism.

12.3.1 Phytotoxins

Phytotoxins are products of parasites that induce few or none of the symptoms caused by the pathogen. They are non-specific for the host and there is no relationship between the toxin production and virulence of the pathogen. Some examples of phytotoxins are given below:

Lycomarasmin: This compound is a product of *Fusarium oxysporum* f. sp. *lycopersici* and is a dipeptide. It is responsible for the occurrence of necroses at the tips and periphery of tomato leaves attacked by this systemic wilt producing fungus. Two other phytotoxins are also produced by this fungus: *Fusaric acid* and *Vasin fuscarin*. Both of these compounds seem to induce vascular browning.

Alternaric acid: It is produced by *Alternaria solani* and is an unsaturated dibasic acid whose empirical formula is $C_{21}H_{30}O_8$. It is highly phytotoxic. It inhibits the germination of seeds of members of the Solanaceae family. The toxin, when introduced into the tissues of tomato and potato plants, induces some of the symptoms caused by *Alternaria*, that is chlorosis and necrosis. However, there is no correlation between the virulence of *Alternaria* and the amount of alternaric acid synthesized by the fungus.

12.3.2 Vivotoxins

Vivotoxins are compounds shown to produce at least a portion of the disease syndrome. The toxic principle must be isolated from the diseased plant and chemically characterized to be classified as a vivotoxin. Toxins that fall into this category are usually non-specific.

Piricularin: The causal organism of paddy blast, *Pyricularia oryzae*, produces an active semispecific toxin called piricularin. It is more toxic to the fungus that produces it than to the host itself. The fungus, however, is able to produce a protein that inactivates the antifungal properties of the molecule, but the phytotoxic properties are retained.

Ipomeamarone: This vivotoxin is produced by sweet potatoes in response to infection by *Ceratocystis fimbriata*, the causal agent of black rot, and *Helicobasidium mompa*, the causal agent

of violet root rot. Ipomeamarone is the best example of a pathogen – induced production of a toxin by the host plant.

12.3.3 Pathotoxins

Compounds that play a real causal role in plant disease symptom production are called pathotoxins. Pathotoxin was proposed as a broad generic term for a toxic product of a pathogen, of a plant, or of a plant-pathogen interaction. They are able to produce in susceptible plants all or most of the symptoms characteristic for pathogen-infected plants. Pathotoxins are separated into two main classes on the basis of origin (Table 11.1). Subclasses are based on whether the pathotoxin acts selectively or non-selectively when applied to plants which are susceptible and resistant to the disease involved.

Table 11.1 Classification of Pathotoxins

Class	Subclass	Producing organism
1. Pathogen produced	**A. Selective**	
	Victorin	*Helminthosporium victoriae*
	T-toxin	*H. maydis*, race T
	HC-toxin	*H. carbonum*
	Phyto alternarin	*Alternaria kikuchiana*
	PC-toxin	*Periconia circinata*
	B. Non-selective	
	Tentoxin	*Alternaria tenuis*
	Tabtoxin	*Pseudomonas tabaci*
	Syringomycin	*P. syringae*
	Phaseotoxin	*P. phaseolicola*
	Fumaric acid	*Rhizopus* spp.
2. Plant or Plant – pathogen produced	**A. Selective:** Amylovorin	*Erwinia amylovora* on Rosaceae
	B. Non-selective: Juglone	*Juglans migra*

Pathogen-produced substances which are selectively toxic to plants susceptible to the pathogen have been termed host-specific toxins. Actually, so-called host-specific toxins are those which at certain concentrations damage only susceptible plants but at higher concentrations also damage those that are resistant. These toxins exhibit selectivity rather specific activity.

Victorin is by far the most potent and selective pathotoxin known. It is polypeptide in nature and is produced by *Helminthosporium victoriae* causing Victoria blight of oats. Since changes caused by victorin are remarkably similar to those which occur in plants infected by *H.*

victoriae, Victorin has provided a valuable model for investigations of the nature and sequence of events that occur during pathogenesis.

Three main lines of evidence have been used to implicate **nonselective toxins** in plant disease. These are: reproduction by the toxin of distinctive early disease symptoms, correlation of toxin production and pathogenicity and recovery of the toxin from diseased plants in quantities sufficient to account for symptoms development.

Tentoxin is produced by *Alternaria tenuis* and both the toxin and the pathogen cause striking variegated chlorosis in seedlings of cucumber, cotton, citrus and other plants. Tabtoxin, formerly known as the wild-fire toxin, is produced by *Pseudomonas tabaci* which causes wild-fire of tobacco. The toxin has been identified chemically as β-lactam threonine.

Fire-blight of apples and other species of Rosaceae is a highly destructive disease caused by *Erwinia amylovora*. The toxin, amylovorin, is considered to be a product of an interaction between the plant and the pathogen. The leaves, bark and green fruits of black walnut (*Juglans nigra*) trees contain large quantities of a non-phytotoxic compound, hydrojuglone. This compound is readily oxidized to juglone (5-hydroxy-1,4-naphthoquinone) upon exposure to air. Juglone is highly toxic to a number of plants and is believed to be responsible for failure of such plants to grow in the vicinity of walnuts.

12.4 PHYTOALEXINS

Phytoalexins are a special group of post infectional phenolics that are antibiotic. They are most commonly produced as a result of the interaction of two metabolic systems, host and parasite, and inhibit the growth of microorganisms pathogenic to the plant. To date, several of these compounds have been isolated and identified. They are associated with the metabolism of aromatic compounds in the plants.

The original observations by Muller that led to the phytoalexin concept were made on the potato late – blight fungus *Phytophthora infestans*. The term phytoalexin, coined by Muller and Borger (1940) was originally narrowly defined as a chemical produced only when living plant cells, invaded by a parasite, are undergoing necrobiosis with the further requirement that the chemical be responsible for the death of the parasite. Failure over a period of three decades to identify a chemical which met all of these requirements led to redefinitions. Phytoalexins are now generally defined as antibiotics produced in plant-pathogen interactions or as a response to injury or other physiological stimuli (Kuc, 1972).

Most known phytoalexins are toxic to and inhibit the growth of fungi pathogenic to plants, but some are also toxic to bacteria, nematodes and other organisms. More than 300 chemicals with phytoalexin like properties have been isolated from plants belonging to 30 families. Some commonly studied phytoalexins are **phaseollin** in bean, **pisatin** in pea, **glyceollin** in soybean, alfalfa and clover, **rishtin** in potato, **gossypol** in cotton and **capsidiol** in pepper.

Phytoalexin production is stimulated in a host by the presence of certain pathogen substances called *elicitors*. Most phytoalexin elicitors are generally high-molecular weight substances that are constituents of the fungal cell wall, such as glucans, chitosan, glycoproteins and polysaccharides. Although most phytoalexin elicitors are thought to be of pathogen origin, some elicitors, for example, oligomers of galacturonic acid are produced by plant cells in response to infection or are released from plant cell walls after their partial break down by cell-wall degrading enzymes of the pathogen.

To sum up, the phytoalexins as a group of antimicrobial compounds have the following characteristics:

a) Fungistatic and bacteriostatic and active at very low concentrations.

b) Produced by the host plant in response to infection or in response to the metabolic byproducts of microorganisms.

c) Absent in the healthy cells, or present only in extremely minute quantity.

d) Usually remain close to their site of production.

e) Produced in larger quantities in response to weak pathogens and non-pathogens than to virulent pathogens.

f) Produced relatively quickly by the cells, usually within 12 to 14 hours, reaching a peak around 24 hours after inoculation.

g) Host-specific, rather than pathogen-specific; the basic response leading to their formation is more related to the host than to the pathogen.

12.5 PHYSIOLOGICAL CHANGES IN DISEASED PLANTS

When a plant is diseased, many or all of its critical biochemical functions may be affected. Some of these critical functions include the fixation of CO_2, photosynthetic phosphorylation, starch metabolism, respiration, oxidative phosphorylation and sugar metabolism. The most commonly known and widespread physiological imbalances in the diseased plants are discussed below:

12.5.1 Changes in Respiration

Changes, usually increases, in respiration are characteristic of most diseased plant tissue. Early investigations with powdery mildews and rusts indicated an initial rise in respiratory activity at or just prior to the development of visible symptoms. Respiration continued to rise with disease

development and usually reached a maximum 2 to 4 times higher than that of uninfected tissues at the onset of sporulation. Later the respiratory rate declined.

Experiments in which the pathogen was at least partially removed by brushing the leaf surface or stripping off the epidermis indicated that the major portion of the increased respiration was mediated by the plant. This conclusion was strengthened when respiratory increases were found in pathogen-free areas adjacent to lesions produced by non-biotrophs, in plant tissues infected with certain viruses, and in those treated with pathotoxins.

Some of the hypotheses which have been advanced to account for pathological increase in respiration include: The uncoupling hypothesis and involvement of the pentose phosphate pathway.

The uncoupling hypothesis: The hypothesis states that pathological increases in respiration result from an uncoupling of phosphorylation from electron transport. Attempts to test the uncoupling hypothesis have yielded mixed results. Rusted wheat leaves failed to respond to the uncoupling agent 2,4-dinitrophenol (DNP) as would be expected if uncoupling had already occurred. The diseased leaves were also insensitive to malonate (a competitive inhibitor of succinic dehydrogenase) but remained sensitive to fluoride (an inhibitor of enolase in the glycolytic pathway) and to azide (an inhibitor of metal-containing oxidases) as healthy leaves. Homogenates of diseased leaves also showed a marked increase in ascorbic acid oxidation. These data suggested that uncoupling followed by a shift from an iron – to a copper-containing enzyme pathway of oxidation might account for pathological increases in respiration.

Further work with rusts, victorin-treated oat leaves, and other diseased tissues has cast considerable doubt on the validity of the uncoupling hypothesis. With tissues uncoupled by DNP, the respiratory quotient (R.Q.) rises from a normal value of 1.0 to above 2.0. No such effect is found with diseased tissues. Instead the R.Q. remains near 1.0 or drifts slightly lower. Uncoupling is not ruled out by these results because the expected rise in R.Q. may be masked by increased dark CO_2 fixation and a shift to oxidation of organic acids.

Increased synthetic activity and, in some cases, increases in growth in diseases caused by biotrophic fungi provide the most convincing evidence against the uncoupling hypothesis. On the whole, uncoupling does not appear to be responsible for increases in respiration during early stages of pathogenesis but it may be involved later during tissue degeneration.

Involvement of pentose phosphate pathway: Experiments with victorin-treated tissues and plants infected with fungal and viral pathogens indicate that an increase in both the glycolytic – TCA sequence and in the pentose phosphate pathway contribute to pathological increases in respiration. The decrease in C_6 / C_1 ratio, increase in asymmetrically labelled phenolics, and abolition of an apparent Pasteur effect are all consistent with an increase in the pentose phosphate pathway.

12.5.2 Effects on carbon dioxide fixation

Chlorosis resulting from loss of chlorophyll is a common symptoms in plants infected with foliar pathogens. In general, the rate of photosynthesis in infected leaves begins to decline at about the same time that respiration starts to rise. As the disease progresses, photosynthesis continues to decline until eventually the quantity of CO_2 given off in respiration by the plant and pathogen exceeds the amount fixed by the plant. In view of such a net deficit, it is not surprising that infection often results in a 50% reduction in total dry weight and even greater reductions in grain yields.

Changes in starch content following infection have been observed in many foliar diseases. The general pattern is an initial decrease followed by a marked increase with heavy accumulations around the margins of lesions. Still later, the starch content again declines. Work with wheat leaves infected with *Puccinia striiformis* led to the suggestion that changes in the activity of ADP-glucose pyrophosphorylase during disease development might account for the pattern of changes in starch content.

Autoradiographs of leaves exposed to $^{14}CO_2$ in the dark provided the initial evidence for increased dark CO_2 fixation in diseased plants. Plant infected with rust or powdery mildew fixed the greatest amount of CO_2 in the dark at the time of sporulation. Moreover, fixation was concentrated in the area of sporulating lesions. This suggested that much of the increase was due to the ability of the fungus to fix CO_2. Bean and cereal rust uredospores possess the malic enzyme which catalyzes the reversible reaction, Pyruvate + CO_2 $\longleftrightarrow$ malate in both directions. This enzyme may be responsible at least in part for increased dark CO_2 fixation in diseased leaves.

Evidence for increased dark CO_2 fixation in diseased plant tissues in the absence of a living pathogen was also provided. The HC-toxin produced by *Helminthosporium carbonum* applied to maize causes increases in dark CO_2 fixation similar to those obtained when oats are treated with victorin.

12.5.3 Nucleic acids and Proteins

In many diseased tissues, especially in those characterized by overgrowths, galls or other growth abnormalities, increased metabolic activity is accompanied by marked increases in nucleic acid and protein synthesis.

Nucleic acid metabolism: Infection of susceptible plants with rust or powdery mildew fungi usually results in an increase in RNA which begins with and parallels the rise in respiration. No such increase in RNA occurs in resistant reactions, and little or no change in DNA is observed in either susceptible or resistant tissues in some cases.

Very large increases in nucleic acid content have been found in gall cells induced by fungi, bacteria, or nematodes. In cabbage club root gall cells which contained large plasmodia of the pathogen *Plasmodiophora brassicae* nucleolar volumes were 30-fold higher and DNA contents 16-fold greater than in non-infected cells.

Changes in Proteins: The total protein content, when both that of the plant and the pathogen are included, usually increases in early stages of infection. When the contribution of the pathogen is taken into account, little change or, in some cases, decreases in plant protein are found in most diseased tissues. Some however, crown gall tumors for example, contain much more protein than could be expected from synthesis by the pathogen. In late degenerative stages of disease, large decreases in protein, some times accompanied by increases in free amino acids, are often found.

Changes in increased levels of proteins and RNA are often accompanied by marked increases in the activity of phenylalanine ammonia lyase, polyphenol oxidose, peroxidase and a number of enzymes involved in glycolysis and the pentose phosphate pathway. Changes in peroxidase isoenzymes have been found in plants infected with bacteria, fungi, viruses and in those treated with pathotoxins. In all cases, isoenzyme patterns in diseased tissues differed markedly from those in healthy controls.

Changes in enzymes, particularly peroxidase, play a key role in disease reactions. Increased peroxidase activity has been correlated with resistance to a number of fungal, bacterial and viral pathogens. Resistance to the wild-fire disease, induced by injection of heat-killed cells of *Pseudomonas tabaci* into tobacco leaves has been reported to be accompanied by marked increases in peroxidase activity.

12.5.4 Phenol metabolism

Biosynthesis of phenolic compounds in plants may proceed via one or more of three different pathways; the shikimic acid pathway, acetate malonate pathway and acetate-mevalonate pathway. The most important of these appears to be the shikimic acid pathway. In a number of plant tissues, phenylalanine ammonia lyase (PAL) activity is greatly increased by injury or infection (PAL is a key enzyme in the shikimate pathway). Increased PAL activity induced by injury or infection usually reaches a peak after 24 to 48 hrs and then declines sharply.

Current interest in phenol metabolism stems from the identification of a large number of compounds synthesized via the shikimik acid or acetate pathways which accumulate following infection or injury. Many of these compounds, termed phytoalexins, have antibiotic properties and are thought to function in disease resistance.

12.6 SUMMARY

Tissue destruction by the pathogen implies that the pathogen possesses enzymes that degrade the structural components of the host. For this to occur, an enzyme or battery of enzymes produced by the pathogen must be extracellular. Many plant pathogens are known to produce

several enzymes such as cutinases, pectinases, cellulases, hemicellulases and proteases in order to gain entry as well as for tissue destruction.

Toxins are injurious substances produced by organisms. They can be categorized into phytotoxins, vivotoxins and pathotoxins. Phytotoxins are products of parasites which induce few or none of the symptoms caused by the pathogens. Vivotoxins are compounds shown to produce at least a portion of the disease syndrome. The toxic principle must be isolated from the diseased plant and chemically characterized to be classified as a vivotoxin. Compounds that play a real causal role in plant disease symptom production are known as pathotoxins. They can produce all or most of the symptoms characteristic for pathogen-infected plants.

Phytoalexins are defined as antibiotics produced in plant-pathogen interactions or as a response to injury or other physiological stimuli. They play an important role in disease resistance. In many plants, compounds with phytoalexin properties accumulate as a response to infection or injury. Chemical, physical and biotic agents which induce resistance to disease also stimulate the synthesis of phytoalexins. In general, phytoalexins accumulate more rapidly and in grater quantities in resistant than in susceptible reactions.

When a plant is diseased, many or all of its biochemical functions may be affected. Changes, usually increases, in respiration are characteristic of most diseased plant tissues. The rate of photosynthesis in infected leaves begins to decline at about the same time that respiration starts to rise. In many diseased tissues, increased metabolic activity is accompanied by marked increases in nucleic acid and protein synthesis. Increase in phenols in tissues following infection or injury reveals their function in disease resistance.

12.7 MODEL QUESTIONS

1. Discuss the role of toxins in disease development.
2. Explain the physiological changes in diseased plants.
3. Write short notes on:
   a) Phytotoxins
   b) Pathotoxins
   c) Role of enzymes in pathogenesis

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Unit-III

Lesson 13

FACTORS AFFECTING OUTBREAK OF PLANT DISEASE; PLANT DISEASE INDEXING AND FORECASTING

13.0 **OBJECTIVE:**

In this lesson, factors affecting disease development; plant disease indexing and forecasting are discussed.

13.1 INTRODUCTION

13.2 AERIAL ENVIRONMENT

13.3 SOIL ENVIRONMENT

13.4 BIOTIC FACTORS

13.5 PLANT DISEASE INDEXING

13.6 FORECASTING OF PLANT DISEASES

13.7 SUMMARY

13.8 MODEL QUESTIONS

13.9 REFERENCE BOOKS

13.1 INTRODUCTION

Whether in any one given situation, a plant disease will develop is dependent upon three things. First of all a susceptible host is required. In conjunction with this, the seasonal development of the host is important, since within certain periods of a plant's development it may be more susceptible than in others. Next, a virulent pathogen is required. Virulence is measure of the pathogen's ability to cause disease. The prevalence of the pathogen is also important. Finally, a favorable environment of sufficient duration must be present to allow infection by the pathogen and subsequent development of the disease. The factors that influence the severity of an epidemic, or epiphytotic are listed below:

1. Susceptibility of the host

a. Seasonal development

b. Inherent susceptibility

2. Inoculum potential of pathogen

a. Prevalence of pathogen
b. Virulence of pathogen

3. Impact of environment

a. Severity of environment
b. Duration of infection period

Plant diseases develop when a virulent pathogen attacks a susceptible host within a favorable environment. The environment can affect the perpetuation of the pathogen from one growing season to the next, the build up of the primary and secondary inoculum, the dissemination of inoculum and the germination and host penetration of inoculum. The environment can affect growth and development of the host prior to being infected in such a way as to affect its susceptibility. Finally, environment can affect the actual development of disease after the host has become infected.

13.2 AERIAL ENVIRONMENT

Moisture: Many pathogens attacking stems and leaves are intimately affected by moisture, either as free water on plant surfaces or as water vapor in the air. Some pathogens do not germinate and thus can not penetrate the host in the absence of free water. Moisture also affects the build up and spread of inoculum.

Moisture content of the air is usually referred to as "relative humidity". It is defined as the ratio of the actual amount of moisture in the air compared to the amount of moisture the air could hold if the air was saturated at a given temperature. Moisture is important for sporulation and take off of spores for secondary spread in many diseases.

The geographic distribution of many diseases of plants is determined by moisture. Late blight of potato is of rare occurrence in dry areas. Brown and Yellow rusts of cereals and downy mildew of grape vine are more serious in wet than in dry areas.

In blast disease of rice, conidia of *Pyricularia oryzae* are not produced below 88% relative humidity and at least 90% saturation is essential for their abundant production. Dispersal of conidia is highest during night hours when relative humidity is 86 to 98%, temperature in the range of 25 to 27°C and the wind is calm. A minimum night temperature range of 20-26°C in association with a relative humidity range of 90% and above lasting for a period of a week or more during any of the susceptible stages of crop growth, viz., seedling stage, post transplanting tillering stage and at neck emergence stage, is ideal for out break of the disease in any area.

The powdery mildew fungi are a group of pathogens that are affected by moisture in an unusual manner. Germination of the conidia of the lettuce powdery mildew fungus (*Erysiphe cichoracearum*) is greatly decreased at 100% relative humidity and germinates best at 93% RH.

The spores of these fungi contain a very high proportion of moisture and are not dependent on external moisture for germination. Free moisture is actually inhibitory to some powdery mildews.

Temperature: The temperature of the air can affect plant pathogens directly or indirectly. Seasonal and regional occurrence of plant diseases is, to a great extent, determined by temperature. Certain plant diseases occur during winter, others during summer and for many diseases rainy season is most favorable.

The late blight of potato (*Phytophthora infestans*) develops better in areas or seasons when the temperature is low. High temperature is harmful for the fungus and unfavorable for disease development. Yellow rust of wheat and stripe disease of barley are other examples where the disease development is favored by low temperature.

Many plant diseases and their causal agents are favored by high temperatures and distribution of such diseases is also common in the warm climate of tropics and sub tropics. *Pseudomonas solanacearum* causing brown rot of potato is most severe in warm regions. It does not affect the potato crop in the plains of India which is raised during winter but may occur when the crop is planted in January-February. Soft rot of potato caused by *Erwinia* is also common in warm, humid seasons.

The late blight of potato is reported to be unknown in areas where the mean atmospheric temperature exceeds 25°C. Temperature affects the mode of germination of sporangia and thereby determines the number of infective units. The optimum for germination of sporangia by zoospores is 12-13°C and by germ tube 24°C.

Wind: The role of the wind in disease development is mainly one of dissemination of plant pathogens or their vectors. It may also be important in determining the length of time plant surfaces are wet with dew, the dew disappearing faster under windy conditions.

Light: Light is necessary for photosynthesis, and thus the general well-being of the plant is affected by both the quantity and quality of light. Several virus diseases are known to be affected by light intensity. For instance, tomato plants will survive infection by sugar beet curly top virus when they are grown under conditions of low light intensity, that is shaded, whereas in full sunlight they will die.

Several fungus diseases are also affected by light, usually the intensity of light being the important factor. Powdery mildews often develop better under shaded conditions. The development of rust diseases, however, is generally favored by high light intensities.

Aerial gases and Particles: Oxygen is required by most, if not all, plant pathogens for growth. It is doubtful if oxygen concentration in the air ever becomes a limiting factor in disease development. Sugar beet leaves covered with cement-kiln dust are perhaps more susceptible to leaf spot (*Cercospora beticola*).

13.3 SOIL ENVIRONMENT

Since the soil is the medium in which a plant is anchored and from which it obtains nutrients and water, the various factors that make up the soil environment can also be importance to diseases of the plant parts below ground as well as to those of the aerial position of the plant.

Moisture: Some pathogens are dependent on an abundance of water in the pores of the soil for movement, an example being the zoospores of various *Phytophthora species* that attack roots. Thus, diseases such as *Phytophthora* root rot of alfalfa and safflower are most severe in very wet soils.

Low soil moisture can also favor certain diseases such as scab of potatoes (*Streptomyces scabies*). Under most conditions scab is more severe in dry soils, particularly sandy soils. The reason for this type of response is not clear but may be related to the fact that the pathogen is a strict aerobe, and high moisture levels might result in a low oxygen content in soil pores, thus retarding the growth of the pathogen. For most diseases, however, infection and disease development take place over a wide range of soil moisture conditions.

Temperature: The soil temperature can affect disease development by one or more of three ways: by affecting the host, pathogen, or the host-pathogen interaction. Seedling blight of corn and wheat is caused by *Gibberella zeae*. It is found that the disease is most severe on corn at temperatures below 13°C while on wheat it is most severe at soil temperatures above 13°C. Since growth of wheat is favored by cool temperatures and growth of corn by warm temperatures, the disease is most severe at temperatures unfavorable for growth of the host. Thus soil temperature in this case appears to affect disease development by affecting the host's susceptibility.

In the case of *Verticillium* with of cotton, the disease development is most severe at soil temperatures most favorable for growth of the pathogen, temperatures of 20-30°C. The fungus will not grow at temperatures above 30°C and hence there is no infection at temperatures of 33-35°C.

Fertility: The effect of soil nutrients on disease development is again an area where few generalizations can be made. If an effect is observed it is usually on the host and its reaction to the pathogen rather than a direct effect on the pathogen. Sugar beets planted on land cropped continuously to sugar beets showed a high incidence of seedling disease caused by *Aphanomyces cochlioides*. These soils also proved to be deficient in nitrogen and phosphorus. Tests indicated that by increasing the fertility of the soil, the seedling incidence could be cut from 80-90% to 15%. These tests show that the addition of either N or P helps to reduce the disease incidence but a combination of both is best.

Potassium is another element that affects the development of certain diseases. The lack of potassium coupled with high available nitrogen and phosphorus results in corn that is more susceptible to the various stalk rot fungi. Many other studies show an association between soil fertility and disease incidence. The responses are quite varied from one crop to the next and from one disease to the next.

Soil reaction or pH: It has been shown that the concentration of hydrogen ions in the soil has an affect on disease development. The action is probably directed at the pathogen, affecting its growth and perhaps its ability to survive or cause infection. Another possible effect would be on the availability to the plant of various nutrients, which could then possibly influence disease development.

Club root of cabbage (*Plasmodiophora brassicae*) is favored by a pH of 7 or less. Liming of the soil to raise the pH above 7.0 provides some relief from this disease, presumably by inhibiting the germination of the spores of this pathogen since germination is poor above pH 7.0.

Potato scab, on the other hand, appears to develop best when the pH is above 5.2. Attempts to control this disease by acidifying the soil with sulfur have met with some success.

Gases: The two gases in the soil other than water vapor that could affect plant diseases are O_2 and CO_2. Oxygen concentration is probably sufficient for growth of most soil-borne plant pathogenic fungi and bacteria except under conditions where the soil pores are completely filled with water.

The spores of fungi such as *Phytophthora* sp. and *Thielaviopsis basicola* can germinate at oxygen levels down to 0.1-1%. Thus low concentrations of O_2 probably have little effect on the pathogen but may weaken the host and make it more susceptible to infection.

The concentration of CO_2 in the soil atmosphere is probably higher than that in the above ground atmosphere. Increased concentration of this gas inhibits growth of *Rhizoctonia solani* in soil. The take-all disease of wheat (*Gaumannomyces graminis*) is more prevalent in sandy alkaline soils due to low carbon dioxide concentration in soil atmosphere.

13.4 BIOTIC FACTORS

Since atmosphere contains many ypes of living organisms, both plant and animal, it might be expected that products given off or exuded by these organisms might have some effect on plant pathogens and/or disease development. Although there are many reports of suppression of infection by pathogens such as rusts, by phyllosphere microflora, the actual mechanism has not been explained. It can be assumed that intensity of phyllosphere microflora may induce antagonism to the invading pathogen. This may include competition for chemicals that stimulate spore germination and germ tube growth, production of antibiotics and direct parasitism

Rhizosphere is the zone of influence of plant roots in the soil. The microbial population and activity in immediate vicinity of roots (the rhizosphore) is always higher and more complex than in soil away from roots. The rhizosphore may influence disease by one or more of the following mechanisms:

a) Availability of inorganic nutrients to the plant and the pathogen

b) Production of beneficial or harmful microbial metabolites affecting the plant and/or pathogen.

c) Interaction with symbionts and parasites.

Sterols of fungal, plant and animal origin are reported to induce -- a) sexual reproduction in species of *Pythium* and *Phytophthora*, b) formation of large zoosporangia in *Phytophthora parasitica*, c) increased tolerance to temperature in *Pythium* etc. Root exudates of sugar beet have been found to attract zoospores of *Aphanomyces cochlioides*. Organic acids (especially gluconic acid) and neutral fractions of the root exudates attract zoospores but do not influence their germination and development, while amino acid fraction some times helps in germination and growth.

Antagonistic effects are also common in biotic environment. Some saprophytic fungi such as *Trichoderma viride* are known to produce antifungal materials that inhibit the growth of various plant-pathogenic fungi.

Environmental factors are often directly related to the out break of plant disease epidemics. Epidemics can be localized and occur in one or a few fields or they can be widespread over a large area. For a whole population of plants to become diseased over a wide geographic area - an "epidemic" - the following conditions are necessary:

1. An abundance of viable inoculum must be located near the plants early in the growing season and this inoculum must be disseminated to the host plants at the time when environmental factors are favorable to infection.

2. If physiological races of a pathogen are known, the most virulent ones should be present in large number.

3. Following the initial infection of a crop, there must be a rapid and wide spread dissemination of inoculum.

4. Further more, there should be extensive and concentrated plantings of susceptible varieties of plants that are in their most susceptible stage when the inoculum arrives.

5. Environmental conditions, particularly temperature and moisture, should be optimum for infection, disease development and continued production of more inoculum.

As can be seen from the above, an epidemic usually involves a series of events. The speed at which these events occur varies from one pathogen or disease to the next. For many soil-borne diseases the development of an epidemic may take place over a period of 5-10 years when inoculum build up and spread is slow. Once the soil of a field has enough inoculum to produce an epidemic, the epidemic may continue year after year as long as a susceptible host is planted in that field. That has been the case with *Verticillium* wilt of many crops, but particularly with tomatoes and cotton.

For the disease of above ground portion of the plant where the inoculum is disseminated by aerial means - wind, rain and vectors, the development of an epidemic may be very rapid. Under optimum conditions the late blight fungus can infect a plant, grow in the plant, and then sporulate to produce more inoculum in as short a time as two and a half days. Thus in a period of several weeks this pathogen could destroy an entire field, as has happened many times in the past.

13.5 PLANT DISEASE INDEXING

The methods of measuring disease intensity vary with the nature of disease, and there can be no single method applicable to all diseases. On the basis of net effect of disease on plant, two general methods have been employed for measuring disease intensity:

In those diseases which kill plants rather quickly or which cause the same amount of damage to all the infected plants (vascular wilts, systemic viruses, damping-off, ergot, smut etc.), recording the percentage of diseased plants and organs, is a direct measure of crop loss involved.

Such direct counts are not applicable to diseases in which different plants show different amounts of infection. Leaf spots vary in intensity from plant to plant and from leaf to leaf on the same plant. For such diseases where the amount of disease varies greatly on different plants in the population (rusts, mildews, leaf spots e. any arbitrary indices and ratings had been in practice. They have now been replaced by percentage scales and standard area diagrams of disease intensity.

Percentage scales and assessment keys: In percentage scale methods, usually the number of plants or organs falling into known percentage disease groups are recorded. The disease groups are categories distinguished on the basis of per cent damage seen by human eye. A 12-grade scale was suggested by Horsfall and Barratt (1945). The relationship between per cent damage and grade scale is as follows:

Scale	Disease (%)	Scale	Disease (%)
1	0	7	50-75
2	0-3	8	75-87
3	3-6	9	87-94
4	6-12	10	94-97
5	12-25	11	97-99
6	25-50	12	100

A system using percentage scale developed by the British Mycological Society is given below:

Late Blight Key (Anon, 1947)

Blight (%)	Nature of infection
0.0	No disease observed
0.1	A few scattered plants blighted; no more than 1 or 2 spots in 10-metre radius
1.0	Upto 10 spots per plant; or general light infection
5.0	About 50 spots per plant; upto 1 in 10 leaflets infected
25.0	Nearly every leaflet infected, but plants retaining normal form; plants may smell of blight; fields look green although every plant is affected.
50.0	Every plant affected and about 50% of the leaf area is destroyed; field appears green, flecked with brown
75.0	About 75% of leaf area is destroyed; field appears neither predominantly brown nor green
95.0	Only few leaves on plants, but stems are green
100.0	All leaves dead, stems dead or dying

The first standard area diagram for a plant disease was given by N. Cobb for leaf rust of wheat, dividing the disease intensity into five grades: 1, 5, 10, 20 and 50 per cent of the leaf area occupied by the visible or sporulating rust pustules. The highest grade (50 per cent) represented about the maximum possible cover. A modified Cobb's scale was proposed by Melchers and Perker (1922) for the estimation of stem rust of wheat. In this scale, maximum rust cover was arbitrarily taken as that occurring when 37 per cent of the leaf or stem area was occupied by the pustules and this was labelled 100 per cent. Diagrams representing 5, 10, 25, 40, 65 and per cent stem rust on this basis were then prepared by copying Cobb's diagrams for leaf rust. Peterson *et al.* (1948) proposed further modifications suggesting 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 per cent cover with rust pustules of various sizes.

One of the most practical set of area diagrams was prepared by James (1971) for Canada Department of Agriculture and is being widely used. Actual area of leaves, stems, pods and tubers occupied by lesions in terms of per cent area covered are shown. Disease assessment key diagrams are presented in Fig. 13.1 indicating host surface area covered by the disease in terms of percentages. These keys are used for comparing the samples collected in the field and calculating the mean percentage area damaged by the disease. It can also be used for calculating **Infection index** or other factor as disease intensity, severity index etc. by converting the keys into grade cards i.e. giving a number to each range of percentage area affected as follows.

In a foliar disease if the assessment keys give 1,5,25,50 per cent area covered, the grade numbers can be given as 0 for no infection; 1 for up to 1% area covered; 2 for up to more than 1% and less than 5%; 3 for more than 5% and less than 25% area covered; 4 for more than 25% and less than 50% area covered and 5 for more than 50% area covered. 0 grade can be eliminated and if so, the grading becomes a 5-point scale. Growth stage at the time of sampling must be recorded according to standards prescribed and the samples must represent the total field area under inspection.

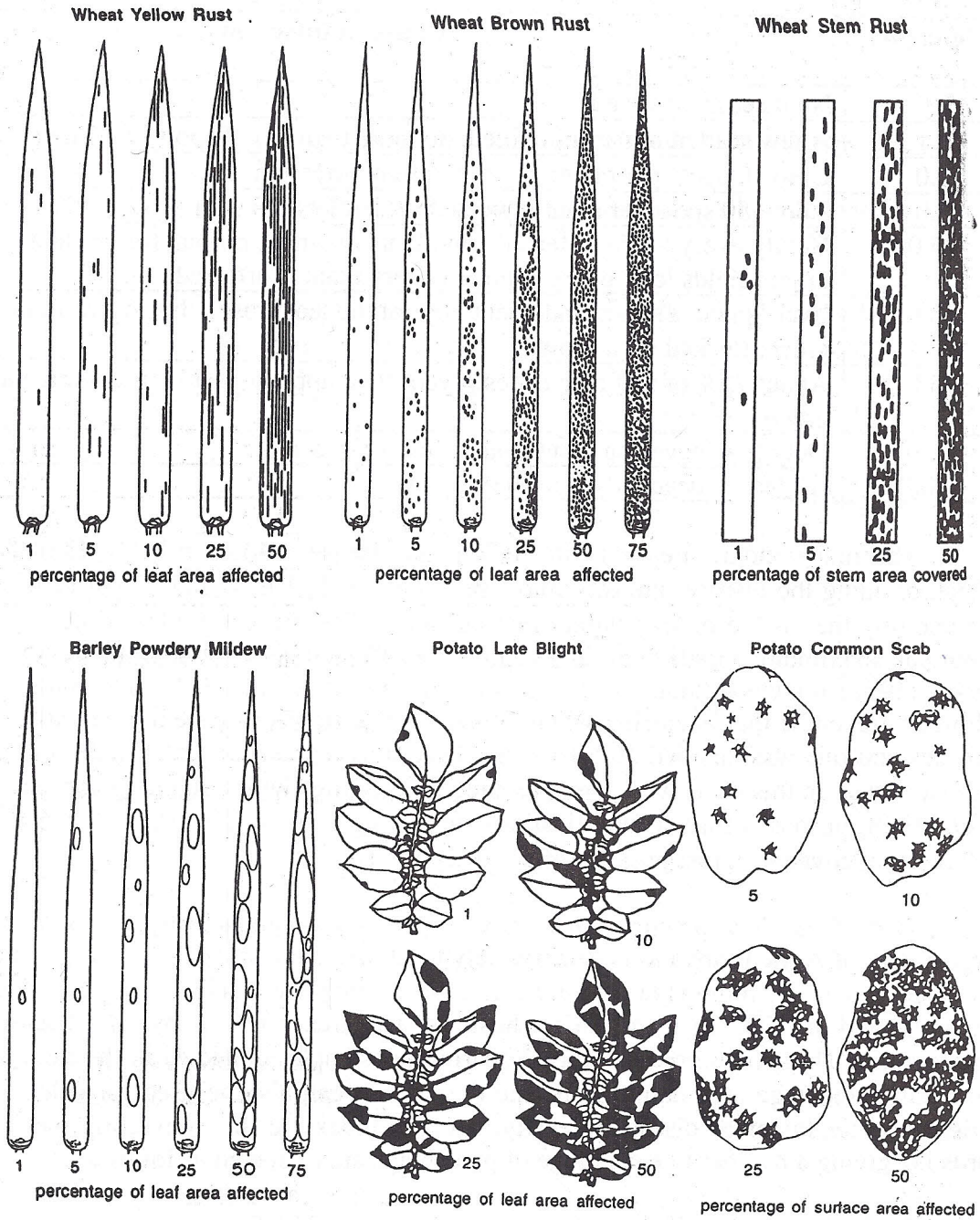

Fig. 13.1 Disease assessment key diagrams for different types of plant diseases.

The following example explains the method of calculating the infection index derived from the records of assessment with the key given above.

Host: Rice

Disease Leaf blast (*Pyricularia oryzae*)

Stage of growth: Post-transplanting tillering stage

No. of samples collected from one hectare field: 50 tillers with total of 200 leaves

% leaf area covered	Grade	Number of leaves in the grade (ratings)	Disease ratings	
0	0	75	0	
1	1	25	25	Sum of disease
10	3	45	135	ratings = 495
25	5	30	150	Total ratings=200
50	7	20	140	Max. disease grade=9
more than 50	9	5	45	

A common formula used to calculate the average infection or infection index is:

$$\% \text{ infection index} = \frac{\text{Sum of all disease ratings}}{\text{Total number of ratings} \times \text{max. disease grade}} \times 100$$

Thus, the index in the above example will be:

$$\frac{495}{200 \times 9} \times 100 = 27.5\%$$

Severity estimates from fairly small areas can be combined to cover large areas – villages, district or states and maps of disease incidence over large areas can be prepared. This overall index can be obtained by using the formula:

$$\frac{\text{Field rating class} \times \text{number of hectares in the class}}{\text{Total number of hectares}}$$

Remote sensing: Aerial photography can detect objects on land over a large area. Colwell (1956) showed that infrared aerial photography could be used to detect rusts and virus diseases of small grains and certain diseases of citrus. Later Infrared Photography was used in England for potato late blight disease. The remote sensing by aerial photography has now been tried for many types of assessment and detection work in agriculture including survey of plant diseases.

The main objectives of remote sensing are detection and identification of diseases, their relation to environment, origin and development of epidemics and quantitative assessment of disease. In addition to the informations on epidemiology the technique gives assessment of the disease over a vast area without ground parties visiting these areas. Quantification of the assessment could be done by recording transmittance of the positive transparencies of the photographs with the help of a simple, inexpensive densitometer. The numerical value of transmittance recorded by the densitometer is used as a figure of disease assessment and can be used like the data collected by other methods for estimating losses.

13.6 FORECASTING OF PLANT DISEASES

Forecasting involves all the activities in ascertaining and notifying the farmers in a community that conditions are sufficiently favorable for certain diseases, that application of control measures will result in economic gain, or that the amount of disease expected is unlikely to be enough to justify the expenditure of time, energy and money for control. This requires the complete knowledge of epidemiology, that is, development of the disease in plant populations under the influence of the factors associated with the host, the pathogen and the weather. Forecasting is actually applied epidemiology.

Practical advantages of forecasting: Forecasting is extremely useful to farmers in the practical management of crop diseases. Disease forecasting allows the prediction of probable outbreaks or increases in intensity of disease, and therefore allows us to determine whether, when and where a particular management practice should be applied. In managing the diseases of their crops, farmers must always weigh the risks, costs and benefits of numerous decisions. For example, besides so many other things relevant to cultural practices, they most frequently need the forecasts that will help them determine whether a plant infection is likely to occur so that they can decide whether to spray a crop right away or wait for more days before spray. If disease forecasting allows them to wait, they can reduce the expenditure on chemicals etc. and on labour. Maybe, this operation is not required at all, which would save money substantially.

Forecasting is done in different ways for different kinds of the pathogens and the weather factors that affect disease development. These are as follows:

a) Forecasts based on assessment of initial inoculum: Disease development in some cases may be predicted by assessing the amount of initial inoculum. In Stewarts wilt of corn caused by *Erwinia stewarti*, the pathogen survives off season in the body of the vector, the corn flea beetle. Thus knowing the number of vectors surviving previous season, would give an idea of the magnitude of initial inoculum available in next seasons.

In pea root rot caused by *Aphanomyces* and other diseases caused by soil-borne fungi, the severity of disease in the field can be predicted by off season tests in the green house. Susceptible plants are planted in green-house taking soil from the field in question. If plants show severe root

rot, the field is not planted with the crop. Initial inoculum of soil-borne fungi and nematodes can also be assessed directly by isolating fungal pathogens and nematode cysts.

b) Forecasts based on weather conditions favoring development of secondary inoculum: For such polycyclic diseases, such as late blight of potato and tomato that have a small amount of initial inoculum but many infection cycles, disease development can best be predicted by assessing the rate of occurrence of the infection cycles. In such cases, regular monitoring of weather factors is thus required.

Late blight epidemic can be predicted with reasonable accuracy if the moisture and temperature conditions in the field remain within certain ranges favorable to the fungus. When constant cool temperatures between 10 and 24°C prevail, and the relative humidity remains over 75% for at least 48 hr or is at least 90% for 10 hr, each day for 8 days, infection will take place, and a late blight out break can be expected from 2-3 weeks later.

Computerised predictive systems have now been developed for epidemics of late blight of potato and several other diseases. In such systems, for example BLITECAST for late blight, FAST (for forecasting *Alternaria solani* on tomato), TOMCAST for tomato early blight, *Septoria* leaf spot and anthracnose, moisture and temperature are monitored continuously.

c) Forecasts based on amounts of initial and secondary inoculum: For diseases in which both the amount of initial inoculum and the number of disease cycles are large (as in apple scab and leaf and stem rusts of wheat), both types of inocula are assessed along with continuous monitoring of weather factors. In the wheat leaf and stem rusts, short (1-2 weeks) forecasts of subsequent disease intensity can be obtained by taking into account disease incidence, stage of plant growth and spore density in the air.

13.7 SUMMARY

Plant disease develops when a virulent pathogen attacks a susceptible host within a favorable environment. The environment can affect the perpetuation of the pathogen from one growing season to the next, the build up of both primary and secondary inoculum, the dissemination of inoculum and the germination and host penetration of inoculum. Several factors such as moisture, temperature, light, soil fertility, soil pH and biotic factors influence the outbreak of disease.

The methods used for measuring disease intensity vary with the nature of disease and there can be no single method applicable to all diseases. Recording the percentage of diseased plants and organs is a direct measure of crop loss. Such direct counts are not applicable to diseases in which different plants show different amounts of infection. In such cases percentage scales and standard area diagrams of diseases intensity have been used.

Forecasting of plant diseases requires the complete knowledge of epidemiology, that is, development of the disease in plant populations under the influence of factors associated with the host, the pathogen and the weather. Forecasting is extremely useful to farmers in the practical management of crop diseases.

13.8 MODEL QUESTIONS

1. Discuss in detail about the factors which affect the out break of plant disease.

2. Write short notes on:
   a) Plant disease indexing
   b) Forecasting of plant diseases

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LESSON 14. FUNGAL DISEASES OF CROP PLANTS – 1

OBJECTIVE: To study the pathology of three important fungal diseases of crop plants viz. Club root of crucifers, damping of vegetables and powdery mildew of cucurbits.

CONTENTS:

14.1. Introduction
14.2. Club root of crucifers
14.3. Damping off of vegetables
14.4. Powdery mildew of cucurbits
14.5. Summary
14.6. Model Questions
14.7. Reference books

14.1. INTRODUCTION

Crop plants are attacked by a number of microorganisms causing disease, and some of the diseases occur in very severe form reducing the yield. Of the various groups of microorganisms that cause disease in crop plants, fungi are the most important. Among the fungi all major groups of fungi from slime moulds to basidiomycetes attack the crops causing epidemic diseases. Among the myxomycota (slime moulds) group of fungi that do not form true mycelium with cell walls, *Plasmodiophora brassicae* attacks a number of cruciferous hosts like cabbage, cauliflower etc. causing a debilitating disease called club root disease, characterized by abnormal growth of affected roots. Among the zoosporic fungi of Mastigomycotina, *Pythium* species are the major pathogens attacking germinating seeds and seedlings causing a disease termed damping off. Among the members of ascomycotina, group of fungi, poderymildew caused by species belonging to erysiphaceae are most conspicuous. These are ectoparasites on aerial parts of the crop plants that rapidly spread through air and build up rapid epidemics. In this lesson, the pathology of club root disease of crucifers caused by *Plasmodiophora brassicae* damping off disease of vegetable crops caused by species of *Pythium* and other fungi, and powdery mildew disease of cucurbits caused by *Erysiphe cichoracearum* and *Sphaerotheca fuliginea* are explained.

14.2. CLUB ROOT OF CRUCIFERS:

One of the most destructive diseases of cabbage, cauliflower, radish and other cultivated cruciferous hosts is club root disease caused by *Plasmodiophora brassicae*. The disease is also known as finger-toe disease. It is world wide in distribution. The disease is prevalent in India, nearly whole of Europe, USA, Canada, Australia, S.Africa etc. The disease became so widespread and destructive in the region of St. Petersburg in Russia during 1860's, that the Russian Gardener's Association offered a prize for its study in 1872. As a result Michael Stepanovich Woronin, a well-known Russian plant pathologist and a student of Debary, under took the work on the disease and published the details of his studies during 1874-78.

14.2.1. Symptoms:

The disease affects the roots and may become apparent at any stage of the development of the host, and often progresses far before the symptoms in the above ground plant parts are visible. Seedlings when infected show flagging and symptoms of chronic water deficiency. The leaves become pale green or yellowish, and plants die soon. In case of later infections, the disease may not cause such severe effect.

An effected plant, when pulled up, shows a grossly distorted mass of hypertrophied roots described as clubs. In cabbage and close relatives, individual infections on roots progress in both directions parallel to the axis, and spindle shaped clubs are formed (Fig 14.1). Multiple infections bring about a variety of modifications of the spindle type.

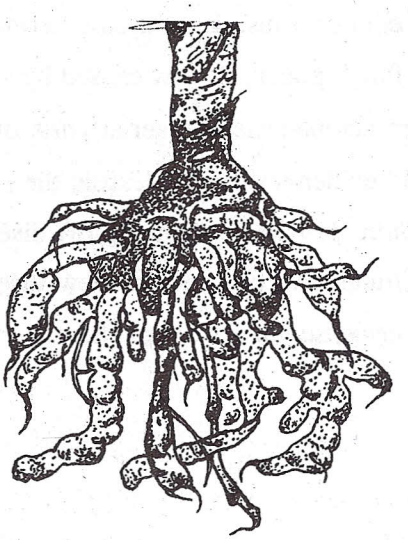


Figure 14.1. Club root of crucifers

The type of hypertrophy of the root system varies is different susceptible hosts. Six general types are recognized.

1. Complete clubbing of main and lateral roots. eg. *Brassica oleracea*
2. Clubs on main roots, laterals free. eg. *Sisymbrium altissimum*
3. Clubs on lateral roots, main roots free. eg. *S. officinale*
4. Clubs on main and lateral roots with club free root lets above the diseased portions eg. *Lepidium sativum*
5. Clubs as tumors of the root eg. *Raphanus sativus*
6. Dark, decomposing spots on the root eg. *R. sativus*

As result of severe malformations of roots, absorption and translocation of water and nutrients to the aerial parts of the plants is greatly affected. The above ground parts show symptoms of wilting and malnutrition, heads are not formed and even if formed remain very small. The swollen distorted roots undergo much decay due to the invasion of bacteria and soil inhabiting fungi at a later stage.

14.2.2. Etiology:

The causal organism of club root disease of crucifers is *Plasmodiophora brassicae*. It belongs to the family plasmodiophoraceae, order plasmodiophororales, class plasmodiophoromycetes of Division Myxomycota.

In the absence of the crop, the pathogen survives is the soil as resting spores. The resting spores are hyaline spherical, up to 4 μm in diameter. After a prolonged resting period, the spores germinate in the root zone of susceptible plants, probably stimulated by host root exudates. The spore swells to several times its original size and release a single naked biflagellate primary zoospore. Both the flagella are of whiplash type but one is longer than the other i.e. anisokont. The primary zoospore after a period of motility loses its flagella and enters the root hair by dissolving root hair cell wall to form a hole. Within the root hair, it grows into a plasmodium.

After completely occupying the root hair cells, the plasmodium is transformed into a sporangium or a number of sporangia. In each sporangium the protoplast cleaves to produce a large number of uninucleate zoospores, which are released by lysis of the infected cells. These zoospores are called secondary zoospores and resemble the primary zoospores except in size. The

secondary zoospores are much smaller than the primary zoospores. It is believed that these secondary zoospores act as gametes and unite in pairs producing diploid zygote. The zygotes infect the roots and enter into the cortical cells. The presence of the pathogen in the cortical cells result in the hypertrophy and the infected cells greatly enlarge to form a giant cell. The cells surrounding the infected cell also become hypertrophied, thus resulting in a group of giant cells, described by Woronin as 'krankheitsherd'. The IAA content of the infected root increases to as much as 50-100 times more than that in normal roots. The plasmodium is believed to migrate from cell to cell as amoebae or small plasmodium. However, some believe that the fungus is relatively immobile and distributed into a number of cortical cells by repeated division of the infected cell or cells. When the contents of the infected cells are completely consumed, the nuclei of plasmodium is believed to under go meiotic division forming haploid nuclei. A small bit of cytoplasm surrounds each haploid nucleus, rounds up, produce a dark chitinous wall and transform into resting spores. The resting spores are not organized into groups but remain free from one another and fill the entire lumen of the cell. The resting spores are released into soil on decomposition of the infected roots.

There is much difference of opinion in the interpretation of different stages in the life cycle the fungus. The major steps in the life cycle as shown in the figure 14.2

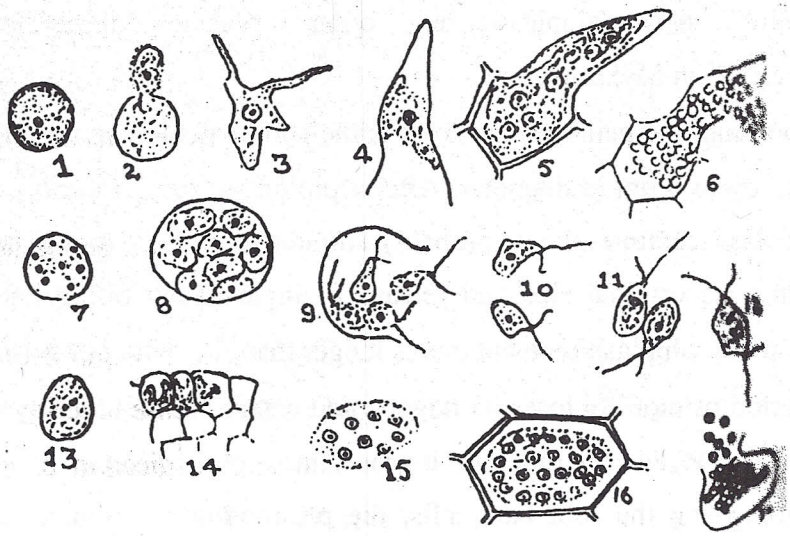


Figure 14.2. Some major steps in the life cycle of *Plasmodiophora brassicae* From resting spore (1) to release of resting spores

14.2.3. Factors effecting:

Soil Temperature: Infection occurs over a range of 9 to 30°C soil temperature. Soil temperatures ordinarily do not limit the infections.

Soil moisture: The disease is more severe in low-lying water logged areas than in well drained dry soils. Low moisture, below 45 percent of water holding capacity limits the infections. Such dry soils are also not favourable to plant growth.

Soil pH: The disease is found to occur only in neutral to acidic soils where pH ranges from 5.0-7.0. This is because the spores of *P. brassicae* can and germinate at this pH range and are highly sensitive to alkaline pH.

Soil fertility: Low potassium and low phosphorus in soil reduce the disease, while low or high nitrogen and high potassium increase the disease incidence.

Spread of the disease: The organism is disseminated locally with drainage water, farm implements, wind borne soil, roving animals and transplants. It does not gain access to the seed and disease is not seed borne. Transplants comprise the chief means of wide spread distribution.

Age of the host: The host is susceptible at all stages of growth, but disease severity is more if the plants are infected early rather than late in the season.

Survival: The pathogen survives in the soil as resistant resting spores lying free or in crop debris. They retain viability for long periods (up to 10 years).

Host range: All cultivated crucifers are susceptible to the disease. Wild crucifers as well as some non-cruciferous plants like *Lolium perenne*, *Trifolium pratense*, *Papaver rhoeas* are also susceptible.

Effect of root exudates: The root exudates of susceptible hosts stimulate the germination of resting spores.

14.2.4. Control: As the disease is a soil borne disease, it is very difficult to control once the infection occurs. Hence, preventive measures are to be taken to reduce the disease severity.

14.2.4.1. Sanitation: Since the pathogen is having a wide host range, eradication of crucifer weeds and other weeds help to reduce the inoculums load in the field.

14.2.4.2. Seedbed management: Since the disease is severe in young seedlings, seedbeds must be prepared carefully is disease free are as with proper drainage. If pathogen has already established, it should be destroyed by soil fumigation with vapam, methyl bromide etc.

14.2.4.3. Seedling infection and treatment: As the infected seedlings serve as the primary source for the introduction of the disease into new areas, careful inspection of seedlings used must be undertaken. The roots of seedlings must be dipped in solutions of organomercurial compounds before transplantation.

14.2.4.4. Soil treatment: Since the disease is severe in acidic soil, the soil pH should be raised to 7 or more using lime (calcium carbonate). Liming of soil is one of the long recognized efficient preventive measures. PCNB(pentachloronitrobenzene) formulations such as brassicol, terrador etc. are effective against the pathogen. These fungicides should be added to soil at the rate of 30 kgs/ha.

14.2.4.5. Crop rotation: Crop rotation for long periods, where feasible, should be adopted with non-cruciferous crops to starve out the pathogen.

14.2.4.6. Resistant Varieties: Using resistant varieties is the best method, but very few resistance varieties are available.

14.3. DAMPING OFF OF VEGETABLES:

Damping off is a very common and extremely destructive disease of young seedlings of a large number of crop plants. A number of vegetable crops, which are raised in seeds beds before transplantation in the main field, and also those that are directly sown in the field are affected by damping–off disease and it is especially severe in seed beds. Some of the important vegetable crops affected by damping off are cabbage (*Brassica oleracea* var. *capitata*) cauliflower (*Brassica oleracea* var. *botrytis*), radish (*Raphanus sativus*), tomato (*Lycopersicon esculentum*), brinjal (*Solanum melongena*), chillies (*Capsicum annuum*), sponge gourd (*Luffa acutangula*), bottle gourd (*Lagenaria vulgaris*), lettuce (*Lactuca sativa*), cluster bean (*Cyamopsis tetragonaloba*) etc.

14.3.1.Symptoms: The symptoms of damping off disease are similar in all affected plants. The diseases of germinating seeds and of seedlings are collectively called damping off. Hence two stages are recognized in the disease 1. Pre emergence damping off and 2. Post emergence damping off.

14.3.1.1. Pre emergence damping off: In this phase, the young seedlings are killed before they reach surface of the soil. They may infact be killed even before the hypocotyls has broken the seed coat. The radical and plumule when come out of the seed, undergo complete rotting due to the

attack of pathogenic fungi. Since this happens under the soil surface, the disease is not recognized at all by the farmers, who attribute the failure in emergence of seedlings to poor quality of the seed.

14.3.1.2 Post emergence damping off: This phase of the disease is characterized by toppling over of the inflected seedlings, any time after they emerge from the soil until the stem has hardened sufficiently to resist invasion by the pathogens. Infection usually occurs at or below the ground level and the infected tissue at hypocotyl region appears soft and water soaked. As the disease advances the stems become constricted at the base and the seedling collapse. Generally the cotyledons and the young leaves wilt slightly before the seedling is prostrated, although sometimes they remain green and turgid until collapse occurs.

The disease is most severe in nurseries or seedbeds, and the disease usually radiates from the initial foci of infection. In highly susceptible varieties seedling losses of 25 to 75% occur, and most of the loss is due to preemergence damping off.

14.3.2. Etiology: The most common cause of damping off of vegetable crops is infection by *Pythium* species like *P. aphanidermatum, P. debaryanum, P. myriotylum, P. ultimum P. butleri, P. arrhenomenes* etc, and *Rhizoctonia solani*. The species of other fungal genera are also occasionally involved and they belong to *Phytophthora, Sclerotinia, Botrytis, Fusarium, Phoma, Sclerotium, Colletotrichum* etc.

Pythium aphanidermatum, P. debaryanum and *Rhizoctonia solani*, which are the most important pathogenic fungi causing damping off of vegetable crops are briefly described.

Pythium is a zoosporic fungus belonging to the family pythiaceae, order peronosporales, class oomycetes of sub division Mastigomycotina.

14.3.2.1. *Pythium*: In *Pythium*, mycelium is composed of coenocytic, hyaline, freely branching thick hyphae. Septa may be formed to separate reproductive structures and in old hyphae. It is a facultative parasite and can freely grow is soil. In the infected tissues of the host, hyphae grows both intercellularly and intracellularly. When intracellular, the hyphae sends in haustoria into the host cells. But haustoria are lacking in intracellular mycelium.

In *P. aphanidermatum*, the most common cause of damping off of vegetables, sporangia are irregular, lobed and intercalary (Figure 14.3)

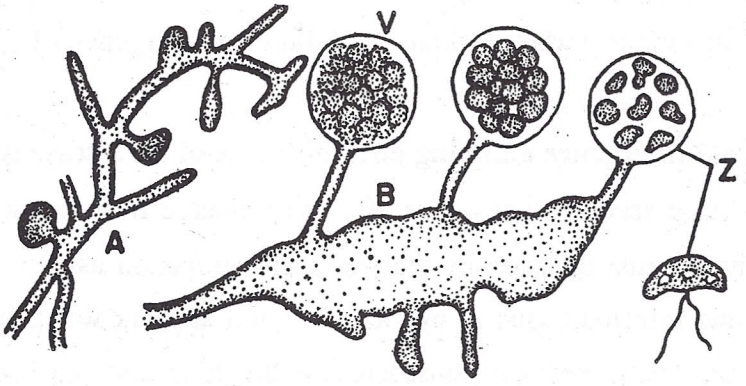
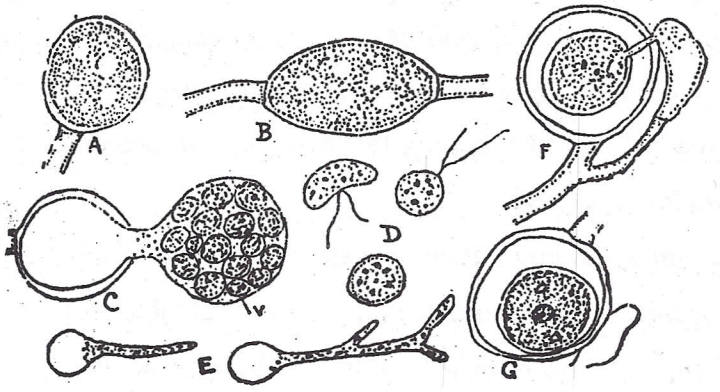


Fig.14.3. *Pythium aphanidermatum.* A. Mycelium with sporangial swellings and hyphae, B Germinating sporangium with vesicle (v) and Zoospores (z)

In *P. debaryanum*, sporangia are globose and terminal. Occasionally intercalary sporangia are also formed (Fig 14.4)



14.4. *Pythium debaryanum.* A terminal sporangium B. intercalary sporangium C – vesicle D – Zoospores, E – germinating Zoospores F,G- Sexual Production.

The sporangia of Pythium species is hyaline with thick granular protoplasm. They germinate by a tubular structure which ends in a vesicle. The vesicle is spherical, thin walled, hyaline, and the protoplasmic contains of the sporangium pass into the vesicle. In the vesicle, the protoplasm cleaves into several equal units and each unit develops into a kidney shaped zoospore without any definite cell wall but with two laterally arranged equal but heterokont flagella,

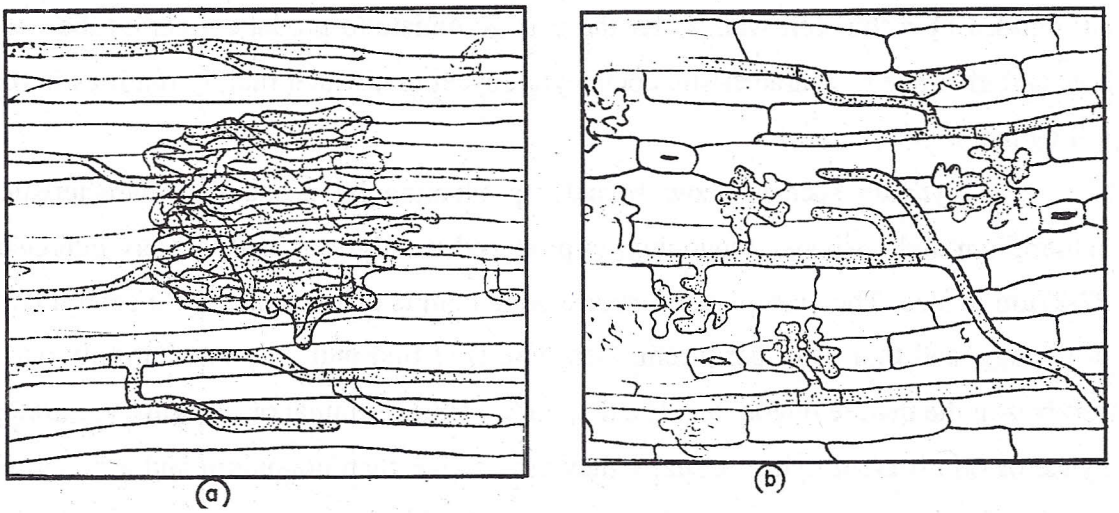
Zoospores measure 7-12 µm in diameter. The vesicle bursts to release the zoospores, which swim around in water film. In about half a hour, they shed their flagella, become spherical and encysted, surrounded by a thin cell wall. Later the cyst germinate to produce a germ tube which enter the host and give rise to characteristic coenocytic mycelium, which rapidly ramifies in the host tissue killing them.

Pythium species show sexual reproduction by producing characteristic oogonium, antheridium and oospores. Oogonium is produced mostly terminally, rarely intercalary, measure 22-27µm in size. The protoplast in mature oogonium is differentiated into central dense oosphere or the egg, and thin outer periplasm. Oosphere is at first multinucleate but only one egg nucleus survives in the mature oogonium. The antheridia, either terminal or intercalary, develop from same hyhae or on different hyhae. Usually they are smaller than oogonium and are club shaped. They attach to oogonium and produce fertilization tube which releases the contents of the antheridium or its nuclei into oosphere. Only one antheridial nucleus units with oosphere nucleus and rest of the antheridial nuclei degenerate. Fertilized egg develops a double wall and transform into a thick resting structure called oospore. Germination of the oospore may be by germ tube or by formation of a vesicle in which zoospores are differentiated.

14.3.2.2. *Rhizoctonia solani*: It is a common soil borne fungus belonging to family agonomyceteaceae, order Agonomycelats, class Hyphomycetes of Deuteromycotina. The fungus donot produce any asexual spores. The perfect stage of the fungus is observed in cultures, but not in nature, and identified by *Thanetophorus cucumeris* belonging to basidiomycotina.

The mycelium of *R. solani* is composed of freely branching, septate, dark brown hyphae. It produces small dark brown to black sclerotial bodies in culture.

R. solani is having a wide host range and attacks more than 350 host plants. On some hosts (e.g. Radish), the mycelium of *R. solani*, as it grows on to the host surface, produce a thick aggregate of hyphae called 'infection cushion' and enter the host tissue by penetration from infection cushions. On some hosts (e.g. tomato), the mycelium forms a large number of lobate appresoria, from which infection pegs enter the host (Figure-14.5).



14.5.*Rhizoctonia solani*. a. Formation of infection cushion on radish hypocotyls
b. production of lobate appressoria on tomato hypocotyls.

14.3.3. Disease cycle: The damping off fungi are mainly soil borne. *Pythium* species persists as oospores or as actively growing mycelium. *Rhizoctonia solani* persists in soils as actively growing saprophyte, and form sclerotia under adverse conditions.

The pathogenic fungi are attracted towards the germinating seeds and young seedlings, attack them, rapidly kills the host seedlings, and reenter the soil to resume free living stage.

14.3.4. Factors effecting disease development: A number of host factors and soil factors effect the disease development and severity.

14.3.4.1. Age of the host: Parasitism of damping off fungi is limited to germinating seeds and seedlings. The plants become progressively resistant to infection with age. The disease is severe in the first week of growth, infections disease in the 2nd week and by the end of 3rd week the

seedlings almost become resistant to damping off because of the development of mechanical tissues.

The rate of seedling emergence is inversely related to pre emergence damping off. i.e. damping off is most severe when conditions favour the growth of fungi but not the host

14.3.4.2. Effect of root exudates: The exudates from germinating seeds and from the roots of young seedlings affect the behaviour of fungi involved in damping off. The exudates may (1) stimulate the germination of resting spores and sclerotia (2) attract motile spores or mycelium to the roots and (3) growth of fungi at root surface enhanced.

14.3.4.3. Soil types: Soil types as such do not affect much the occurrence of damping off, but the disease is most severe in ill aerated, ill drained soils. Such conditions are common is compact heavy soils. In loose soils, having a good proportion of sand, the disease is less severe.

14.3.4.4. Soil moisture: The effect varies with pathogen. Damping off caused by *Pythium* species is severe at high moisture levels and these conditions are favourable for growth and essential for zoospore production.

The disease caused by *Rhizoctonia solani* increase with soil moisture to approximately 2/3 saturation, but declines at moisture levels above this. The growth of *R. solani* in soil was poor at high moisture levels.

14.3.4.5. Soil temperature: A temperature range of 24-28°C is optimum for damping off caused by *Pythium* and other fungi.

14.3.4.6. Soil pH: Though disease occurs over a wide range of pH (4-8), it is usually favoured by slight acidity.

14.3.4.7. Seeding rate: The disease is usually severe in nurseries where seeding rate is high and over crowding of seedlings occur.

14.3.5. Control: Damping off disease is very difficult to control, once the pathogens are established in the field various measures have to be taken to reduce the disease severity.

14.3.5.1. Seedbed management: Good seedling vigour is an important factor in escaping disease, and hence the primary approach in control should be good seedbed management. Providing better drainage by improving soil texture and by preparing raised seed beds with free drainage all around is important. There should be thin seeding to avoid over crowding of seedlings. Fertility level, especially nitrogen content of seedbeds should be high to promote rapid vegetative growth. Light irrigation at frequent intervals, rather than heavy watering leading to water logging, must be given.

14.3.5.2. Soil sterilization: Once damping off becomes trouble some on a site, steps should be taken to reduce inoculum level in the soil. This is achieved by partial soil sterilization either by burning trash on the field soil surface or drenching the soil with 2% formaldehyde to soak the soil up to a depth of at least 10 cm. One gallon is required for 2-3 sq. ft. of soil.

Methyl bromide and chloropicrin are also widely used for soil sterilization. Methyl bromide penetrates well and is particularly effective against fungal sclerotia but is highly volatile. Chloropicrin is less volatile and, as it is immiscible with water. Water is often applied to soil surface to act as a seal after treatment with chloropicrin.

14.3.5.3. Seed treatment: Preemergenc damping off can be controlled by treating seeds with seed dressing fungicides such as organomercurials (ceresan, arasan, semesan, agrosan etc) and other fungicides like captan thiram, blitox etc. Brassicol (PCNB) is particularly effective against *Rhizoctonia*. Dexon is toxic to *Pythium* and zoosporic fungi.

14.3.5.4. Biological control: A possible method of controlling damping off is to make use of microorganisms antagonistic to the pathogenic fungi. However, introduced organisms rarely become dominant to suppress the native pathogenic fungi. Use of organic materials such as chitin, grass meal etc. will stimulate the development of a wide range of saprophytic organisms, some of which may be antagonistic to the pathogens. Use of organic compost also enhance soil microflora and reduce the severity of soil borne diseases including damping off.

14.4. POWDERY MILDEW OF CUCURBITS:

Powdery mildew is a serious disease of cucurbits n 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.

14.4.1. Symptoms: Powdery mildews are caused by obligate ectoparasites whose mycelial growth appear as white patches or powdery flour on host surface, and the symptoms are almost similar on all susceptible hosts. Usually all aerial parts of the host plant are attacked but symptoms on leaves are predominant. They appear on both upper and lower surfaces of the leaves.

The symptoms first appear as tiny white to dirty grey patches (some times with reddish tinge), which become powdery as they enlarge. In the early stages, colonies of the fungus appear as distinct compact white patches on leaves of some hosts (e.g. *Momordica*) while on others they appear as diffuse white powdery flour with no definite colony appearance from the beginning (e.g. *Lagenaria*). The superficial powdery mass ultimately covers entire leaf surface.

Black pin point bodies, representing the ascigerous stage of the fungus appear rarely in the late season. They have been formed during winter months is North India, and they rarely appear in South India.

14.4.2. Etiology:

Powdery mildew on cucurbitaceous vegetables is caused by two fungi belonging to Erysiphaceae. They are *Erysiphe cichoracearum* and *Sphaerotheca fuliginea.*

14.4.2.1. *E. cichoracearum*: Mycelium is superficial, well developed, consists of a network of branched, septate, hyaline hyphae. The hyphae send in haustoria into the epidermal cells of the host to obtain nourishment. A few days after infection, mycelium produce long, hyaline, erect conidiophores in large numbers. Conidia are formed in long chains and mature from top. The terminal cell of the conidiophore first matures in to a conidium and drop off, then the lower cell transform into conidium and so on. The conidia are single celled, hyaline, ellipsoidal to barrel shaped and vary in size from 25-45 x 14-26 µm.

The ascocarps are formed towards the end of the crop season. They are gregarious or scattered, globose 90-135 µm in diameter. Peridium is distinct with polygonal cells and posess no ostiole. Appendages are numerous, basally inserted, mycelioid, interwoven with vegetative mycelium, hyaline to dark brown, 1-4 times as long as the diameter of the ascocarp rarely branched. Each ascocarp contains 10-25 asci, which are ovate to broadly obovate, rarely subglobose, more or less stalked, 60-90 x 30 -50 µm in size. The number of ascospores formed in

each ascus is usually two and rarely three (fig. 14.6). They are hyaline, oval or sub cylindrical and measure 20-30 x 12-18 µm. At maturity ascocarp peridium cracks to expose asci, and ascospores are released by bursting of the ascal apices.

The conidia or ascospores, when deposited on the host leaf surface, germinate to give rise to a long germtube which forms a globose, thin walled appressorium. From the appressorium the infection pegs arise which penetrate epidermal cells to form haustoria, thus establishing the pathogen on the leaf surface.

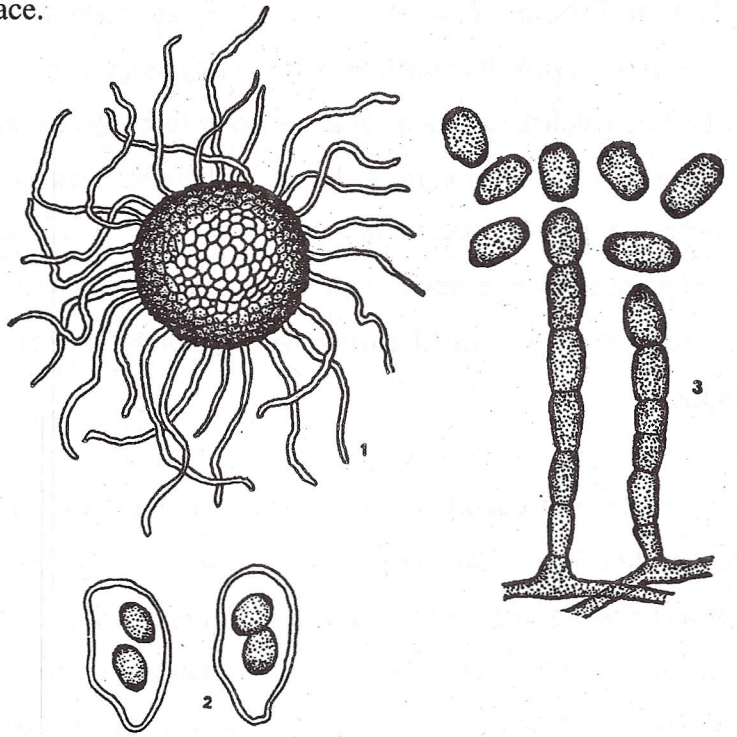


Fig 14.6. *Erysiphe cichoracearum*. 1. Ascocarp with hyphal appendages 2. Asci with ascospores 3. Conidiophores and conidia

14.4.2.2. *Sphaerotheca fuliginea*: It is similar to *E. cichoracearum* in most of the morphological characters, and hence the confusion in identification. It differs from *E. cichoracearum* mainly in the following characters.

The powdery patches (symptoms) formed by *Sphaerotheca* are not pure white but have reddish brown tinge, because mycelium, though mostly hyaline, show reddish brown tinge when old.

The conidia on germination form a characteristic forked germ tube, while those of *E. cichoracearum* produce appresoria on unbranched germ tube.

The ascocarps of *Sphaérotheca* contains only one ascus with eight ascospores (14.7) while in *E.cichoracearum* ascocarps contain large number of asci, with each ascus containing only two ascospores. This is the most important character to distinguish the two pathogens.

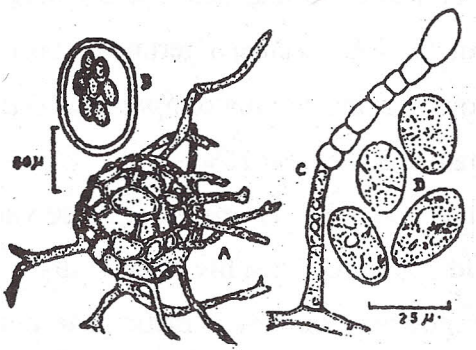


Fig 14.7. *Sphaerotheca fuliginea* A. ascocarp B. ascus C. conidiophore D. conidia.

14.4.3. Epidemiology:

14.4.3.1. Survival: The conidia are relatively short lived, and the method of survival or perpeputation of the pathogens between the two crop seasons is not very clear. Where ascocarps are formed they can help in perennation of the fungus from one season to the next. It is also possible that wild cucurbits harbour the conidial stage of the fungus, and release conidia for primary infection of the spring or summer sown cucurbits. Further, cultivation of cucurbit vegetables (one or the other) is continuous throughout the year, at one place or the other, and this continuous cropping helps in perpetuation of the pathogens.

14.4.3.2. Environmental factors:

Cucurbit plants are susceptible to powdery mildew disease at all stages of growth. Hence, the environmental factors play an important role is disease severity. The disease is usually severe in post-rainy season and cool, dry periods are favourable.

Temperature: As a group, the powdery mildews have an optimum temperature of 21°C for germination and growth and the range varies for the individual pathogens.

E. cichoracearum can germinate at 5°C but will not infect until the temperature is close to 10°C. the fungus can germinates at 33°C but maximum temperature for infections is 27°C. Thus the conidial germination can occur between 5 to 33°C but infection can occur only between 10-27°C, and optimum range is 20-25°C. *Sphaerotheca* is having much lower optimum of 15-20°C.

Moisture: In general, free moisture is inhibitory to powder mildews. Not only germination is inhibited but growth of mycelium is also abnormal (short and enlarged) when a moisture film exists on leaf surface. The conidia of *E. cichoracearum* germinate best at near saturation, but can germinate at low RH range also. However, the conidia of *Sphaerotheca* require high humidity for germination, and they fail to germinate at RH below 75%.

The conidia of *Sphaerotheca* and *E. cichoracearum* lose viability within 1 to 3 hrs, if immersed in water, but floating conidia germinate readily after 24 hrs.

Light: The direct effect of light on powdery mildew is difficult to assess. That shading is more favourable for powdery mildews has been noted by a number of workers. Light effects formation and maturation of appresoria. Maturation was favoured by low light intensities and inhibited by high light intensities. Germ tubes are negatively phototropic to white light at low light intensities. This would tend to favour infection in low light, but excellent infection takes place in complete darkness.

14.4.3.3. Dispersal: The conidia are readily dispersed by air, and they are perfectly suitable for aerial spread, as they are foliar ectoparasites producing numerous dry conidia. They are mainly dispersed during daytime and spore concentrations in air increase with increasing wind speeds. They are passively liberated and no active mechanism is involved.

14.4.4. Control: Field sanitation and fungicidal sprays are recommended for control of powdery mildew disease of cucurbits. Roguing of infected plant parts helps in reducing the inoculum density in the field. Effective control can be achieved by spraying fungicides at frequent intervals. Sulphur dusting is a very common control measure against powdery mildews. Spraying of wettable sulphur is more effective than sulphur dusting. Spraying of copper sulphate is also found to be effective. Dinocap, an aromatic compound marketed under the trade name Karathane, is the most popular, widely used and effective fungicide for control of powdery mildews. Spraying with 0.05 to 0.2% Karathane is recommended for control of powdery mildews.

14.5 SUMMARY:

Club root disease is a serious disease of cabbage, cauliflower, radish and other cultivated crucifers, characterized by abnormal swelling of roots and making them non functional. It is caused by *Plasmodiophora brassicae*. The fungus survives in the soil as resting spores, which germinate to give rise to primary zoospores that attack the root hairs of susceptible crops. The root hair infection leads formation of zoosporangia and zoospores. These zoospores apparently behave like gametes and fuse in pairs. The resulting zygotes infect cortical cells, which become swollen resulting in characteristic symptoms. In the cortical cells, the mature plasmodium produce resting spores, and the nuclear division before resting spore formation is believed to be meiotic. The plants are susceptible at all stages, and disease is severe in acidic soils. Since it is a soil borne disease, it is difficult to eradicate and to reduce disease severity field sanitation, raising soil pH by liming, and crop rotation should be followed.

Damping off disease is a severe disease of vegetable crops belonging to various families. The disease of germinating seeds and young seedlings are collectively called damping off and two phases viz. pre emergence damping off of germinating seeds, and post emergence damping off of young seedlings are recognized. The second phase is most distinctly observed stage and it occurs before the seedlings develop mechanical tissue. It is characterized by toppling over of the infected seedlings with in 2-3 days. It is caused mainly by species of *Pythium*, a zoosporic fungus, and *Rhizoctonia solani*, a deuteromycotina fungus. Both are soil borne facultative pathogens. The disease is severe in seedbeds and for reducing the severity management practices such as seedbed management; soil sterilization and seed treatment are suggested.

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.

14.6. MODEL QUESTIONS

Essay type questions

1. Discuss the symptoms, etiology and control of club root disease of crucifers
2. Describe the symptoms, causal organisms and factors effecting damping-off disease of vegetable crops
3. Give an account of pathology of powdery mildew disease of cucurbits.

Short answer questions

Plasmodiophora brassicae

Post emergence damping off

Epidemiology of powdery mildews

Control of damping off

Erysiphe cichoracearum.

14.7. Reference Books

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2. Rangaswami, G. and Mahadeven, A.1999. Disease of crop plants in India 4th edn., Prentice Hall of India Pvt. Ltd., New Delhi
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PROF. K.V.MALLAIAH

LESSON 15: FUNGAL DISEASES OF CROPS PLANTS –2

OBJECTIVE: To study the pathology of three important fungal diseases of crop plants viz. rust disease of ground, whip smut of sugarcane and blast disease of rice.

CONTENTS:

15.1. Introduction
15.2. Rust disease of groundnut
15.3. Whip smut of sugarcane
15.4. Blast disease of rice
15.5. Summary
15.6. Model questions
15.7. Reference books

15.1. INTRODUCTION

Rust is an important disease of crop plants which rapidly spread with in a short period, causing serious yield losses. There are about 4000 species of rust fungi distributed among 100 genera, and all the rust fungi belong to the order uredinales of class teliomycetes of Basidiomycotina. Rusts are obligate parasites. The pathology of rust fungi is explained taking the example of groundnut rust, which became a serious disease of the crop since 1970s in different parts of the world.

The fungi belonging to ustilaginales of teliomycetes produce smut diseases on higher plants. There are about 75 genera and 1100 species of smut fungi. Smut fungi commonly attack reproductive structures of the plant, and black spore mass is formed on the infected part. Hence the symptom is described as smut, meaning soot or black powder. Smut disease is very prevalent on a number of crop plants, and one of the most conspicuous smuts of crop plants is whip smut of sugarcane, in which infection results in transformation of the entire inflorescence into a long whip like structure. The pathology of smut diseases is explained taking the example of whip smut of sugarcane.

A large number of deuteromycotina fungi like species of *Alternaria, Cercospora, Colletotrichum, Drescshlera, Helminthosporium, Pyricularia* etc cause serious diseases of crop plants. Blast disease caused by *Pyricularia oryzae* is the most important disease of rice crop and the details of this disease are also explained.

15.2 RUST OF GROUNDNUT

The rust disease of groundnut (*Arachis hypogaea* L) caused by *Puccinia arachidis* became a serious disease of the crop, wherever the groundnut is grown since 1970s. Prior to 1969, the disease was largely confined to south and central America. The disease was first described by Spegazzini in 1884 on the leaves collected from Uruguay and Paraguay. In India it was first recorded by Chahal and Chohan in 1969 from Punjab.

15.2.1. Symptoms: The rust of groundnut is typically postulate (fig.15.1.) The disease appears in the field when the crop is about 6 weeks old and rapidly increases under favourable weather conditions. The pustules first develop on the lower side of the lower leaves and then spread other aerial parts. The infections first appear as white flecks on the lower surface of the leaflets, which turn yellowish green flecks with next 24 hours. Uredinial pustules become visible by rupturing the epidermis with in whitish flecks on lower surface and slowly enlarge. Pustules on upper surface appear opposite some of those on the lower surface a short time later. Individual pustules are very small, 0.3 to 0.6 mm in diameter, circular and often surrounded by leaf tissue that is dull green to light brown. Coalescence of infection sites is common leading to elongate or variously irregular patches of sori. Erumpent pustules vary in colour from dark orange when young to dark reddish sites at maturity. The plant tissue surrounding the visible sites of infection becomes necrotic and dessicated in irregular patches and eventually leaflets curl and drop off. Leaflet necrosis is usually more prominent on the upper surface. In severe cases, the pustules also develop on petioles and stems.

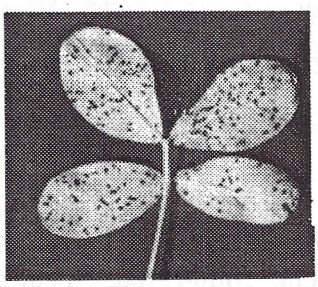


Figure 15.1. Groundnut leaf with rust pustules

15.2.2. Etiology: The rust on groundnut is caused by *Puccinia arachidis* which belongs to the family puccinaceae, order uredinales, class teliomycetes of subdivision basidiomycotina.

Only uredinial and telial stages of the pathogen were discovered so far, and in most of the groundnut growing countries only uredinial stage occurs. The telial sage occurs very rarely.

The pathogen is obligate and mycelium is internal, intercellular sending in haustoria into the host cells. Hyphae are septate without clamp connections. Each pustule is formed on a limited mycelium, which gathers below the epidermis. Urediniospores are formed on delicate stalks that dehisce leaving the spores free. Urediniospores are produced in large numbers, and eventually the epidermis ruptures, due to the pressure from within, exposing the spore mass. Urediniospores are broadly ellipsoidal or ovoid 16-22 x 23 to 29 µm (average 21-25 µm) in size (Fig. 15.2). The spores are thick walled, and the wall is brown in colour and 1.2 to 2.2 µm thick, moderately echinulate, with 2 nearly equatorial germ pores.

Figure 15.2. Urediniospores of groundnut rust

Telial stage of the rust is rarely formed, and where formed the telia are hypophyllous, small 0.2 to 0.3 mm in diameter, scattered, chestnut brown and ruptured epidermis appears prominently. Teliospores are smooth, oblong or ovate, often with 3 to 4 cells, chestnut brown, 14 to 16 x 38 to 42 µm, rounded or obtuse above and some what narrowed below, slightly constricted at the septum (fig. 15.3.).

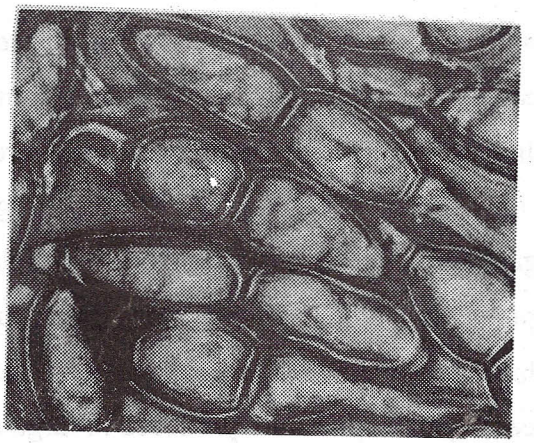


Figure 15.3. Teleutospores of groundnut rust

Groundnut rust exclusively occurs in uredinial stage in most countries, and the role of teliospores in disease cycle is not known.

The urediniospores are dispersed aerially and when they are deposited on the host leaf surface, they ge minate by give rise to a delicate germ tube. Free water is essential for germination and temperatures in the range of 20-25 °C are optimum. The germ tubes produce a globose, thin walled appressorium, and infection pegs arising from the appresoria enter the leaf either penetrating through stomata or directly between the epidermal cells within the leaf, the mycelium grows, exclusively in the intercellular spaces sending in haustoria into the host cells. The pustules are formed first on the lower surface of the leaf, irrespective of the surface of penetration. The incubation period is 6 -7 days under favourable conditions.

15.2.3. Epidemiology:

15.2.3.1. Survival: Groundnut rust is known almost exclusively by its uredinial stage and there are only a few records of the occurrence of telial stage. The urediniospores on infected crop debris lose viability within four weeks. Hence, the survival of the fungus in the absence of crop, and primary source of inoculum remains as an unsolved problem. Subrahmanyam and McDonald (1984) postulated that in India the practice of continuous cultivation of groundnut is an important factor in perpetuation of groundnut rust. The overlapping crop seasons in India (Figure 15.4) helps in perpetuation. Mallaiah and Rao (1980) observed that groundnut crop cultivated in summer usually do not show rust disease but rust appears severely within a few days on summer crop after first monsoon rains. Basing these observations they postulated that rust survives as mycelium in

the host tissue without forming symptoms during hot summer. Similar situations may be occurring in other countries also.

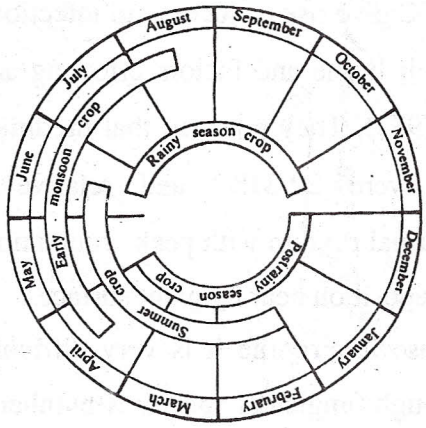


Figure 15.4. Continuous cropping pattern of groundnut in India

The possibility of long distance dispersal of groundnut rust urediniospores was postulated by Van Arsdel and Harrison (1972). They reported that initial infections in groundnut fields in Texas, USA arose from urediniospores originated in Mexico. They trapped the spores in rainwater during July-August 1970, and observed that rust appeared in the fields 10-15 days later. At that time rust was prevalent in a region of Mexico 1290 km distant, and meteorological conditions, especially wind direction and speed are favouring the long distance dispersal of the spores.

15.2.3.2. Factors effecting: A number of factors effect the disease incidence and spread.

Age of the host: Groundnut plants are susceptible to rust at all ages. Inoculation experiments have shown that coalescence of the pustules and early leaf fall occurs when the plants are 10-30 days old, and number of pustules formed is always higher on plants of 40-50 days old.

Surface of the leaf: The number of pustules formed and their size are always more on lower surface of leaf than on upper surface. The pustules on upper surface always appear later and smaller, and they are usually opposite to those on lower surface. Irrespective of surface of inoculation, pustules always appear first on lower surface. Inoculated area of the leaflets turns yellow on upper surface, while such yellowing was rare on lower surface. These observations indicate that the lower surface is more susceptible than upper surface.

Effect of weather: The optimum temperature for germination of urediniospores is 20°C and for germ tube growth 25°C. The urediniospores do not germinate at RH below 100% and free water is

essential. Hence rust is more severe during rainy season. Night temperatures of 22-25°C and day temperatures ranging from 30-43°C give rise to successful infections.

Secondary spread: The rust is air borne and factors effecting actual spread of groundnut were reported by Mallaiah and Rao (1982). They reported that urediniospore incidence in the air was maximum when temperatures were 29-31°C and relative humanity 75-85%. Airborne urediniospores showed a clear diurnal rhythm with peak around noon. Further, it was reported that around 20% of air borne spores deposit on near by plant surfaces.

15.2.4. Control: Since the disease is airborne it is very difficult to control. The only method available is chemical control through fungicidal sprays. A number of fungicides were found to be effective against urediniospore germination, but relatively few fungicides are used at field level. These include dithane M-45, dithane Z-78, calixin(tridemorph), chlorothalonil etc. Fungicidal sprays at 2-3 week intervals starting from 40-50 days after sowing give reasonable protection.

Using resistant varieties is the best method, but most of the commonly acceptable cultivated varieties are susceptible. However, some resistant genotypes like EC 76446(292), NCAC 27090, PI 259747, PI 314817 etc are recognized, and breeding programmes are going on at ICRISAT and other centers to develop commercially acceptable varieties using rust resistant genotypes.

15.3 WHIP SMUT OF SUGARCANE

Sugarcane (*Saccharum officinarum* L) is one of the most important cash crops of India. A number of diseases were reported on the crop, and of all diseases, whip smut of sugarcane is the most common and conspicuous disease, as the inflorescence is transformed into a long whip like structure due to the attack of a smut fungus *Ustilago scitaminea*. The disease was reported from various countries including India, West Indies, South Africa, Philippines, China, Italy etc. it is primarily a disease of wild canes and those sugarcane varieties which nearly approach wild canes are most susceptible to the disease. This disease is a systemic disease and main symptom appears at the time of inflorescence emergence stage.

15.3.1. Symptoms: This is one of the most easily recognizable disease of crop plants. The affected plants are stunted and the central shoot is replaced by a long whip-like dusty black structure, several feet in length (Fig. 15.5). At first the mass of smut powder on this out growth is covered by

a thin silvery membrane made up of host epidermis. It soon ruptures exposing the dense black powdery mass consisting of the smut spores. The spores are easily dispersed even by a gentle breeze.

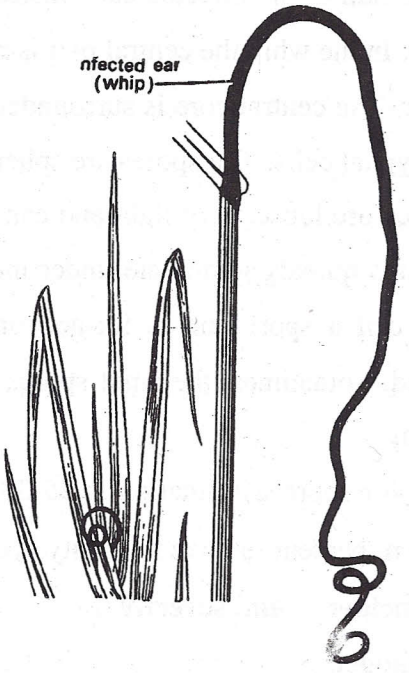


Figure 15.5. Whip smut of sugarcane

From the lower part of the systemically infected canes, lateral buds sprout into lateral shoots which also produce smutted whips. The production of these lateral shoots is stimulated, if the whip at the top of the main shoot is cut-off.

Since the affected plants are greatly stunted and became thin, the black whips stand out prominently in the field, and can be easily identified from a distance.

There are two main flushes of the production of whips, the first during May-June period and the second during October-November period. The presence of infection in seed pieces result in first flush while secondary infections results in appearance of whips late in the season.

15.3.2. Etiology: The disease is caused by *Ustilago scitaminea*. In India, Mundkar (1939) had identified two varieties in the species viz. *Ustilago scitaminea* var *sacchari-barberi* and *Ustilago scitaminea* var *sacchari officinarum*, on the bases of the size, colour and markings or the spore wall.

The fungal mycelium is formed abundantly in the infected tissues of the cane. The hyphae are septate much branched without clamp connections and spread in the intercellular spaces

sending in haustoria into the host cells. The fibro vascular bundles are not attacked. As the cane grows, the mycelium in the lower parts tend to disorganize and accumulate at the growing tips. At the time of flowering, the terminal bud of the infected cane instead of producing an inflorescence produce a long whip like structure. In the whip the central part is made up of vascular fibrous mass of host cells, held closely together. The central core is surrounded by a thick mass of smut spores formed by the transformation of hyphal cells. The spores are spherical, light brown, echinulate and measure 5-10 μm in diameter. They are loose, very light and can be easily disseminated by wind. On a suitable substratum, the spores quickly germinate under moist conditions producing 3 or 4 celled promycelium. From each cell a sporidium is formed on short stalks. The sporidia are elongate, hyaline and single celled. Sometimes, the smut spores may germinate to give rise to a germ tube rather than promycelium.

The optimum temperature for spore germination is 25-30°C, the maximum 36°-40°C and minimum 5°-9°C. Free water or cent percent relative humidity is essential for spore germination.

15.3.3. Factor effecting disease incider and severity

15.3.3.1. Perpetuation of the pathogen.

The sugarcane crop has no dead season and hence, survival is not a problem for the pathogen. Field experiments have shown that the pathogen perpetuate and spreads in many ways.

1. **Planting of setts of smutted canes:** The diseased setts containing living mycelium when used for planting give rise to smutted plants.
2. **Spores borne on buds:** If setts with buds carrying spores are planted the disease may be produced in the canes developing from such buds.
3. **Infection of buds on standing crop:** During the growing season, spores from smutted whips may fall on the exposed buds. Some of these infected buds may give rise to smutted whips in the same season, while others carry the dormant mycelium until setts bearing them are planted in the next crop season.
4. **Ratooning of the crop:** The ratooned stumps of diseased canes harbour the mycelium of the fungus, and fresh shoots arising from these stumps are invariably smutted.

15.3.3.2. Secondary spread in the field: The spores of smutted whips produced in the first flush, are easily disseminated by air and some of the spores are deposited on the near by canes. The spores

deposited at the junction of the leaf and leaf sheath travel down the sheath and reach the tender nodal region. At the nodal region surrounded by leaf sheath sufficient moisture accumulates and facilitates the spore germination. The spore germinates within 24 hours at an optimum temperature of 25-30°C. The diffusates from the buds may stimulate the spore germination. Copius mycelium can be seen in the growing point of nodal buds, and the buds are easily infected. These nodal buds give rise to branches bearing smutted whips. The nodal buds are stimulated to grow, when the whips formed on the main branches are pruned.

15.3.4. Control:

Prevention rather than cure is the best method of controlling the sugarcane smut. Preventive measures should be taken from planting of the seed setts to harvesting of the cane.

15.3.4.1. Selection and treatment of seed setts: Setts for sowing should be selected from only healthy fields without disease. The setts before sowing should be treated with disinfecting fungicides like organomercurials. A 5 minute dip in 0.1% mercuric chloride solution or 0.25% solution of Methoxy ethyl mercuric chloride (MEMC) is recommended. The mercurials inactivate inoculum on the surface only. If presence of internal mycelium is suspected, the setts should be treated with systemic fungicides like vitavax, benlate, bavistin etc.

15.3.4.2. Avoid ratooning: The practice of leaving cane stumps to raise the next crop is called ratooning. Since rationing of the infected stumps invariably give rise to smutted whips, the practice of ratooning should be avoided.

15.3.4.3. Roguing of smutted whips and canes: When the disease is sporadic in the filed, a careful watch must be kept, and as soon as a whip is spotted, it must be removed carefully without allowing the spores to shed on to the ground or disperse to the neighbouring canes. The plant itself must be up rooted and burnt, as it may be a potential source of infection.

15.3.4.4. Soil sterilization: After harvesting the trash may be burnt in the field so that the soil is partially sterilized. Such a practice is carried out by farmers routinely in the rural areas.

15.3.4.5. Crop rotation: If the disease becomes serious in the field, crop rotation should be followed to starve at the spores in soil.

15.3.4.6. Use of resistant varieties: A number of cultivated varieties with resistance to smut are released in India. They include Co (coimbattage) varieties like 300, 301, 311, 336, 475, 508, 975 etc and BO varieties like BO 11, 22, 24 and so on.

There is frequent failure of resistance in sugarcane varieties. Hence, it has been recommended that is a particular locality even a resistant variety should not be grown continuously for many years.

15.4 BLAST DISEASE OF RICE:

Blast is one of the earliest known and most widely distributed diseases of rice crop occurring in more than 80 countries. It is known to occur in Japan in 17th century. It was first described by Cavara from Italy in 1891. In India, it was first recorded in 1913, and a devastating epidemic occurred in 1919 in Tanjavore delta of Tamilnadu. Losses up to 75% were recorded in epidemic years, and even in non-epidemic years losses are considerable.

15.4.1. Symptoms:

By definition, blast disease means sudden death of flower buds or inflorescences. In rice also, the panicle is affected causing great loss of yield and hence, the disease is called blast of rice. The disease is also common on leaves and stems.

The leaf spots are typically elliptical or spindle shaped with more or less pointed ends. The centre of the spot is usually greyish or white and the margin is usually brown or reddish brown. A diffuse yellow halo may occur around the spots.

Both the shape and colour of the spots vary depending upon the environmental conditions, age of the spots and degrees of susceptibility of the rice cultivars. The sots usually begin as small, water soaked, whitish or greyish or bluish flecks. They enlarge rapidly under moist conditions on susceptible cultivars and attain a size of 1-1.5 cm long and 0.3-0.5 cm broad, and develop a brown margin with greyish centre. On resistant cultivars, the spots do not enlarge much and remain as minute brown flecks. On cultivars with intermediate reaction, small round or short elliptical lesions, a few mm long develop. The small brown lesions are called 'chronic spots' and greyish spots which rapidly enlarge are called 'acute spots' (fig. 15.6).

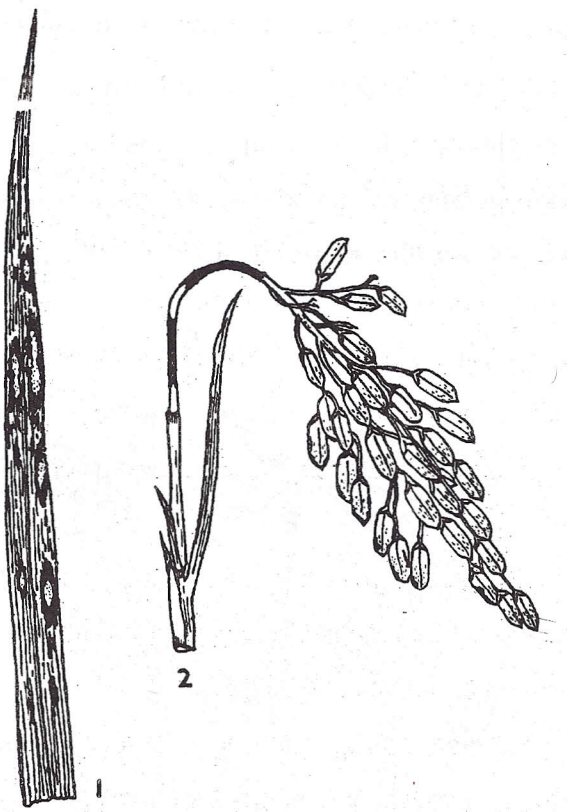


Figure 15.6. Leaf spot (1) and neck blast (2) symptoms of blast disease of rice

When the node is infected, the sheath pulvinous rots and turn black, and often breaks part. All parts above the infected node die.

Any part of the panicle may be infected producing brown or black lesions. Areas near the panicle base are often attacked causing rotten neck or neck rot symptoms. It is also called neck blast. If the infection has occurred much before the grain formation, the grains are not filled and the panicle remains erect, but if the attack takes place after some grains have formed, the panicle hangs down and fall off, as the rachis breaks at the infected region. This stage of the disease causes maximum damage.

15.4.2. Causal organism: The pathogen of blast disease of rice is *Pyricularia oryzae*. It is an imperfect fungus belonging to the family dematiaceae, order moniliales and class hyphomycetes. The perfect stage of the fungus is not known to form in nature. Fungus is heterothallic. However, when strains from widely different geographical zones were brought together in culture, the

fungus produces ascigerous stage and the perfect stage belongs to ascomycete fungus *Ceratosphaeria grisea.*

Mycelium is internal, consists of septate, mostly uninucleate, branched hyphae. Conidiophores arise in singles or in fascicles and emerge through stomata. They are simple, rarely branched, septate, slender, geniculate and greyish in colour. Conidia are produced in succession one at a time at the tips of the conidiophores (fig.15.7).

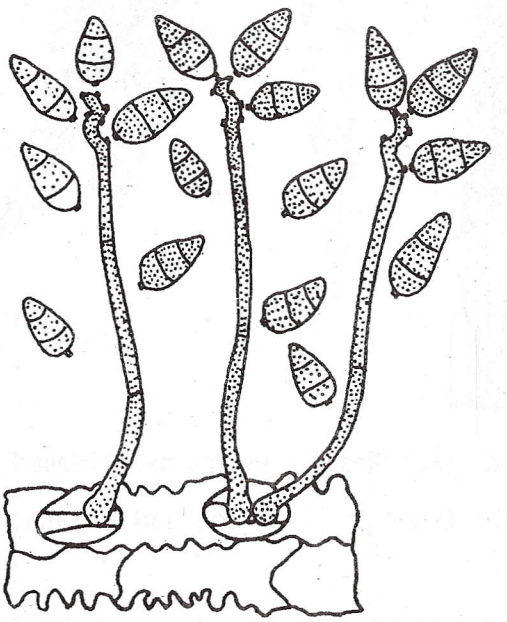


Figure 15.7. Conidiophores and conidia of *Pyricularia oryzae*

One to 20 conidia may be formed on a single conidiophores. Conidia are pyriform to obclavate, base round, apex narrowed, usually 2-septate, some times slightly constricted at the septa. They are hyaline to pale olive in colour and measure 19-23 x 7-9 µm in size with a small basal hilum of 2 µm diameter. The conidia are released from the conidiophore and disseminated by air currents. On a suitable substratum, conidia germinate within 3 to 4 hrs from the apical or basal cell and rarely from middle cell. The germ tubes form appressoria from which infection pegs arise and penetrate the host cells. After infection, the lesions appear in about 4 days and sporulation starts in 6-7 days.

15.4.3. Factors influencing disease development:

15.4.3.1. Pathogen factors: Spore production, dissemination, deposition, germination and infection are important pathogen factors in disease cycle.

15.4.3.1.1. Spore production: Conidia are produced on lesions in about 6 days after infection. The rate of sporulation increases with increase in R.H. Below 93% RH no conidia are produced. A typical lesion produces 2000-6000 conidia each day for about 14 days. Conidial production reaches peak 5-8 days after appearance of leaf lesions.

15.4.3.1.2. Spore release and dissemination: Most the spores are produced and released during night, particularly between 2 and 6 a.m. Presence of moisture on leaf surface is essential for spore release. When treated with water, most spores are discharged within 2 minutes, especially during the initial 30 seconds. The spore release is also triggered either by sudden increase or sudden decrease in humidity. Spores are also released by strong winds. Ingold (1964) reported that the conidium is probably violently discharged, although to a very short distance, by the bursting of the minute stalk cell attaching it to the conidiophore.

15.4.3.1.3. Dispersal: Under relatively calm conditions with heavy dew or, moisture, which favour spore release, dispersal occurs within field around the plants. In stronger winds the conidia are dispersed to longer distances.

15.4.3.1.4. Spore deposition: The number of spores deposited on a leaf differs greatly according to the position of the leaf and the angle between the leaf and the stem. More spores are deposited on 3rd leaf than on second, and still fewer on the top leaf. Usually around 20% of the airborne spores are deposited on the leaf surfaces.

15.4.3.1.5. Germination and infection: Free water is required for germination and high relative humidity near saturation in necessary for infection. Infection follows germination of conidia, formation of appressoria, and production of infection pegs from appresoria which penetrate the host directly through epidermis or through stomata. Optimum temperature for germination and appressorial formation is 26-28°C. Maximum infection occurs at temperature of 24-28°C and with 16-24 hrs of continuous wetting. Infection occurs most readily in darkness and suppressed under diffused light.

15.4.3.1.6. Incubation period: The incubation period varies with temperature: 13-18 days at 9-10°C, 7-9 days at 17-18°C, 5-6 days at 24-25°C and 4-5 days at 26-28°C.

15.4.3.2. Host factors: Blast reaction on the host varies according to varieties. Within the same variety, severity varies with age of the plant, host nutrition and predisposition due to meteorological factors such as temperature and moisture.

15.4.3.2.1. Age of the host: There are three distinct stages of plant growth at which rice is highly susceptible to infection to blast pathogen. Seedling stage and rapid tillering stage (15-30 days after translocation) for foliar infection and ear emergence stage for nodal neck infections. The upper surface of the leaf is more susceptible than the lower surface.

15.4.3.2.2. Host nutrition: Heavy nitrogenous manuring increases the susceptibility of the plants. Neither phosphorous nor potassium have any effect on blast incidence on susceptible varieties. However, plants growing in soils deficient of phosphorous show more disease, especially at lower temperatures. This is because phosphorous occurs in bound (insoluble) form at low temperatures and in readily available (soluble) form at high soil temperatures.

Silence content of the leaves is having important effect on disease incidence. Silicon content and susceptibility of the plant are inversely related. When silicon uptake is more, it combines with one or more constituents of cell wall to form complex substances resistant to digestion by enzymes produced by the fungus. Under higher nitrogen content of the soil, less silicon is taken up by the plant. When plants are growing in nitrogen deficient medium, there is greater absorption of silicon, which imparts resistance to the fungus in the plant.

15.4.3.2.3. Effect on environmental factors on host susceptibility: Infection occurs most severely on plants growing at 20°C or below and disease incidence progressively decreases with increase of temperature up to 30°C.

The rice plants growing in dry soils are highly susceptible to blast, moderately resistant in moist soil and resistant under flooded conditions.

Slight shading at the early stages of lesion development favour extension rapidly while further development is favoured by high light intensities.

The role of nyctotemperatures (night temperatures) on host susceptibility was stressed by research works at Madras University. They observed that at a night temperature of 30°C the number and size of lesions is small on susceptible varieties while resistant varieties remain free

from infection; at 20°C the susceptible varieties show a 3-fold increase in number of lesions and increase in size of the lesion, while on resistant varieties the lesions are few and small; at 15°C both susceptible and resistant varieties show high infection with large lesions. This was attributed to the effect of Nycto temperatures on metabolic pattern of the host. At low temperatures, the absorbed nitrogen tends to accumulate as soluble nitrogen which favours nitrogen metabolism and better utilization of nitrogen, while high night temperatures favour secondary metabolism leading increased mechanical resistance and formation of phenols associated with active resistance.

15.4.4. Disease forecasting: Effective forecasting systems are not available in most of the rice growing countries. However, in Japan, a very elaborate forecasting system was developed. Suziki (1969,74) observed that the number of airborne spores trapped correlate with degree of blast incidence, and basing on that developed a forecasting system as given in the table 15.1.

15.1. Forecasting system of blast of rice based on airborne conidia

Maximum number of spores per day at periods more than 2 weeks before heading.	Degree of leaf blast severity forecasted	Percentage of panicles with neck rot forecasted
Less than 30*	Slight	Less than 5%
From 30-160	Moderate	20%
From 100-500	Severe	60%
More than 500	Very severe	100%
*Number of spores counted in the area 24 x 18 mm under a cover glass.		

El Refaei (1977) found that the number of lesions was more closely correlated with dew periods than with number of airborne spores. Using both dew period and airborne spores, a fairly good fore casting system, 5 days a head, was developed using regression analysis.

15.4.5. Survival of the pathogen: In temperate regions, mycelium and conidia on diseased straw and seed are the principal over wintering structures. In the fields, commonest source of primary inoculum is the conidia present and formed with in the straw piles.

In tropics, over wintering is not a problem in disease cycle because airborne conidia are present throughout the year. The fungus occurs on a number of weed hosts like *Digitaria*

marginata, Dinebra retroflexa, Leersia hexandra, Echinochloa etc; and their role in survival of the fungus during the absence of the crop is stressed by many researchers. However, they may or may not be the strains pathogenic rice in all cases.

15.4.6. Control measures: Three primary control measures for blast are cultural practices, fungicidal sprays and use of resistant varieties.

15.4.6.1. Cultural practice . Field sanitation, destruction of alternative hosts and seed treatment are precautionary measures.

Since the pathogen is seed borne, seed should be selected from healthy fields. Seed treatment with organomericurials such as agrosan is effective in eliminating externally seedborne inoculum. A mixture of 20 ppm aureofungin and 20 ppm copper oxychloride is very effective for seed treatment.

Early sowing of short duration varieties, often escape disease severity, and must be followed wherever possible.

15.4.6.2. Chemical control: Blast can be controlled in the nurseries and main fields by fungicidal sprays at frequent intervals.

Copper fungicides are effective in controlling blast but tend to be phytotoxic, especially on high yielding varieties.

Organophosphorus fungicides such as kitazin and Hinosan have been used successfully in recent years. Hinosan (Edifenphos) is especially the fungicide of choice against the disease.

Systemic fungicides especially benzimidazole formulations like carbendazim, benlate, MBC etc. are also effective in controlling blast disease.

Antibiotics are extensively used in Japan. The common antibiotics used are Blasticidin-S (Bla-S) and kasugamycin (kasumin). They are very effective at 20-ppm concentration.

15.4.6.3. Resistant varieties: Use of resistant varieties is the best method to control the disease.

International cooperative studies have shown that usually exotic varieties show field resistance. Thus, many japonica varieties are resistant in South Asia (India, Pakistan, Srilanka), while indica varieties are resistant in temperate Asia (Japan, Korea). However, Taichung native and other similar varieties introduced into India for their high yield were found to be susceptible.

Breeding for disease resistance and release of new resistant varieties is going on both at National and International level. Breeding for disease resistance in India is going on since 1920s.

Central Rice research Institute (CRRI) at Cuttack at National level, and International Rice Research Institute (IRRI) at Manila, Philippines at International level, are premier Institutions for Rice research.

Cultivars like Jaya, Vani, Akashi, Rasi, IR-8, IR-36 are some of the promising varieties released in India.

Varieties like Bharani and Vaigai exhibit high field resistance but need chemical protection in epidemic years.

15.5. SUMMARY

The rust disease of groundnut caused by *Puccinia arachidis* is typical pustulate rust that spreads very rapidly attacking leaves, petioles and stems. The disease appears in the field when the crop is about 4-6 weeks old and becomes very severe when the crop 70-80 days old. Only uredinial stage of the rust is recorded in most parts of the world and telial stage was reported only occasionally. The urediniospores of the rust spread aerially and there is evidence of long distance dispersal also. The rust is very difficult to control but the severity can be reduced by fungicidal sprays. Growing resistant varieties is the best method.

Whip smut of sugarcane is caused by *Ustilago scitaminea.* The smut infections are systemic but produce no symptoms in the vegetative parts, but at the time of inflorescence formation, the infected branches give rise to a long smutted whip like structure rather than normal inflorescence. The disease spreads in the field by aerial dispersal of the smut spores. When the spores deposit on nodal buds, they become infected and give rise to smutted whip. The infected seed setts used for planting is the main source of inoculum to the new crop. Ratooning of the crop invariably gives rise to smutted whips. The disease is very difficult to control, but severity can be reduced by proper cultural methods.

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. Nodal infection result in breaking of the parts above to the infected node. Neck rots result in dropping of the inflorescence. The fungus survives in the infected straw and

also seedborne 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. Nyctotemperatures (night temperatures) also play an important role on disease development. In Japan forecasting system was developed basing on the concentrations of airborne conidia. 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.

15.6. MODEL QUESTIONS:

Essay type questions

1. Discuss the symptoms, etiology, epidemiology and control of blast disease of rice.
2. Describe the symptoms, causal organism and control measures to be taken with respect to whip smut of sugarcane
3. Discuss the symptoms, etiology and epidemiology of groundnut rust.

Short answer type questions.

4. Groundnut rust
5. Whip smut of sugarcane
6. Symptoms of rice blast
7. Epidemiology and control of rice blast.

15.7. REFFERENCE BOOKS:

1. Singh, R.S. 1998 plant diseases 7th Edn. Oxford & IBH publishing Co. Pvt. Ltd, New Delhi.
2. Ranga swami, G. and Mahadevan.A. 199. Diseases of crop plants in India 4th Edn. Practice Hall of India Pvt. Ltd, New Delhi.
3. Mehrotra, R.S. 1983. Plant pathology. Tata McGraw Hill publishing Co. Pvt. Ltd, New Delhi.
4. Mc Donald *et al.* 1987. Groundnut Rust disease. Published by ICRISAT, Hyderabad.

PROF. K.V.MALLAIAH

LESSON 16: PLANT DISEASES CAUSED BY BACTERIA AND VIRUSES

OBJECTIVE: To study the pathology of some important crop diseases caused by bacteria and viruses

CONTENTS:

16.1. Introduction
16.2. Leaf blight of rice
16.3. Citrus canker
16.4. Brown rot of potato
16.5. Little leaf of brinjal
16.6. Mosaic disease of tobacco
16.7. Tungro disease of rice
16.8. Summary
16.9. Model questions
16.10. Reference books

16.1. INTRODUCTION

A limited number of bacteria and a large number of RNA viruses are associated with plant diseases. T.J. Burrill of Illinois University, USA was the first to report that a bacterium later named as *Erwinia amylovora*, cause fire blight disease of apple. Later, a large number of diseases were diagnosed as caused by bacteria. A part from *Erwinia* spp,

a large number of species of *Xanthomonas* and *Pseudomonas*, and *Agrobacterium tumefaciens* also cause wide spread plant diseases. A few species in other bacterial genera such as *Corynebacterium, Streptomyces, Bacillus, Clavibacter, Rhodococcus, Mycoplasma* and *Spiroplasma* also cause plant diseases. Leaf blight of rice, citrus canker, brown rot of potato and little leaf of brinjal are some of the important diseases of crop plants caused by bacteria, and pathology of these diseases are explained

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. Two of the most important viral diseases of crop plants are tobacco mosaic disease and tungro disease of rice. The details of these two viral diseases are also explained in this lesson.

16.2. BACTERIAL LEAF BLIGHT OF RICE:

Leaf blight of rice, caused by *Xanthomonas campestris* pv. *oryzae*, is a wide spread disease of the crop in all rice growing countries, especially in Asia. It is known to occur in Japan since 1881, and in India it was first reported from Maharastra by Srinivasan *et al.* (1959), and now occurs in all rice growing regions of the country.

16.2.1. Symptoms: The blight pathogen causes two types of symptoms on the host viz. 1. Leaf blight, which is most commonly visible and 2. Wilt or kresek, which is most destructive.

16.2.1.1. Leaf blight phase: Leaf blight phase of the disease is characterized by linear, yellow to straw coloured stripes with wavy margins, generally on both edges of the leaf, rarely on one edge. These blighted patches start from the tip and extend downwards. This is followed by drying and twisting of the leaf tip and rapid extension of marginal blight lengthwise and crosswise to cover large areas of the leaf. Sometimes linear blight patches may develop anywhere on the lamina or along the midrib with or without the marginal blight. The blighting may extend to the leaf sheath and culms, killing the tiller or the whole culm. In dry weather opaque and turbid drops of bacterial ooze, which dry into yellowish beads, can be seen on the leaf surface (fig. 16.1). The blight phase of the disease usually appears 4-6 weeks after transplantation, and progressively increase.

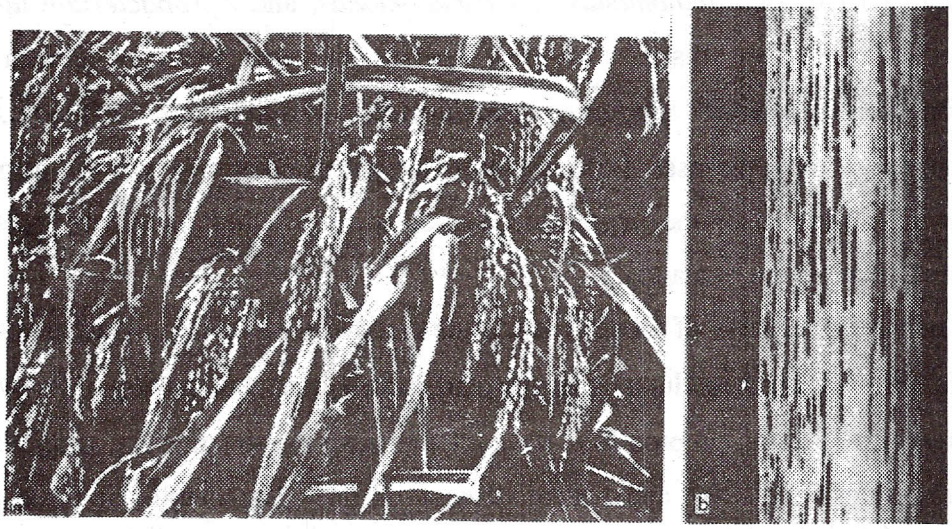


Figure 16.1. A. Rice plants showing blight symptoms B. bacterial ooze seen on the surface of the infected leaves

16.2.1.2. Wilt or kresek phase: The wilt phase of the disease is most destructive and known since long time in Indonesia by the name 'kresek'. It occurs in all tropical countries and result from early systemic infection. The leaves roll completely droop, turn brown or gray and ultimately the tillers wither away. In severe cases the affected stool may be completely killed. It commonly occurs within 3-4 weeks after transplantation of the crop.

16.2.2: Causal organism: The casual organism is *Xanthomonas campestris* pv. *oryzae*. It belongs to the family pseudomonadaceae of gram-negative bacteria group. It is a rod shaped bacterium with rounded ends, measure 1-2 x 0.8-1.0 μm in size, motile by single polar flagellum of 6-8 μm. It is aerobic, gram negative and nonspore forming. The cells are surrounded by a mucous capsule, and joined to form an aggregated mass, which is relatively stable in wate.

The pathogen can grow on ordinary bacteriological media, and on nutrient agar compact yellow colonies are formed. The yellow pigment is insoluble in water. The optimum temperature for growth is 25-30 °C and pH 7.2.

Different strains of the pathogen differ in their virulence and most of the tropical strains are virulent forms, capable of causing considerable damage and kresek symptoms.

16.2.3. Disease cycle: The pathogen can survive in the absence of the crop in many ways. It is seed borne. It is soil borne and occurs in the rhizosphere zone of many grasses. It also infect a number of other grass hosts like *Leersia hexandra, Cyprus rotundus, Panicum repens, Paspalum scrobiculatum* etc. with or without producing symptoms. It may even survive on inflected rice straw.

The primary infection occurs quite early in the season after transplantation or in the seedbeds either through the roots or through leaves. The secondary spread is brought about by entry through wounds or stomata by bacterial cells disseminated by windborne rain drop splashes, by irrigation water, or rain water coming from infested fields or by contact between diseased and healthy leaves. The insects like leaf hopper (*Nephotettix viriscens*) and grasshopper (*Hieroglyphus banian*) can transmit the bacterium mechanically. After the entry the bacterium multiplies in the intercellular spaces of the host parenchyma.

The favourable conditions for disease incidence are rainy weather, dull windy days and an atmospheric temperature of about 25 °C. Plants in shade and closely planted crops supplied with high doses of nitrogen show more disease incidence.

16.2.4. Control:

16.2.4.1. Seed treatment: Since the disease is seed borne, the seed should be treated before sowing. Seed treatment with agrimycin, streptomycin or organomercurials like ceresan can effectively control seed borne inoculum.

16.2.4.2. Chemical sprays: Since the disease has many sources of survival, the disease appears even in the fields sown with disinfected seeds, and chemical sprays are essential to reduce disease severity. Spraying with copper fungicides alternatively with streptocycline (250 ppm) is reported to be effective in controlling the disease.

16.2.4.3 Disease tolerant varieties: Most of the cultivated varieties are susceptible. Some varities like TKM6, IR42 etc are tolerant to the disease and should be used wherever possible.

16.3 CITRUS CANKER

Citrus canker, caused by *Xanthomonas campestris* pv *citri*, is a wide spread severe disease throughout the citrus growing countries of the eastern hemisphere and particularly serious in India, China, Indonesia, Japan, Philippines and other countries. It is thought to have originated somewhere in south East Asia or India, and spread throughout the world.

Almost all the known species of *Citrus* are susceptible, but not equally so. *Citrus limon*, *C. grandis, C. auruntium* and *C. aurantifolia* are among the most susceptible ones. In India, acid lime and lemon have been found to be severely infected, while mandarin and sweet oranges are much less affected.

16.3.1. Symptoms: Canker affects all aerial parts of the plants and particularly prominent on leaves, twigs, young branches and fruits. On the leaves, canker first appears as small, watery, translucent spots, usually of a darker green colour than the surrounding tissues, with a raised convex surface. As a rule, the spots first become evident on the lower surface, and as the disease advances, the surfaces of the spots become white or grayish and finally rupture, exposing a light brown, spongy central mass developed in a crater-like formation. These spots usually become

surrounded by a yellow halo which persists in very old lesions. Older lesions become co. y and brown (fig. 16.2).

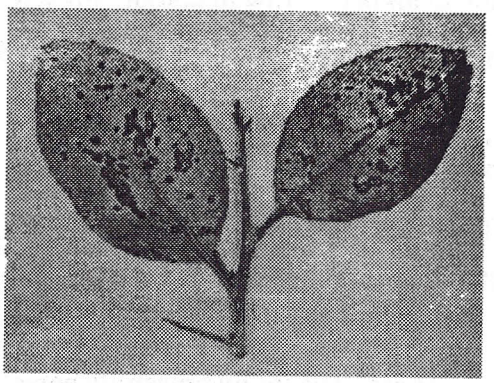


Figure 16.2. Citrus leaves showing canker symptoms

Lesions on twigs are common on the more susceptible species of *Citrus*, and on young twigs they are similar to those on leaves, but on older twigs they are more irregular in shape.

The canker lesions on the fruits have much the same appearance as on leaves, except that the yellow halo is usually absent and the crater like a rance is more prominent. The spongy, rough eruptions may be scattered over the surface, or several may coalesce forming an irregular mass. The lesions on fruits are only skin deep and do not effect the pulp or juice.

In severe infections, the leaf drop is heavy and fruits tend to be smaller. Even though the inner edible or juicy part of the fruit is not affected, the appearance of lesions on fruits reduce their market value.

16.3.2 Causal Organism: The causal organism of citrus canker was originally described as *Xanthomonas citri*, but now it is referred to as *Xanthomonas campestris* pv.*citri*. It belongs to the family Pseudomonadaceae of Gram negative bacteria included in Vol. I of Bergey's manual of Systematic Bacteriology.

The bacterial cells are rod shaped 1.5-2.0 x 0.5-0.75 μm in size. The cells are capsulate and may occur singly or in short chains. In dividual cells are motile by a single polar flagellum. They are gram negative in staining property and do not produce endospores. *Xanthomonas* is mainly aerobic and a poorly facultative anaerobe.

The organism can grow easily on ordinary bacteriological media. The colonies on nutrient agar medium are circular, straw yellow to amber yellow in colour, slightly raised and glistening.

A number of pathotypes with in the pathovariety (pv) *citri* were recognized by host range, serology, bacteriophage typing and other characters. Pathotypes B, C, and D are common on lemon (*Citrus lemon*) and lime (*C. aurantifolia*)

16.3.3 Disease Cycle: Citrus is a perennial plant, and pathogen survives during unfavourable weather on infected plant parts on standing crop. It does not survive in soil or on infected plant parts fallen on the ground for long.

Spread of the pathogen in the orchards occurs by splash dispersal. Rains driven by wind velocity, in excess of 8 m/sec., aid in dispersal. Light rains spread over long periods favour the disease spread than short but heavy rain.

Insects such as citrus leaf miners (*Phyllocnistis citrella* and *Thosconyrsa citri*) also disperse the bacterium. The insect transmission is purely mechanical but not biologic.

The bacterium enters the host thro ·h natural openings (stomata) and through wounds such as those caused by insects, movement .iorns etc. It multiplies in the intercellular spaces, dissolves the middle lamella, and establishes in the cortical region.

The disease is favoured by mild temperatures and wet weather. Temperatures between 20 ^{0}C and 30 ^{0}C with good evenly distributed rains are most favourable for disease development. Presence of free moisture on the host surface for atleast 20 min. is essential for successful infection.

16.3.4. Control: Since Citrus has no dead season, it is very difficult to control the disease. The control measures recommend are many.

16.3.4.1 Eradication: The only effective method of control of citrus canker is complete destruction of the affected trees by burning. Although costly this method has proved its efficiency in USA. In India however, the disease is well established in most of the orchards and eradication of trees is not feasible.

16.3.4.2. Pruning: In old orchards pruning of the affected twigs and spraying with copper fungicides at periodic intervals will reduce the disease.

16.3.4.3. Disease free stock: Use of disease free nursery stock for planting new orchards is the preliminary precaution.

16.3.4.4. Chemical sprays: Spraying of antibiotics like streptomycin (100-500 ppm) or phytomycin (2500 ppm) were reported to be effective. The antibiotic is absorbed by the leaves and translocated, thus functioning as a systemic bactericide.

Spraying of copper fungicides like bordeaux mixture was also reported to be effective.

16.3.4.5: Use of resistant varieties: It is the best method, but most of the commercial varieties are susceptible.

16. 4. BROWN ROT OF POTATO

The bacterial brown rot of potato is common in tropical and subtropical regions and also in some warm temperate regions of the world. In India, the disease is destructive in the mid hills and plateau region of West Bengal, Karnataka and other regions. The disease is essentially a wilt disease and losses up to 70% are reported.

16.4.1. Symptoms: The characteristic symptoms of the disease are stunting, yellowing of the lower foliage, sudden wilting and finally collapse of the entire plant. In the hilly regions of India the bacterial wilt appears in July, a fortnight after the start of the monsoon. The brown rot refers to the browning of xylem in the vascular bundles. This browning is often visible from the surface of infected stems as dark patches or streaks. The tubers are also affected. In the tubers, a brown ring is formed due to the discoloration of vascular bundles (Fig.16.3). The skin of the tubers is often discoloured and eye buds are also blackened.

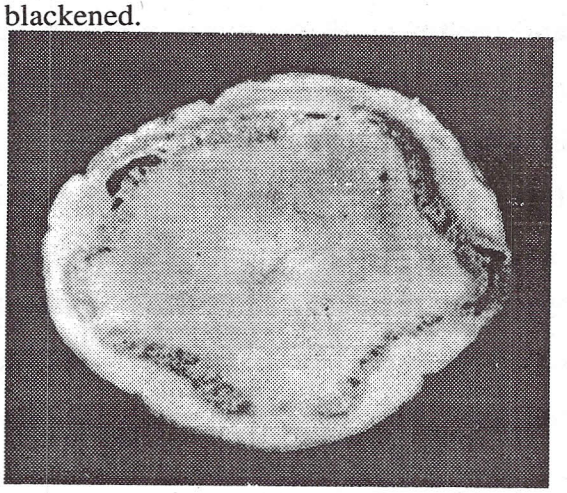


Figure 16.3. Brown rot symptom of the potato tuber

16.4.2. Causal organism: The brown rot disease is caused by *Burkholderia solanacearum*. The pathogen is originally placed in the genus *Pseudomonas*, but in recent revision the genus, it is

placed in the genus *Burkholderia.* It belongs to the family Burkholderiaceae, order Burkholderiales and class β-Proteobacteria.

The bacterium is typically rod shaped, measuring 1.5-2.5 x 0.5-0.7 µm. It is motile by 1-4 polar flagella. The cells are gram negative, non-spore forming and surrounded by a thick polysaccharide capsule. It can grow on many bacteriological media, and on nutrient agar the colonies are either smooth elevated and mucilaginous, or rough, dry and flat. The colonies are usually yellowish green and produce a soluble fluorescent pigment. Optimum temperature for growth is 35-37 ^{0}C and optimum pH 7.0 to 7.2.

16.4.3. Disease cycle: The organism persists in soil for more than 12 months. It may live saprophytically on plant residues or in the tubers left over in the field. It can also perpetuate through diseased tubers when used as seed material. When diseased tubers in a lot of seed material are cut with a knife before planting, the cutting knife spreads the bacteria to healthy tubers. In the field the infection may takes place through incidental wounds caused to the plants at the time of various cultural operations. The bacteria may spread in the field through irrigation and rainwaters, implements and tools and by various other means. The disease is favoured by relatively high temperature (30-35°C) and high soil moisture.

16.4.4 Control: Since the disease is soil borne and tuber borne, various measures recommended for control include seed selection and treatment, field sanitation, and crop rotation.

16.4.4.1. Selection and treatment of seed tubers: Healthy seed tuber should be selected only from disease free crops, and tubers should be treated in 0.02% steptocycline for 30 minutes. Knives used for cutting tubers should be properly sterilized.

16.4.4.2. Field sanitation: Fields should be properly prepared with good drainage facility and kept clean by weeding.

16.4.4.3. Crop rotation: A crop rotation to avoid potato, tomato and brinjal in the field for not less than two or three years would help in starvingout the pathogen.

16.4.4.4. Resistant varieties: Growing resistant varieties is the best method.

16.5. LITTLE LEAF OF BRINJAL

Brinjal (Solanum melongena) is one of the popular vegetables grown all over India and neighbouring countries. Of the various diseases reported on the crop, little leaf disease is the most

severe disease which causes almost 100% yield losses. All cultivated varieties of brinjal are susceptible to the disease. The disease was first reported from Coimbatore region of Tamilnadu by Thomas and Krishnaswami in 1939, a graft transmissible virus disease. Since then it was reported from all brinjal growing areas. Varma et al. (1969) showed that it is not a virus disease, but caused by Mycoplasma like organism (MLO).

16.5.1. Symptoms: The main symptom of the disease is production of very short leaves by the affected plant. The petioles are very much reduced in size and the leaves appear sticking to the tem. Such leaves are narrow soft, smooth and yellowish in colour. Newly formed leaves are further reduced in size. The internodes of the stem are shortened, and at the same time a large number of axillary buds are stimulated to grow into short, erect, thin branches with small leaves. This gives the plant a bushy appearance (Fig. 16.4). Usually such plants fail to produce flowers. Even if flowers are formed they remain green. Fruiting is rare.

Fig. 16.4. Symptoms of the little leaf disease of brinjal caused by mycoplasma.

16.5.2. Casual organism: Until 1969, the causal organism is considered as a virus. Since the discovery by Doi et al. in 1967, that Mycoplasma like organisms (MLOs) cause some of the diseases like 'yellow diseases', the cause of various diseases, including little leaf of brinjal, was investigated. Varma et al. (1969) showed that Mycoplasma like bodies (MLBs) are present in the phloem cells of the diseased plants. These bodies are spherical to ovoid and measure 40-300 nm in diameter and devoid of a rigid cell wall, but surrounded by triple layered membrane, rich in

sterols. Hence, the disease is considered as a Mycoplasma disease. Presently, the mycoplasmas causing plant diseases are considered as belonging to the genus *Phytoplasma*. It is placed in the order Mollicutes, and treated as a separate section, along with gram negative bacteria in Bergey's Manual of Systematic Bacteriology Volume-I.

16.5.3. Transmission: The disease is graft transmissible. It is also transmitted from plant to plant by insects vectors. *Hishimonas phycitis* is the major insect vector for transmission of the pathogen.

16.5.4 Collateral hosts: The same pathogen also causes disease in *Datura fastuosa* and *Vinca rosea* in nature. It has been transmitted by artificial inoculation to tomato, potato and tobacco. In the absence of brinjal crop in the field, these collateral hosts may help in the perpetuation of the pathogen. and provide primary source of inoculum to new crop of brinjal.

16.5.5. Control: There are no effective measures to control the disease. Mycoplasmas in general are susceptible to tetracyclines, and tetracycline susceptibility is considered as a major biochemical character. Hence, spraying of tetracyclines help in reducing the diseases incidence. Spraying of ledermycin at 500 ppm has shown beneficial effects by suppressing the symptoms. Temporary masking of symptoms was also reported with sprays of achromycin, terramycin and aureomycin.

Since the pathogen has a number of weed hosts, field sanitation to remove the collateral hosts helps in reducing the disease in the main crop.

Since the insects are known to transmit the pathogen, spraying of insecticides during cropping season reduces the chances of transmission.

Use the resistant varieties like BB-7, BWR-12, Pant Rituraj and H-8, is the best method to control the disease.

16.6. TOBACCO MOSAIC DISEASE

Tobacco mosaic disease is the first recognized virus disease of plants and is world wide in distribution. It is the most common disease on tobacco crop in India, appearing in almost every tract of the country, causing heavy yield loss. More than 20 different virus diseases occur on tobacco but mosaic disease is the most important one. The same virus infects a large number of other crops and weeds.

16.6.1. Symptoms: The first symptom is light discoloration along the veins of the youngest leaves. Soon the leaves develop a characteristic light and dark green pattern. The light and dark green patches are distributed randomly on the leaf surface (fig. 16.5), and hence the name mosaic. The dark green areas are usually associated with the veins.

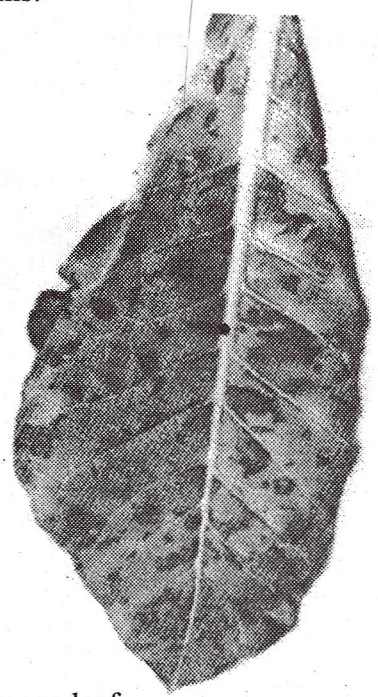


Figure 16.5. Symptoms of mosaic on tobacco leaf

The typical mosaic symptom is sometimes accompanied by a blistered appearance of the leaf due to more rapid growth of the dark green tissue. At times leaf spots also appear. The plants that are infected early in the season are usually very much stunted, while those infected late in the season show little reduction in size. In severe infections, the leaves are narrowed, puckered, thin and in general malformed beyond recognition.

It is easy to recognize the disease in the main field, but almost impossible in nursery or seed beds. The disease causes extensive damage by way of reduction in yield and quality of the product.

16.6.2. Causal virus: The virus that cause tobacco mosaic disease is the first in identified virus, and it is named as Nicotiana virus-I. It is commonly referred to as tobacco mosaic virus (TMV).

The virus particle is rod shaped, the rods measure 280-300 nm is length and 15-18 nm in diameter with a central hallow tube of about 20 °A in diameters. Its mol. wt. is 40 millions.

Each virus particle is made up of centrally placed genome (RNA) covered with a protein coat (capsid). The genome is a single stranded RNA of mol. wt. 2.4 millions. It consists of 6,500 nucleotides. It forms a helix within the protein coat. The protein coat, the capsid, is made up of 2,130 identical sub units called capsomeres. Each capsomere is composed of a single chain of 158 amino acid residues of known sequence. The ultra structure of the virus is shown in the figure 16.6.

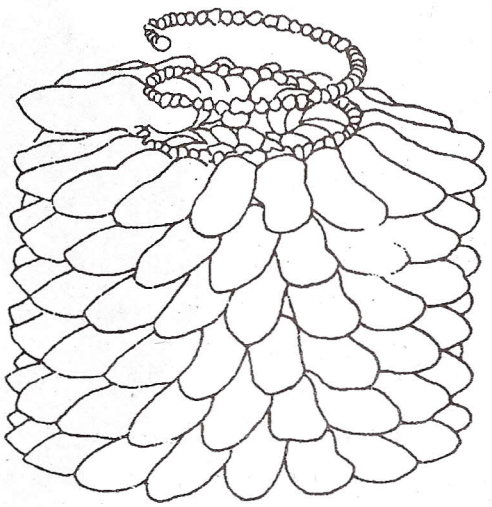


Figure 16.6. Tobacco mosaic virus

The thermal death point of the virus is approximately 90°C for ten minutes exposure, and the dilution end point is about 1:1,000,000. It is capable of remaining alive for over 50 years under dry conditions.

16.6.3. Disease cycle: The virus is sap transmissible, and enters the host through wounds. No insect vector is known. It is most resistant to adverse environmental conditions and hence when it reaches the field it may become infective any time the crop is sown. Besides it has a wide host range affecting nearly 50 plant species belonging to 9 different families the virus is not seed transmitted except as a contaminant on the surface. The virus produces different types of symptoms on several species of *Nicotiana*, tomato, brinjal, chillies, *Datura stramonium*, *Solanum nigra*, *Petunia* and other collateral hosts. In some cases, the host may be a symptom less carrier, making it difficult to effect control measures with such hosts.

The virus is easily transmitted in the field by mechanical means and by wind and water. Various cultural operations cause wounds on the plants which become the portals of entry for the virus. Such operations as topping or clipping the shoots spread the virus from diseased to healthy plants. Even the workers in the field may transmit the virus from chewing tobacco and snuff, to the standing crop. Thus there is unlimited scope for the virus to set in and cause damage to the crop.

16.6.4. Control: Various physical and biological properties which impart highly resistant qualities to the virus, and varying modes of spread of the virus, make the disease control almost impossible. The only method is to grow resistant or at least tolerant tobacco varieties and practice field sanitation. Cultivars GSH-3, L-2663a, MDS-13, L series 687 to 722, V 2809 to 2814 are resistant to mosaic. The field should be weeded thoroughly. After weeding, the workers should wash their hands thoroughly with disinfectant and running water. Regular roguing of diseased plants and weeds in the field should be carried out. Utmost care should be taken while doing so, the person handling the diseased plants should never come in contact with healthy plants. After roguing, the plants should be destroyed by chemical treatment or burning, and the person who handled them must wash his hands thoroughly with soap and water or some disinfectant. These measures help to reduce the disease intensity in the infected fields.

16.7. TUNGRO DISEASE OF RICE:

Tungro is the most serious virus disease of rice, and it was first identified as a distinct virus disease at International Rice Research Institute (IRRI), Philippines in 1963. A disease with similar symptoms was recorded by Raychaudhuri et al. in 1967 in India. The diseases described under different names in different countries such as Penyakit merah in Malaysia, mentek in Indonesia, Bangchee in Thailand etc are now considered as viral diseases caused by different strains of tungro viruses.

16.7.1. Symptoms: Tungro disease is characterized by stunting and yellowish orange discoloration of the leaves. The symptoms may appear at any stage of crop growth, and vary with environment conditions. But, in general, the symptoms appear as yellow to yellow-orange discolouration of the leaves that spread from tip downwards along the margins and in severe cases towards the mid rib. On young leaves mottling is common while on older leaves yellow orange spot may occur. Plants infected in the early stages are severely stunted with reduced tillering. The

affected plants bear poor panicles with empty glumes. Grains are rarely formed and even if formed they appear brown or rusty.

16.7.2. Causal viruses: Tungro is the result of mixed infection by two viruses. One is a single stranded RNA virus and the other a double stranded DNA virus. The RNA virus is spherical in shape and identified as Waika virus. It is called rice tungro spherical waikavirus (RTSV). The DNA virus is a rod shaped or bacilliform virus. It is identified as Badna virus, and commonly called rice tungro bacilliform virus (RTBV). Typical symptoms of tungro are produced by the infection with RTBV alone and they are intensified by the presence of RTSV. The spherical RNA virus may some times occur alone but the infection result only in very mild symptoms.

16.7.3. Vectors: Both the viruses are transmitted by several leaf hoppers, particularly *Nephotettix impicticeps* and *N. viriscens.*

16.7.4. Collateral hosts: The tungro viruses also infect a number of other grass hosts and important ones are *Eleusine corocana, Echinocloa colonum, E. crusgalli* etc.

16.7.5. Control: It is very difficult to control the disease one established in the field. Growing resistant varieties is the best method. Spraying of insecticides reduce the vector population.

16.8. SUMMARY:

Bacterial leaf blight (BLB) is a wide spread bacterial disease of rice, caused by *Xanthomonas campestris* pv *oryzae*, a gram negative, aerobic, non spore forming rod. The pathogen causes two types of symptoms viz. blight and wilt. Leaf blight is the most visible symptom that appear 4-6 weeks after transplantation. Wilt disease, also called kresek, appears due to early seedling infections, and most destructive. The disease is more severe in kharif during rainy season.

Citrus canker is caused by *Xanthomonas campetris* pv. *citri*. It attacks all aerial parts of the plant including leaves, petioles, twigs and fruits. The disease is characterized by thickened, raised, corky dead tissue at the site of infection. The canker on fruit is only skin deep but reduce the market value of the fruits. Lime and lemon are highly susceptible while oranges are less susceptible.

Brown rot of potato caused by *Burkholderia solanacearum* is a wilt disease of the crop and the vascular tissue turns brown due to infection. The brown ring discoloration of cut pieces of potato tuber is very prominent.

Little leaf disease of brinjal is caused by Mycoplasma, a wall less pleomorphic bacterium. The pathogen spread from plant to plant by insects, and systemically infect the plant with organism occurring in phloem. The disease is characterized by extreme reduction of size of the leaves and other aerial parts which gives the infected plant a small stunted bush like appearance.

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.

Tungro disease of rice is characterized by stunting of the plant and discoloration of leaves ranging from various shades of yellow to orange and rusty bloches spreading down wards from the leaf tip. It is transmitted by green leaf hoppers and very difficult to eradicate.

6.9. MODEL QUESTIONS

Essay type questions

1. Discuss the symptoms, etiology and control of bacterial leaf blight of rice.
2. Discuss the pathology of citrus canker.
3. Give an account of the symptoms and pathogens of brown rot of potato and little of brinjal, and suggest control measures
4. Give an account of tobacco mosaic disease and the characters of the pathogen.

Short answer type questions

5. Brown rot of potato
6. Citrus canker
7. Little leaf of brinjal
8. Tungro disease of rice.

6.10. REFERENCE BOOKS:

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 Pvt. Ltd., New Delhi
3. Mehrotra, R.S. 1983. Plant pathology. Tata McGraw Hill publishing Co. Pvt. Ltd., New Delhi.

PROF. K.V.MALLAIAH

LESSON – 17. PRINCIPLES OF PLANT DISEASE CONTROL

OBJECTIVE: To study the principles of the plant disease control viz. plant quarantine, seed treatment, cultural practices, chemical control and use of disease resistant varieties.

CONTENTS:

17.1. Introduction
17.2. Plant quarantine
17.3. Seed treatment
17.4. Cultural practices
17.5. Chemical control
17.6. Disease resistance
17.7. Summary
17.8. Model questions
17.9. Reference books

17.1. INTRODUCTION

The object of plant disease control is to prevent economic loss and increase the yield of the crop. For effective control, the knowledge of the pathogen and factors affecting 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 disease 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, and this is called exclusion, and quarantine measures are taken to 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 result in environmental pollution. Hence, the measures are

taken to increase the resistance in host plants, and it is achieved by breeding for disease resistance. These principles and measures aimed at disease control are explained in this lesson.

17.2. 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, have resulted in the spread of some of the devastating plant diseases. Some important examples of diseases introduced into India from other countries through diseased material are given in the table 17.1.

Table-17.1: Some important diseases introduced into India through diseased plant material.

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 tumefasciens*	1940	England
Bunchy top of banana	Virus	1940	Sri Lanka
Potato wart	*Synchytrium 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 majors ports (sea, air and land) of entry.

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.

Governmental restrictions alone cannot prevent complete exclusion or the spread of airborne diseases likes rusts, unless there are natural barriers to spread like high range mountains, deserts or oceans.

17.3. 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 type viz. seed disinfection, seed disinfestation and seed protection.

17.3.1. Seed disinfection: Seed treatment directed towards the eradication of the pathogen in the seed is referred to as seed disinfection. Hot water treatment is devise 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°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, streptocycline etc. at proper concentrations.

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

17.3.3. 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 protectant fungicides for maize, pea, bean, and other crops. These fungicides are applied to the seed by immersing the seeds 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.

17.4. 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 cultivational practices.

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

17.4.1.1. Eradication of seed borne inoculum: The pathogen may be externally or internally seed borne, and it may be eradicated by seed treatment.

17.4.1.2. Eradication of diseased plants: One of the most extensive eradication operations carried out so for 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 programmes on field scale are often carried out for many soil borne diseases of crop plants.

17.4.1.3. Eradication of alternate hosts: One of the classic example is eradication of barberry bushes in Europe and America to control wheat rust epidemics.

17.4.1.4. 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 remove the primary source of inoculum.

17.4.2. 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 is also an important measure of sanitation.

17.4.3. 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 two important patterns of cultivation for disease control.

17.4.3.1. 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.

17.4.3.2. 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 in creased 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 the other is badly damaged by a disease.

17.4.4. Cultivational methods: The incidence of many diseases on crop plants can be reduced by following proper cultivational practices.

17.4.4.1. 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.

17.4.4.2. 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 do not fall during that favourable period helps in less exposure of the crop to diseases.

17.4.4.3. Soil amendments: Adjustment of soil pH_s balanced use of fertilizers, correction of minor elements are 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.

17.5. 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 plants 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.

17.5.1. 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.

17.5.1.1. 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 flour mixed with inert materials are available. Before mixing, the sulphur is ground into fine particles of about 4μ diameter. For some preparations sulphur is roasted in a furnace and 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 me brane lipids favour the direct uptake of sulphur. The dry spores of powdery mildews contain mo ds, and it may explain their affinity and susceptibility to sulphur.

17.5.1.2. 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 control of plant diseases for 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 commonly used copper fungicides.

Because of the phytotoxicity of copper compounds, its use declined with the advent of organic fungicides, which are better tolerated by the plant.

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

17.5.1.4. Dithiocarbamates: Dithiocarbamates are derivatives of dithiocarbamic acid, and the usefulness of carbamates was first reported by Tisdale in 1931, but they came into wide use after 1960s. The important dithiocarbamates are thiram, ziram, ferbam, nabam, (marketed as Dithane D-14), zineb (marketed a Dithane Z-78) maneb (marketed as Dithane M-22) and mancozeb (marketed as Dithane M-45). The structures of these compounds are shown in the figure 17.1.

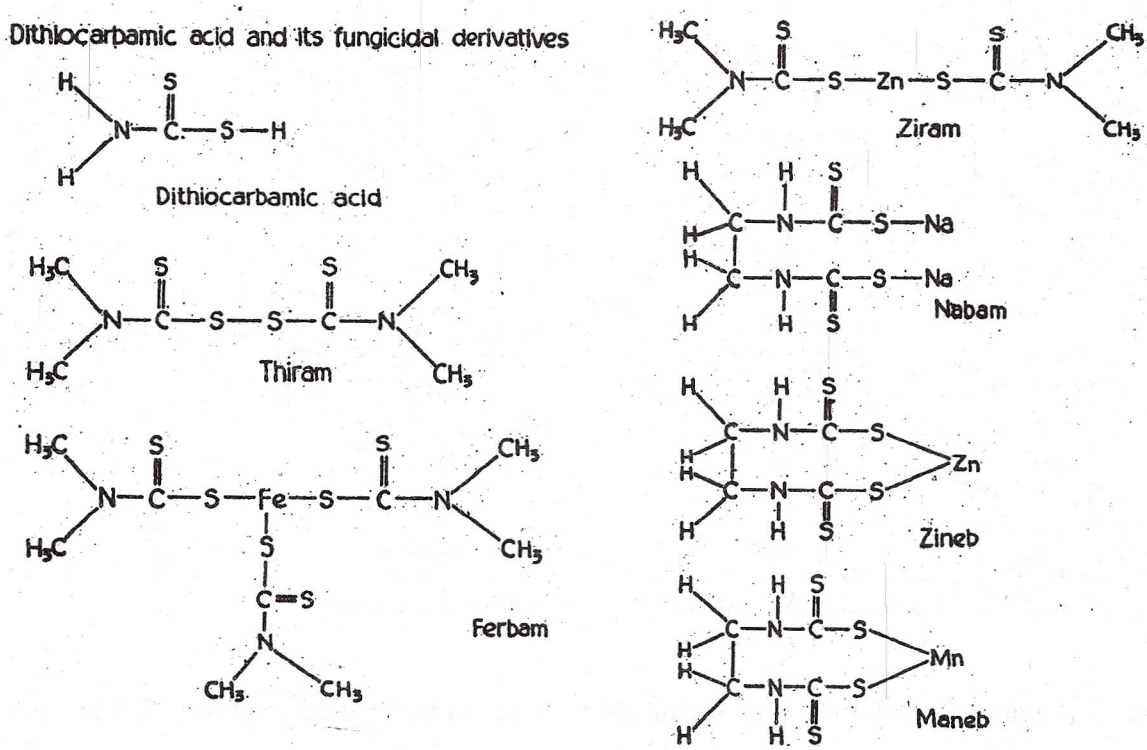


Figure 17.1 The structure of dithiocarbamic acid and its fungicidal derivatives

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.

17.5.1.5 Quinone fungicides: Quinones are naturally present in plants and animals, and are the source of colouration. 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 (Fig 17.2.) are two well known fungicides in this group.

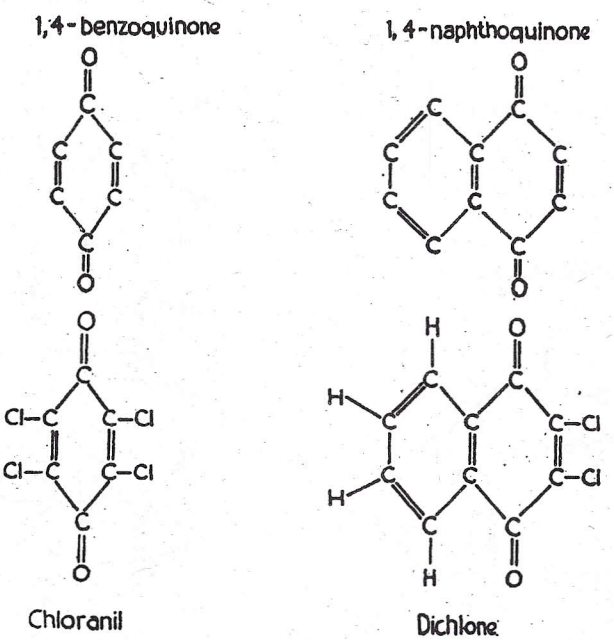


Figure 17.2. The chemical structure of chloranil and dichlone

Chloranil is mainly used for seed treatment against smut of barley and sorghum. Dichlone is used for seed treatment and also as a foliar spray. It is effective against seed rot of bean, apple scab, peach leafcurl etc.

17.5.1.6: Benzene fungicides: Many aromatic compounds have 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 karathane (Fig.17.3).

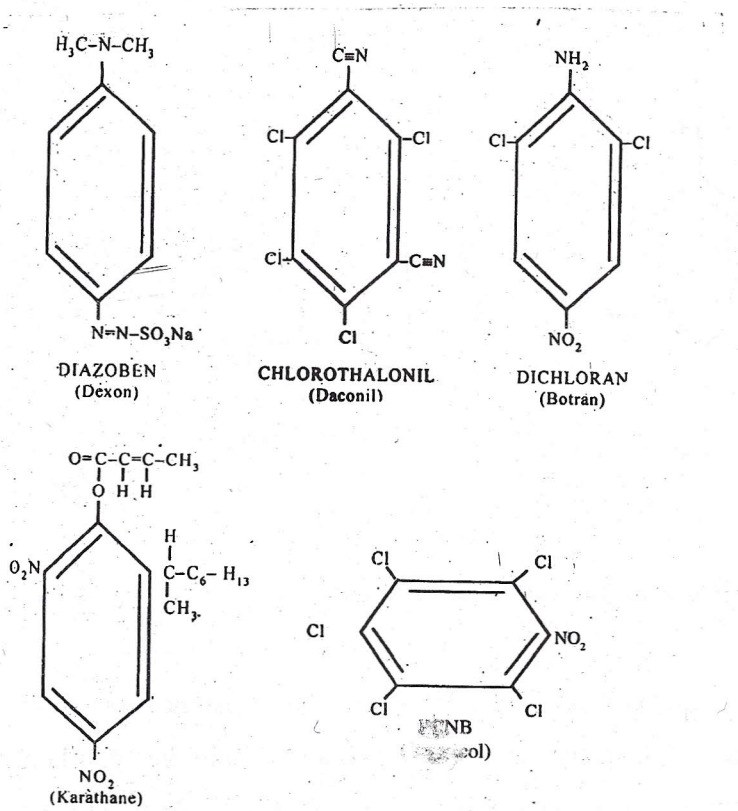


Figure 17.3. Benzene fungicides

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 spray fungicide, and also in soil treatment. Brassicol (Pentachloronitrobenzene, PCNB) is a common fungicide of this group and is effective against many soil borne fungi like *Sclerotium, Rhizoctonia, Sclerotinia* etc. Dinocap (tradename Karathane) is extensively used against powdery mildews.

17.5.1.7 Heterocyclic Nitrogen Compounds: Important fungicides of this group are captan and captatol (Fig 17.4). Captan is the most commonly used seed protectant against *Pythium*, which cause damping off and root rots. It is also used for soil drenching and for foliar sprays. Captatol (trade name difoltan) is an effective foliar spray and seed dressing fungicide against early blight of potato, apple scab etc.

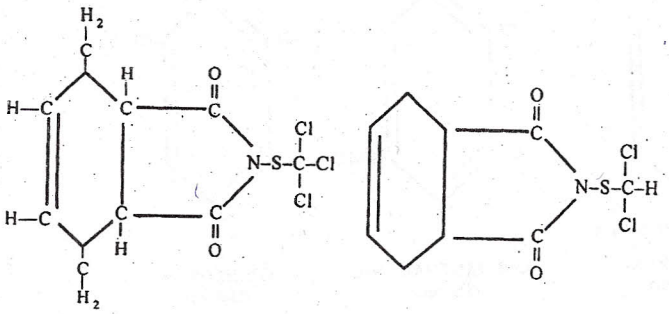


Figure 17.4. The structure of captan and captatol.

17.5.1.8 Organotin compounds: Duter, Brestan and Brestanol are three fungicides based on organic tin compounds. They are very e e against a number of plant pathogenic fungi like *Cercospora, Helminthosporium, Alteraria, Pythium, Phytophthor*a and *Rhizoctonia*. They have antibacterial and anti insect activity also and has some systemic activity. However, due to phytotoxicity to host, these finitudes have restricted use.

17.5.1.9. 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 caused by *Cercospora musae*. Other disease that can be controlled by using oils included greasy spot of citrus (*Cercospora citrigrisea*), angular leaf spot of tung tree (*Mycosphaerella aleuritidis*), leaf spot of celery (*Septoria apii*), wheat rust (*Puccinia graminis tritici*), powdery mildew of rose (*Sphaerotheca pannosa*), downy mildew of grapes (*Plasmopora viticola*) etc.

The oils used in disease control are mainly mineral oils from petroleum, and to a lesser exent 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 it 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.

17.5.2. SYSTEMIC FUNGICIDES:

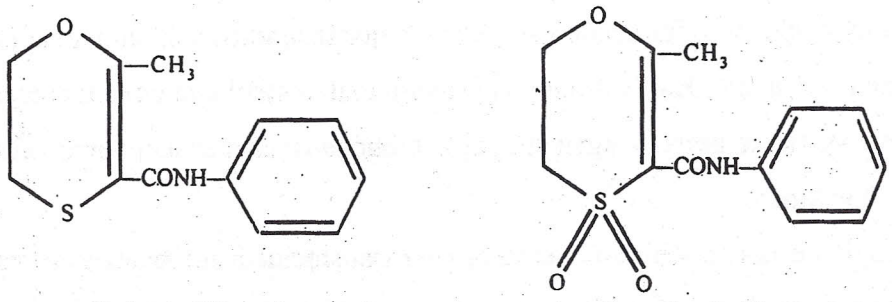
The introduction of systemic fungicides in 1966 is a major landmark in chemical control of plant diseases. Von Schmeling and Kulka in 1966 have shown that synthetic chemical compounds of oxathiin group have systemic activity against fungal pathogens. Later a number of systemic fungicides have been developed.

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 the active ingredient moves along the sap stream. Down ward 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.

17.5.2.1. Oxathiins: Carboxin (trade name vitavax) and oxycarboxin (trade name plantvax) 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 (*Ustilago nuda*) in barley. They were later found to be effective against a number of other diseases, especially those caused by basidiomycetous fungi. The structures of these fungicides are given in the figure 17.5.



Carboxin (Vitavax) Oxycarboxin (Plantvax)

Figure 17.5. The structure of carboxin and oxycarboxin

Vitavax is the most popular fungicide for seed treatment to control loose smut of wheat, bunt of wheat and flag smut. 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.

17.5.2.2. Benzimidazoles: Benomyl, carbendazim and thiabendazoles are important systemic fungicides of benzimidazole group.

Benomyl was discovered by E.1. Dupont de Nemours and Colnc 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, Podosphaera, 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 – 3 – benzimidazole carbamate (MBC) and butyl carbamate, which is converted into a volatile compound -butyl isothiocynate. 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 (abbreviated as TBZ) has a similar fungitoxic spectrum as benomyl, but qualitatively less effective than benomyl.

The structures of benomyl and carbendazim, the two most popular fungicides of the group are shown in the figure 17.6.

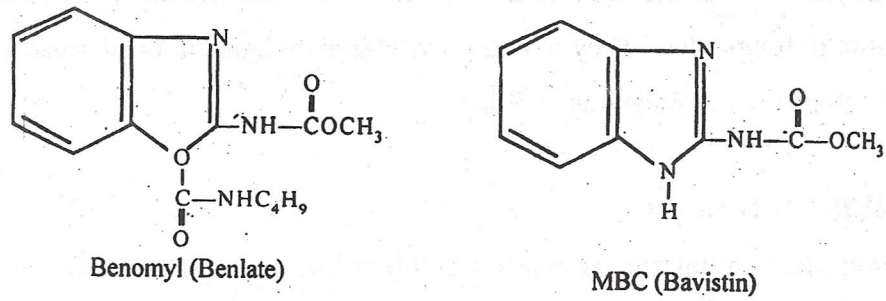


Figure 17.6. The structures of benomyl (benlate) and carbendazim (bavistin).

17.5.2.3. 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 resemble that of benomyl. Two compounds namely thiophanate (commercial name topsin or cercobin) and thiophanate methyl (commercial name Topsin-M or Cercobin M) are commercially available.

17.5.2.4. 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.

17.5.2.5. Pyrimidine fungicides: This group includes diamethirimol (Milcurb), ethirimol (Milstem), bupirimate (Nimred), fenarimol (Rubigan) etc. All these fungicides effects sterol synthesis and effect stability of fungal cells. Diamethirimol is highly effective against powdery mildews and smuts.

17.5.2.6. Triazoles: Some of the best fungicides which inhibit sterol synthesis belong to this group and include triadimenol (marketed as Bayton), bitertenol(marketed as Bacor), boutrizol (marketed as Indar) etc. They show long term protective and curative activity against many powdery mildews, rusts, smuts leaf spots and blights.

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

17.5.2.8. Organophosphates: Kitazin and Hinosan (Ediphenphos) are widely used systemic organophoshate fungicides. They are highly effective against blast disease of rice. In India Hinosan is extensively used against rice blast.

17.5.3. 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.

Some of the important antibiotics used in agriculture are given below.

17.5.3.1. 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 mixed with tetracycline in 9:1 ratio and marketed as streptocycline.

17.5.3.2. Tetracyclines: They are produced by species of *Streptomyces* and also synthesized using natural tetracyclines as base. 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.

17.5.3.3. Cycloheximide: It is produced by *Streptomyces griseus* and *S. noursei*. 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, and it limited its usage.

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

17.5.3.5. 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.

17.5.3.6. Nystatin: It is the first antifungal antibiotic isolated from *Streptomyces noursei*. It is a polyene antibiotic and it is poorly absorbed by plant or 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.

17.5.3.7. Antibiotics used against paddy blast: Blasticidin-S (Bla-S), kasugamycin (kasumin) and a number of other antibiotics were successfully used in Japan against paddy blast caused by *Pyricularia oryzae*.

17.6. DISEASE RESISTANCE:

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 and 3. Breeding.

17.6.1. 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 with in severely infected crops, and by continued planting and selection under conditions favouring disease, resistant varieties were 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.

Since the selected material may not be genetically pure, two types of selections were followed.

17.6.1.1. Pure line selection: In the method seed from each selected plant is collected and raised separately. The stock thus produced is genetically pure.

17.6.1.2. 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 hybrid varieties the method of selection is not generally useful.

17.6.2. 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. herbaceum and arboreum cottons are highly susceptible to wilt disease caused by *Fusarium* in India, however, they were found to 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 into India they were found to be resistant to wilt under Indian conditions.

Thus the problem of cotton wilt is India was solved by introducing the high yielding exotic varieties. However, with it came the problem of bacterial disease of cotton.

17.6.3. 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 programmes for breeding for disease resistance started. In breeding for disease resistance, two important aspects are sources of resistance and breeding methods.

17.6.3.1. Sources of resistance: For successful breeding programme, the sources of resistance must be first identified. Different ways of obtaining sources of disease resistance are 1. germplasm collection 2. wild varieties and 3. induced mutations.

17.6.3.1.1. 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.

17.6.3.1.2. Wild varieties: Non cultivated relatives the crop plants are called 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 not other agronomically acceptable characters. Hence, they cannot replace the cultivated varieties but can be used in breeding programmes to transfer resistance to cultivated varieties.

17.6.3.1.3. 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 ray 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.

17.6.3.2. Breeding programmes:

The technique of breeding varies with the type of plants involved.

17.6.3.2.1. 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.

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

17.6.3.2.3. 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 nonrecurrent 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 programme.

17.7. 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 cause 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, dithiocarbomates, quinines, benzene compounds, heterocyclic nitrogen compounds, oils etc. Systemic fungicide- are taken up by the plant and translocated within the host tissue and they act for a long time. iins, 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.

17.8. MODEL QUESTIONS:

Essay type 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

Short answer type questions

4. Plant quarantine
5. Seed treatment
6. Cultural methods of disease management
7. Contact fungicides
8. Systemic fungicides
9. Antibiotics in plant disease control
10. Development of plant disease resistance

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LESSON 18. BIOLOGICAL CONTROL OF PLANT DISEASES

OBJECTIVE: To study the control of plant diseases by using microorganisms antagonistic to plant pathogens.

CONTENTS:

18.1. Introduction
18.2. Approaches to biological control
18.3. Biocontrol agents.
18.4. Diseases controlled through use of microorganisms
18.5. Viruses as biocontrol agents
18.6. Fungi as biocontrol agents
18.7. Bacteria as biocontrol agents
18.8. Commercialization of biocontrol agents
18.9. Summary
18.10. Model questions
18.11. Reference books

18.1. INTRODUCTION

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 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.

18.2. 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.

18.2.1. 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 microorganisms attack the resting structures or actively growing mycelium of fungal pathogens surviving in soil. For example sclerotia of pathogenic fungi are destroyed by parasitism of *Sporodesmium sclerotivorum*, actively growing mycelium of *Rhizoctonia solani* is destroyed by parasitism of *Trichoderma harzianum*.

18.2.2. 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 attack of a pathogen. The host is not involved in the interactions between the pathogen and the antagonist, though it occurs on host plant surface. Best example for such a protection is ectomycorrhizae. The ectomycorrhizal fungi form a thick sheath of mycelium or mantle on the surface of host root, and it 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.

18.2.3. 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 give 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.

18.3. 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*.

18.4. DISEASES CONTROLLED USING MICROORGANISMS AS BIOCONTROL AGENTS:

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 plants 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 to attack soil borne plant pathogens in their natural environment than antagonists of aerial pathogens. Some of the important plant diseases controlled by biological means are given below.

18.4.1. Take all disease of wheat: Take-all disease of wheat caused by *Gaumannomyces graminis* is a very serious disease of wheat crop and infected plants are completely destroyed and no yield comes from it. 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-di acetyl phloroglucinol etc. and effectively control the disease. Another approach found successful was cross protection with avirulent fungi.

18.4.2. 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 were identified as belonging to *Agrobacterium radiobacter*. These are non pathogenic and produce a bacteriocin that effectively inhibit *Agrobacterium tumefaciens*. The strains of *A. radiobacter* strain K-84 was commercialized in 1973 in Australia

under the trade name Goltrol, Norback and diegall. A derivative strain viz. *A. radiobacter* strain K-1026 was later developed and marketed in 1991 as Nogall.

18.4.3. Stump rot of pines: Freshly cut pine stumps are infected by *Heterobasidion annosum* (= *Fomes annosus*) causing severing rotting and spread to roots. It was particularly serious in USA and Europe. The *Pinus* stump rot was successfully controlled by inoculating the freshly cut surfaces with 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 success stories of biological control in forest pathology.

18.4.4. 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, and the preparation is applied to the seeds of many crop plants before sowing for control of damping off disease.

18.4.5. 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 wilt in standing crop. The bacterial biocontrol agent produce siderophores and reduce the growth of fungal pathogen.

18.4.6. 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 were antagonistic to the pathogen. Spraying of these bacteria on healthy leaves effectively control the incidence of fire blight. *Pseudomonas fluorescenes* strain-506 was commercialized as '**blight ban**' for biocontrol of fire blight of apples and pears.

18.5. 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 way reported in case of *Rhizoctonia solani* due to which damping-off of sugar beet was significantly reduced. Association of mycoviruses with hypovirulent strains of *Gaumannomyces 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.

18.6. 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.

18.6.1. Hyperparasitism: *Trichoderma* species are parasitic on a large number of soil borne fungi and they have been reported to control several soil borne plant diseases. Four species of *Trichoderma* are commonly used as biocontrol agents, and these are *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. *Darluca 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.

18.6.2. 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 *Gaumannomyces graminis* var *tritici* has been extensively worked out. Avirulent fungi used in control of take all disease include *Phialophora radicicola* var *graminicola*, *Gaumannomyces 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.

18.6.3. 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 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.*

18.7. 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 Rhizobacteria, and among rhizosphere bacteria species of *Pseudomonas* and *Bacillus* are predominant. *Pseudomonas* and *Bacillus* species exhibit bio control 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 (DRMOs) 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 siderophores, d). Production of anti fungal metabolites and antibiotics and e). Induced systemic resistance (ISR)

18.7.1. 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. *Pseudomonas* species compete for the substrate with the pathogen and thus protects the sugar beet seedlings from infection.

18.7.2. 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 rot 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 organisms of damping off and root rot of various crop plants, thus reducing the disease incidence.

18.7.3. Production of siderophores: Iron is one of the most abundant minerals on the earth and is an essential micronutrient for living organisms. However, iron in the soil is unavailable for direct assimilation by microorganisms because ferric iron (Fe III) the predominant form in nature, is sparingly soluble in water i.e. its solubility is only 10^{-18} M mol. at pH 7.4. 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 (~400 to 1000) iron binding proteins known as siderophores. The siderophores bind 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 pseudobactin 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 *F.O.* f.sp. *lini*. Similarly wheat is susceptible to *Gaeumannomyces graminis* which cause take all disease, and seed treatment with *P. fluorescence* strain B10 effectively reduce the disease and increase the growth.

18.7.4. Production of antifungal metabalites and antibiotics: Most of the pseudomonas strains, used as biocontrol agents, produce antifungal metabolites which include phenazines, pyrrolnitrin, pyoluteorin, viscosinamide, tensin, 2,4-diacetylphloroglucinol (DAPG), 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 *Agrobacterium tumefaciens*. Agrocin-84 is considered as a bacreriocin. Bacteriocins are molecular weight proteins produced by bacteria and are lethal to other strains of closely related bacteria.

18.7.5. Induced systemic resistance (ISR): Anumber 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 if induced systemic resistance in plants by *Pseudomonas* species are given in the table18.1.

Table-18.1. Induced systemic resistance in plants by *Pseudomonas* spp.

PSEUDOMONAS STRAIN	BACTERIZED HOST PLANT	CHALLENGE PATHOGEN
P. fluorescens WCS417r	Cornation(roots) Arabidopsis(roots)	*Fusarium oxysporum* *Xanthomonas campestris*
P. fluorescens WCS374r	Radish(roots)	*F.oxysorum* *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 or mechanism for induced resistance seems to be multi component. It is postulated hat ISR may be due to production of low molecular weight proteins called pathogenesis related (PR) proteins, siderphores, 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-glucanases 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 proteins include basic β-1,3- glucanases 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 hot cell deterioration.

Defago et al. (1995) demonstrated induced systemic resistance and the accumulation of PR-1 proteins, β-1, 3-glucanase and endichitinases 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.*

Other compounds: Cytokinins and inhibitors of viral replication have also been implicated in ISR to localized tobacco virus infection.

18.8. COMMERCIALIZATION OF BIOCONROL AGENTS:

Though there are thousands of examples in literature where it has been demonstrated that microbes parasitise or antagonize or compete in any other way with pathogens, they are very few cases where this potential has been exploited on commercial scale. Important examples of commercially available biocontrol agents are given in the table-18.2.

Table 18.2. Details of commercially available biocontrol agents.

Biocontrol agent/ Trade name	Disease controlled	Pathogen
Agrobacterium radiobacter (Galltrol, Diegall, Nogall)	Crown gall of apple	*Agrobacterium tumefasciens*
Peniophora gigantean	Stump rot of pines	*Heterobasidion annosum.*
Pseudomonas fluorescens strain 506 (Blight ban)	Fire blight of apple and pears	*Erwinia amylovora.*
Trichoderma harziana	Damping off of croplants	*Pythium* spp. *Rhizoctonia solani, Sclerotium rolfsii*
Bacillus subtilis (QUANTUM 4000)	Wilt of cereals and vegetables.	Fusarium sp. Verticillium sp.

18.9. 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 out side 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 recieving 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 fluorescence* strain 506 (Blight ban) *Bacillus subtilis* (QUANTUM 4000), *Trichoderma harziana*, *Peniophora antea* are important.

18.10. MODEL QUESTIONS

Essay type 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.

Short answer type questions.

4. Biological control of plant diseases
5. Fungi used for biocontrol
6. Bacteria and biological control of plant diseases.

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PROF. K.V.MALLAIAH