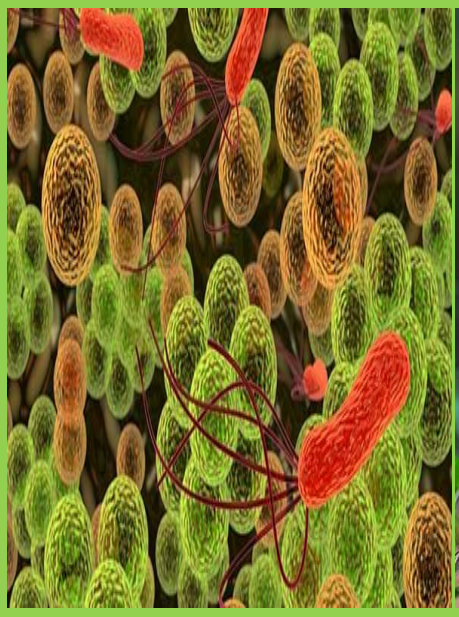
BSCBO- 101

B. Sc. I YEAR

Microbiology, Mycology and Plant Pathology



DEPARTMENT OF BOTANY
SCHOOL OF SCIENCES
UTTARAKHAND OPEN UNIVERSITY

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MICROBIOLOGY, MYCOLOGY AND PLANT PATHOLOGY

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BLOCK-1–GENERAL MICROBIOLOGY

UNIT-1-GENERAL ACCOUNT, DISTRIBUTION AND CLASSIFICATION OF MICROORGANISMS AND MAJOR MICROBES OF FOOD, WATER AND SOIL

1.1 OBJECTIVES

Microbiology is the study of organisms invisible to our naked eye. This branch of science explains the structure, nature, distribution, classification, occurrence, physiology pathogenicity and application of microbes. This unit deals with the introduction, general accounts, distribution, and classification of microbes and also about the soil, water and food microbiology.

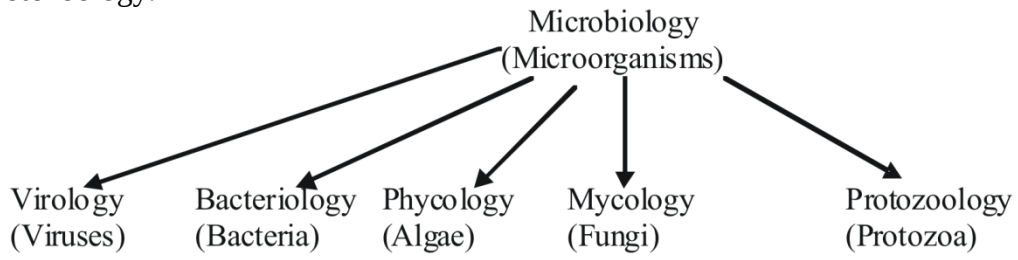
After reading this unit one is able to:

- Know about micro-organisms.
- Learn the variety of microorganisms which occur in the environment surrounding us.
- Understand the existence of minute organisms and realize that these microscopic organisms are living and perpetuate themselves by reproduction.
- Discuss the distinct group of microbes which differ in form and other characters but resemble with each other in their small size and simple structure.
- Study the systematic position, and distribution of micro organisms.

1.2 INTRODUCTION

Microbiology is the study of organisms too small to be clearly seen by the unaided eye. Since objects less than about one millimeter in diameter cannot be clearly seen and must be examined with a microscope, such living objects are collectively referred as microorganisms or microbes. Therefore microbiology is defined as the study of microorganisms. A variety of organisms like bacteria, protozoa, viruses, fungi and algae are included in this category.

Regarding the place of microorganisms in the living organisms, satisfactory criteria were unavailable until late 1940, when more definite observation of internal cell structure was made possible with the aid of the powerful magnification provided by electron microscope. Two cell types were discovered among these microorganisms. In some organisms the cells contained nuclear substance which was not enclosed by a nuclear membrane, while in others, a well-defined nucleus with a nuclear membrane was present. These two patterns were called prokaryotic and eukaryotic respectively. According to these special features of microorganisms, bacteria are prokaryotic and fungi, algae and protozoa are eukaryotic. Viruses are left out of this criteria as they are acellular organisms. Thus microbiology includes five major branches namely, virology, bacteriology, phycology, mycology, and protozoology.



1.3 GENERAL ACCOUNT OF MICROORGANISMS

Antony–van Leeuwenhoek (1632-1723) was the first who studied in detail the microbial content of a variety of natural substances under the microscope. The various natural substances studied by Leeuwenhoek were water from rain barrels, rivers, wells, sea, teeth scrapings and naturally fermented material like vinegar. His observations were confirmed by others, but only in nineteenth century, the extent and nature of microbial forms becames more apparent.

- Microbes are either unicellular or multicellular or non- cellular forms. Protozoa, bacteria and some algae and fungi are unicellular forms and are made up of single cells. While most of the algae and fungi are multicellular forms. Viruses lack a cellular structure and hence they are non-cellular particles and occupy a border line between living and non-living things.
- Based upon the presence or absence of nuclear membrane, microbes are of two types namely Prokaryotes and Eukaryotes. Prokaryotes have incipient nucleus which is suspended in the cytoplasm. This includes bacteria.
- The microbes such as protozoa, algae and fungi are eukaryote which contain a nucleus, with a nuclear membrane and is well separated from the cytoplasm.

General characteristics of Bacteria

1) Bacteria are small microscopic and least differentiated microorganisms. These are believed to be amongst the first primitive organisms on the earth possessing typical prokaryotic cell organization.
2) They are omnipresent, i.e. found in all possible habitats.
3) They are unicellular and may live in association with others in colonies.
4) The size, shape and arrangement of bacterial cells vary and their size is about .5 micron to 3 micron.
5) They exhibit a variety of shapes e.g., spheres (coccus), rods (bacillus), spirals (spirillum), curved (vibrio) etc.

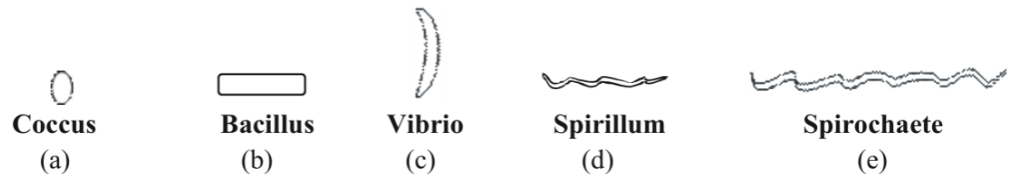


Fig.1.1. Different shapes of bacteria

6) They possess very rigid cell wall, which is not made up of cellulose, characteristic of plant cell walls. It generally contains a peptidoglycan (murein), lipid and lipopolysaccharides. The rigid cell wall determines the shape of bacterial cells.
7) Nuclear material is not enclosed in a nuclear membrane. Nucleolus is absent.
8) An extra chromosomal DNA called plasmid is usually present in the cytoplasm.

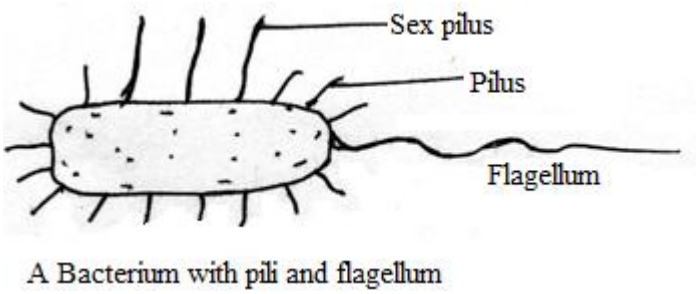


Fig.1.2. A bacterium showing appendages

9) Cell organelles includes 70s type ribosome and mesosome formed by invagination of plasma membrane.Otherorganelles such as mitochondria, lysosomes, Golgi body, endoplasmic reticulum,centriole etc. are absent.

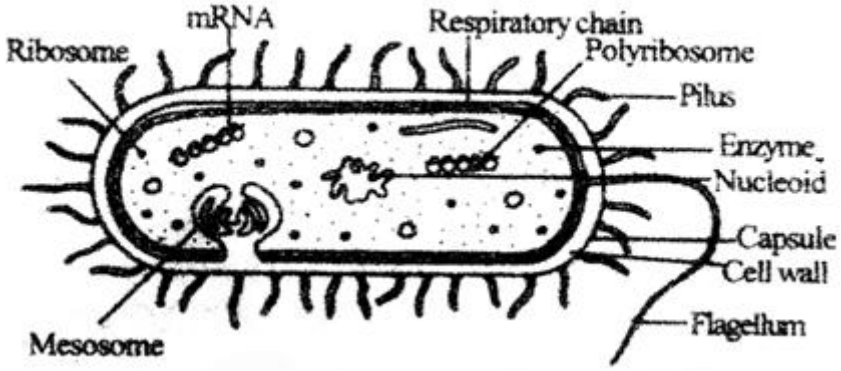


Fig.1.3.Structure of a typical bacterium (E. Coli)

10) Appendages like flagella and pili are present. Some pili are longer in some bacteria and are called as sex pili (**Fig.1.2, 1.3**).
Motility is brought about by flagella. The Bacilli and spirilla are motile and the cocci are non-motile. Thus the bacteria may motile or non-motile.

11) When flagella are absent, the bacterium is called atrichous. In motile bacteria, the number and position of flagella vary. The arrangement may be monotrichous (a single polar flagellum), lophotrichous (a cluster of polar flagella), amphitrichous (Flagella at both the ends either singly or in cluster), cephalotrichous (two or more flagella at one end of the cell), peritrichous (cell surface evenly surrounded by several flagella) (**Fig.1.4)**.

12) The flagella are hair like or helical, consists of a single minute filament which is made up of fibrils of flagellin protein. Unlike hair a flagellum grows at its tip rather than at base.

13) Bacteria are either Gram positive or Gram Negative. Gram positive bacteria retain violet colour on Gram staining while Gram negative bacteria appear in red colour. This is because of the difference in their cell walls. The cell wall of gram positive bacteria contains several layers of peptidoglycan in addition to techoic acid and low quantity of lipoprotein and lipid. In gram negative bacteria, cell wall has thin layer of peptidoglycan and high amount of lipo protein and lipid. Techoic acid is absent in these bacteria.

14) In some bacteria, shorter and thinner hair like appendages are present on the surface of the cell wall. These structures are called pili or fimbrae. There function is to adhere the cell to surfaces and sometimes help in transfer of genome to other bacterial cells. These are called sex-pili.

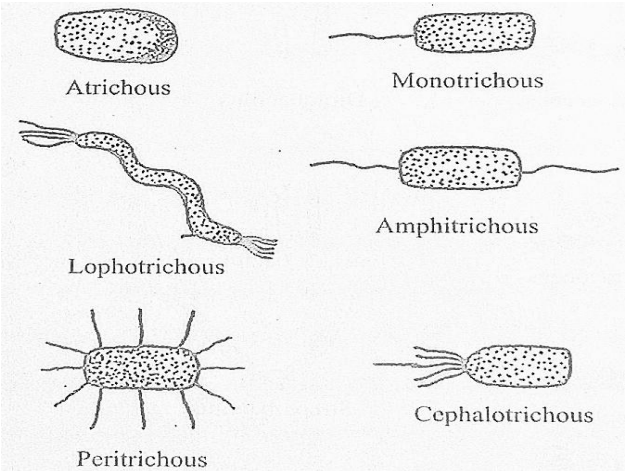


Fig.1.4. Showing flagellation in bacteria

15) A bacterial cell is protected by a cell envelope made up of a capsule, a cell wall and a plasma membrane. The bacteria covered by a capsule are called capsulated bacteria. While the bacteria which do not contain a capsule are called non- capsulated bacteria.
16) Bacteria may be heterotrophic or autotrophic, Heterotrophic may be parasitic, saprophytic or symbiotic. For nutrition, autotrophs use CO_2 as the source of carbon while heterotrophs use organic substances as the source of carbon.
17) Based on temperature tolerance of bacteria they are of three types:-
    - **Mesophilic** bacteria grow well in temperature between 25^0C -40^0C.
    - **Thermophilic** bacteria grow well above 40^0C.
    - **Psychrophilic** bacteria grow well in temperature less than 25^0C.
18) On the basis of availability of O_2 bacteria may be aerobic or anaerobic or facultative anaerobic.
    - Aerobic bacteria use oxygen for respiration.
    - Anaerobic bacteria use CO_2.
    - Facultative anaerobes use oxygen when it is available and use CO_2 when oxygen is not available.
19) Bacteria reproduce by binary fission, budding, fragmentation, endospores, exospores and conidiospores.
20) True sexual reproduction is lacking. However, genetic recombination occurs by conjugation, transformation and transduction.

General Account of Algae

1) Algae are simple, chlorophyll bearing, and unicellular or multicellular microorganisms. Being chlorophyllous these are autotrophs.
2) Algae are heterogeneous groups. They vary in size, habitat and reproductive processes.
3) Algae are ubiquitous and abundantly present in sea water, fresh water, in damp soil, on rocks, stones, barks of trees, on plants and animals.

4) Plant body of algae is called thallus which does not show differentiation into root, stem, leaf and true tissues.
5) Algae are aquatic or terrestrial. But most of them are aquatic. They are either free living or attached forms.
6) A few algae are parasites. Some algae are of specialized habitats, e.g. parasites, symbiotic cryophytes and thermophytes etc.
7) Algae are unicellular like *Chlamydomonas*or multicellular like *Spirogyra*. Multicelluar algae may be in the form of colonies like *Volvox* or in the form of filaments as *Spirogyra*.
8) The algae may be prokaryotes or eukaryotes. All blue green algae are prokaryotes.
9) The cell consists of a cell wall, a plasma membrane, cytoplasm and nucleus. The cytoplasm contains mitochondria, plastids, ribosomes, Golgi complex, endoplasmic reticulum.
10) The plastids in algae, contain pigments which are of three types –
    (a) **Chlorophylls:** Five types chlorophyll (a, b, c, d and e) are found in different algae. Chlorophyll a is present in all the algae.
    (b) **Carotenoids:** These are the yellow and orange pigments (namely carotenes and xanthophylls) and are found in varied quantities in different algae.
    (c) **Biliproteins or phycobilins:** These pigments include phycocyanin (blue in colour) and phycoerythrin (red in colour) and presence of these pigments is the characteristic feature of certain types of algae.
11) The pigments are present in chloroplasts, which are of different shapes in different genera. The chloroplast contains one or more spherical bodies called pyrenoids which are the centres of starch formation.
12) Some algae are motile and possess flagella.
13) Reproduction in algae is of three types, namely, vegetative, asexual and sexual. Vegetative reproduction takes place by fragmentation, fission, budding etc, asexual reproduction by production of asexual spores (motile or non-motile). Asexual reproduction is most common method of reproduction during favorable conditions. Algae reproduce sexually during unfavorable conditions by producing gametes.

General Account of Fungi

1) Fungi are achlorophyllous, non-vascular eukaryotic thallophytes.
2) They are non-green so heterotrophic microbes obtaining their food in a soluble form by uptake through plasma membrane.
3) Being heterotrophic, they live as parasites, saprophytes or symbionts.
4) They are ubiquitous in distribution and occur in any habitat where life is possible.
5) There are about 100,000 species of fungi.
6) Plant body of fungi typically consists of branched filamentous hyphae which form a network called mycelium. The hyphal structure is variously modified.
7) The hyphae are aseptate, multinucleate in lower forms while septate and uni, bi or multinucleate in higher forms.
8) Protoplasm remains surrounded by a distinct cell wall made up of fungal cellulose known as chitin. But in primitive slime moulds cell wall is absent.

9) Fungi are entirely devoid of chlorophyll but carotenoids are normally present. Cytoplasm contains endoplasmic reticulum, mitochondria, Golgi bodies and many non-living substances like reserve food.
10) In lower fungi the reproductive cells (Asexual spores and gametes) are motile (uni or biflagellate). But the higher fungi lack motile cells and show gradual reduction of sexuality.
11) Flagella are two types (i) whiplash (acronematic) flagella are smooth and (2) Tinsel (pentonematic) flagella with numerous minute hairs like structures on their surface.
12) Fungi are heterotrophic due to absence of chlorophyll. So they have to depend for their food on others. Therefore they may beof the following types:
    (a) **Parasites** obtain their nutrition from other living plants or animals. Some of them live only on living protoplasm and are called obligate parasites. Whereas others can also grow on dead organic matter in absence of living host and are known as facultative saprophytes.
    (b) **Saprophytes** obtain their nutrition from the dead decaying organic matter. Among these, some saprophytes such as *Mucor* can obtain their nutrition only from dead organic matter and are known as obligate saprophytes. On the other hand some saprophytic fungi as *Fusarium* have the capacity to invade living organisms and are known as facultative parasites.
    (c) **Symbionts** grow on other living organisms and both are mutually benefited. Such association is known as symbiosis, Lichens and mycorrhiza are common examples of this, in which fungal partner shows mutualistic relationship with alga and roots of higher plants respectively.
13) In unicellular fungi whole vegetative cell is transformed into a reproductive unit such fungi are known as Holocarpic while in most of the fungi only a part of the vegetative mycelium forms reproductive unit and rest remain vegetative. Such fungi are known as Eucarpic.
14) Fungi reproduce by vegetative, asexual and sexual means. Vegetative by fragmentation (e.g., *Rhizopus, Alternaria* etc.) fission (e.g., yeast) and budding (e.g. yeast and *Ustilago*) Asexual reproduction occurs during favourable condition by the formation of a variety of conidia and spores. Spores may be unicellular (e.g. *Aspergillus*) or multicellular (e.g. *Alternaria*). They may be endogenous (developed inside in pycnia or sporangia) or exogenous (developed outside on sporophores or conidiophores). Some common asexual spores in lower fungi are motile known as zoospores. (e.g., Phytophthora), Non motile known as aplanospores or conidia (e.g. *Mucor*, *Rhizopus*). In Higher fungi these non-motilespores are called conidia, oidia or chlamydospores.
15) Except for the class Deuteromycetes, sexual reproduction occurs in all groups of fungi. It is completed in three phases (a) Plasmogamy (fusion of protoplasm of two compatible gametes of sex cells) (b) karyogamy (fusion of two nuclei from two gametes to form Dikaryon). (c) Meiosis (after karyogamy reduction division takes place in diploid nucleus to form haploid stage). The sex organs if present are called gamentangia which may form gametes.
16) The various methods of sexual reproduction (by which the compatible nuclei are brought together for plasmogamy) are as follows:

(a) Planogametic copulation (fusion of two naked motile gametes) May be either Isogamy (fusing gametes morphologically similar) or Anisogamy (fusing gametes are both morphologically and physiologically dissimilar) or Oogamy (fusion between female gamete (egg) and male gamete (antherozoid).
(b) Gametangial contact (Male and female gametangia come in close contact with the help of a fertilization tube).
(c) Gametangial coupulation (Fusion of entire contents of two compatible gametangia) and formation of zygote which develops into a resting spore e.g., *Mucor*, *Rhizopus*.
(d) Spermatization (sex organs are completely absent and the sexual process is accomplished by minute spore like spermatia (malegamete) and specialized receptive hyphae (female gamete) e.g., *Puccinia*.
(e) Somatogamy (sex organs are not at all formed but two vegetative cells or two vegetative hyphae take over the sexual function and fuse together). e.g., *Morchella* and *Agaricus*.

17) The optimum temperature for the growth of fungi is between 20^0C to 30^0C.
18) Although light is not essential for growth, but for sporulation in many species, some light is necessary.
19) There are five basic types of life cycles in fungi as asexual, haploid, haploid-dikaryotic, haploid-diploid and diploid.

General Account of Viruses

1) Viruses are exceptionally simple, filterable, obligate, intracellular particles capable of reproducing inside a living host.
2) These are extremely smaller in size (smaller than bacteria) and it ranges from 20 nm to 300 nm in diameter.
3) Inside a living, the viruses are active and they feed, reproduce, grow and move. But when they live outside, they remain inactive and behave as non- living things. They are also called living chemicals as they behave like chemicals and can be crystallized.
4) Viruses differ fundamentally from cellular organisms in that they contain only one type of nucleic acid either DNA or RNA. The nucleic acid may be single or double stranded DNA or RNA and occur in either linear or circular form.
5) Viruses do not contain cellular structures such as plasma membrane, mitochondria, Golgi complex, lysosomes, ribosomes etc.
6) Their basic structure consists of a protein coat, (capsid) and nucleic acid. The smallest viruses known as virioids, consists of a single strand of naked nucleic acid without protein coat. Capsid is made up of several identical protein subunits known as capsomeres. These subunits are usually arranged in the helical or polyhedral geometric forms which are specific for a particular virus.
7) The capsomeres, forming the capsid (protein coat) of a virus, are of two types: -Pentamer (made up of five identical monomers) and Hexamer(having six monomers). Each monomer is connected with the neighbouring monomers on either side with the help of bonds. Similarly capsomeres are also connected with each other but the bonds between capsomeres are weaker.

8) In complex forms (e.g., influenza and herpes virus and many plant viruses) the virus particles are surrounded by on outer envelope. The envelope is membranous and made up of protein, lipids and carbohydrates. Viruses with envelope are called enveloped and those without envelope are said to be naked (e.g., TMV) (Fig.1.5).

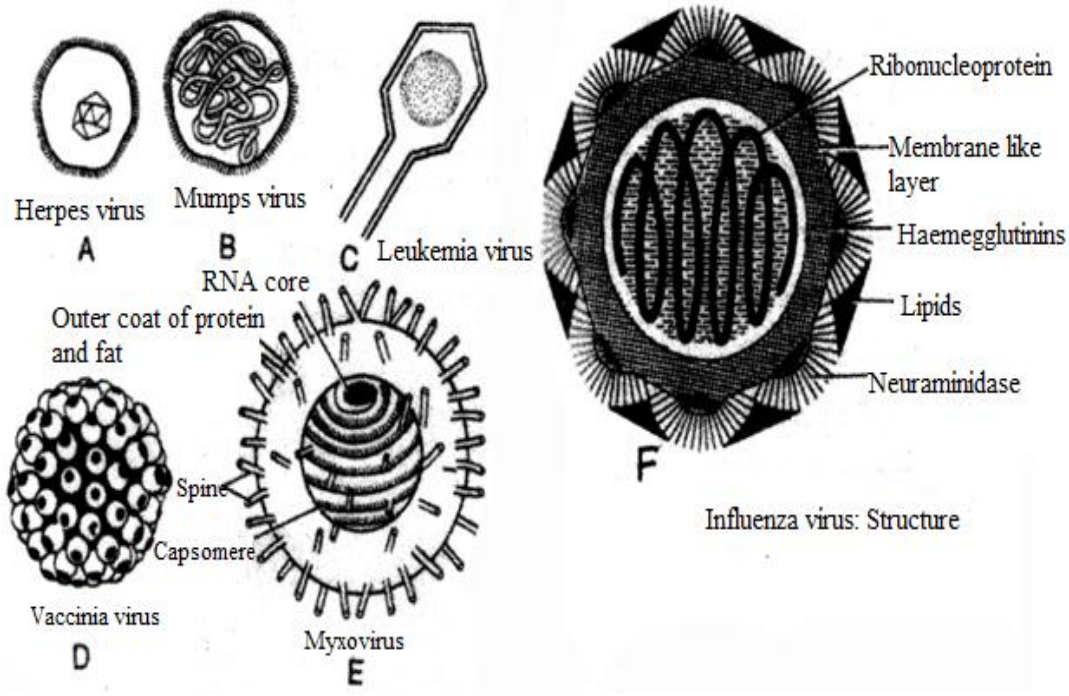


Fig.1.5. Different structures in Viruses

9) Viruses multiply by assembly line method. They do not divide. The cycle of multiplication include :
   - attachment of virus to host cell.
   - penetration by genetic material.
   - production of virus components by the cell.
   - assembly of new virus components by the cell.
   - release from the host cell.
10) They lack enzymes for most metabolic processes.
11) Viruses are also unique microorganisms as they lack machinery for the synthesis of proteins
12) They are obligate intracellular parasites of animals, (protozoainsects,fish, birds, amphibians, mammals and humans) or plants (angiosperms, gymnosperms, ferns, and fungi).
13) Many of the viruses have a close biological relationship with an arthropod or other type of vector on which they are dependent for their transmission from one host to the other.
14) The viruses cause very serious diseases in crop plants, ornamental plants and forests trees and many serious diseases in animals caused by viruses are well known since the time immemorial.

General Account of Protozoa

1) Protozoa are animal like organisms which are motile unicellular, non-photosynthetic eukaryotic microorganisms.
2) They normally obtain their food by ingesting other organisms by a process called phagocytosis.
3) These microorganisms are aquatic (fresh water or marine) or terrestrial (in soil) but majority of them are parasites in other animals including humans.
4) On the basis of the type of movement, the microorganisms are divided into there types:
   (a) Amoeboid protozoa: flagella are absent but a temporary projection of part of cytoplasm called pseudopodium is present.
   (b) Flagellate protozoa: They have either simple flagellum or a very complex flagellar arrangement.
   (c) Ciliaryprotozoa: In some protozoa e.g. *Paramecium*, the surface is covered with these structures and cilia are shorter than flagella and have a co-ordinate motion.
5) The cell is made up of a plasma membrane, cytoplasm and nucleus. The plasma membrane may have outer protective coverings such as pellicles, shell, test or torica.(**Fig 1.6).**
6) The cytoplasm is more or less homogenous substance consisting of protein molecules. It is made up of an outer ectoplasm and an inner endoplasm. Ectoplasm is gel like and endoplasm is more voluminous and fluid.
7) Some protozoa secrete a resistant covering and from a cyst at certain times of their life cycle. This protects organisms against adverse environments. It also functions as a site for nuclear organization and serves as a means of transmission in parasitic species.
8) The nucleus is typical eukaryotic. It has nuclear membrane, nucleoplasm and chromosomes. Normally only one nucleus is present (e.g.,*Amoeba*). But in some protozoa two similar nuclei are found while in others e.g. ciliate species to dissimilar nuclei (one micro and another macro nucleus) are found. The macro nucleus is large and controls the metabolic activities and regeneration process and smaller or micro nucleus is responsible for reproductive activity.
9) On the basis of Nutrition the protozoa are autotrophic, holozoic or parasitic. *Amoeba* uses pseudopodium for gathering food, *Paramecium* uses cilia, and Suctorianus estentacles. The autotrophic forms have chlorophyll for photosynthesis. e.g., *Euglena.* Some protozoa show symbiotic relationship with other organisms.

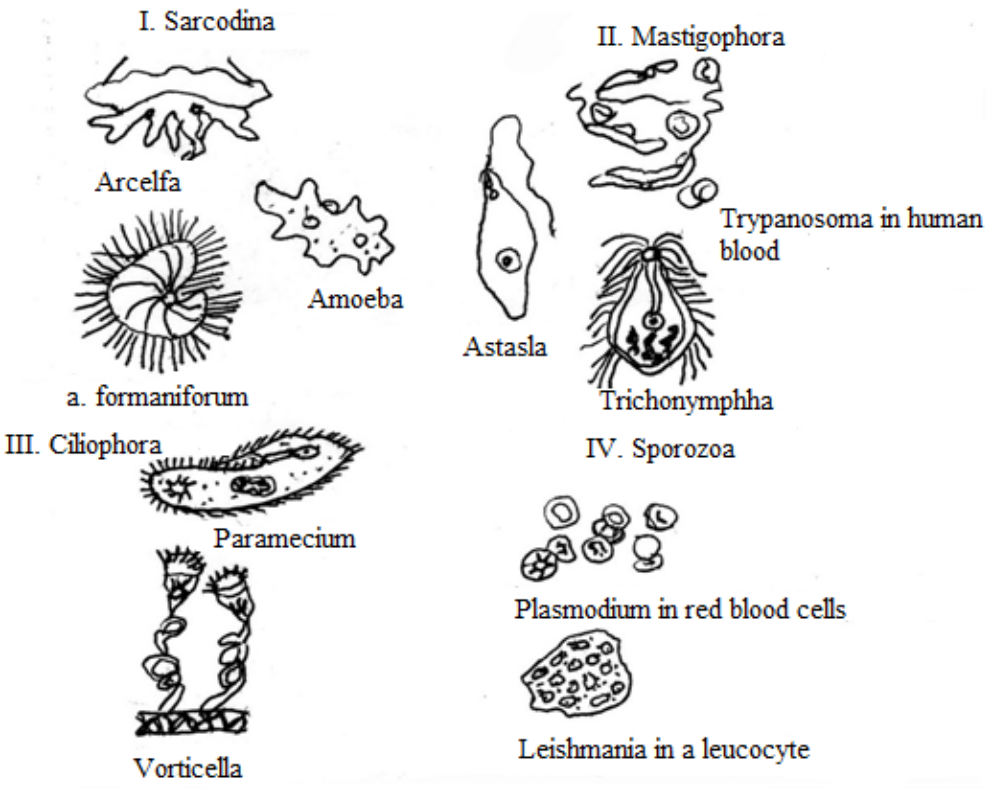


Fig.1.6.Various forms of Protozoa

10) Asexual reproduction is some flagellate and ciliate species is associated with cyst formation.
11) Reproduction takes place by asexual and sexual methods. Methods of asexual reproduction are binary fission (e.g. *Amoeba),* multiple fission (e.g. *Plasmodium*), and budding. Sexual reproduction takes place by conjugation (e.g. *Paramecium*). And isogamy (e.g. *Monocystis*).
12) Protozoa play an important role in food chain and food webs and are of particular importance in the ecological balance of many communities. Some protozoa caused diseases in animals including humans which are chronic and acute. In addition, these microorganisms have also become important research tools for biologists and biochemists.

1.4 DISTRIBUTION OF MICROORGANISM

Microorganisms are cosmopolitan in distribution. They occur in every type of habitat that can support life. This exceptional wide natural distribution is due to physiological diversity exhibited by them. Following physiological characters contribute to their survival in varied habitats:

(i) They can grow in inorganic environments without illumination. (Known as chemolithotrophs).
(ii) They have the capacity for rapid growth.
(iii) They have higher metabolic rates.
(iv) They do not depend on the availability of specific micronutrients in the environment.

(v) Some of them (bacteria and cyanobacteria) can use nitrogen a capability not known to occur in any other group.

Under suitable environmental conditions, the microbes frequently grow multiply and produce spores, cysts and resting cells. We shall discuss the distribution of microorganisms under following heads:

1. Microbes in soil
2. Microbes in aquatic environment
3. Microbes associated with plants
4. Microbes in Air
5. Microbes in Food
6. Microbes in Milk.
7. Microbes of human body.

1. Microbes in Soil

Man depends upon the soil for his food and the soil depends upon the micro-organisms for its fertility. Agriculture would not be possible without microorganisms in the soil. There are five major groups of microorganisms in the soil. They are Bacteria, Fungi, Algae, Protozoa and viruses. One gram of soil has about 200-500 billions of microorganisms.

Microbial Population in a fertile soil

Type	Number per gram
Bacteria: Direct count,	25×10^8
Dilution plate	15×10^6
Actinomycetes	7×10^5
Fungi	4×10^5
Algae	5×10^4
Protozoa	3×10^4

A. Bacteria: Bacteria are the larger group of soil microorganisms. The bacteria which occur in soil are coci, bacilli and spiral forms. Among these, the bacilli are in highest number and they swim actively in the soil solution. Some common soil bacteria are the species of *Pseudomonas, Arthrobacter, Achromobacter, Bacillus, Clostridium, Micrococus, Flavobacterium, Chromobacterium* and *Mycobacterium.* Both autotrophic and heterotrophic bacteria occur in the soil. Different species of *Thiobacillus, Ferrobacillue, Nitrosomonas* and *Nitrobacter*are also found.in the soil as chemosynthetic autotrophic bacteria.

Environmental factors like temperature, moisture, pH and depth of the soil affect the distribution of bacteria in the soil. Certain bacteria like *Mycobacterium* and *Pseudomonas* are commonly found in the soil near the petroleum wells. These bacteria are responsible for oxidizing ethane. *Escherichia* bacteria seldom occur in the soil, many cellulolytic bacteria, such as species of *Cytophaga* and *Sporocytophaga* are found in cellulose rich soil.

B. Actinomycetes: A large number of actinomycetes are present in dry and warm soil. They are particularly abundant in the soil rich in decomposed organic materials. Species of *Streptomyces, Micromonospora* and *Nocardia* are some common actinomycetes which occur in soil. They are responsible for the characteristic musty or earthy smell of a freshly ploughed field and are capable of degrading many complex chemical substances and thus play an important role, in soil environment.

C. Fungi: Several fungi are present in the soil and play an important role in the improvement of soil nutrients in neutral and alkaline soils. Majority of soil fungi grow in acidic soils with aerobic condition. Agricultural practices (crop rotation, use of fertilizers and insecticide etc.) and the depth of the soil also influence the fungal composition. Some important soil fungi are the species of *Aspergillus, Botrytis, Cephalosporium, Penicillium, Alternaria, Monilia, Fusarium, Verticillium, Mucor, Rhizopus, Pythium, Chaetomium,* and *Rhizoctonia,* Yeasts are not very common in the soil except in Vineyard and orchard soils. Some fungi such as species *of Alternaria, Aspergillus, Cladosporim* and *Dematium* are helpful in the preservation of organic materials in the soil. Adding of organic matter to the soil stimulates soil fangal flora. It is to be noted that the mycelium of fungi play an important role in binding the soil particles because of their interwoven hyphae.

Some phytopathogenic fungi also live in soil, often as saprophytes e.g. *Spongospora* of myxomycetes that cause powdery scab of potato tuber; *Phytophthora* and *Alternaria* species cause late blight of potato and early blight of potato respectively.

D. Algae: The algae are widely distributed in the soil even in deserts. Many algae occur on the surface of moist soils where sufficient light is available. The growth of algae is beneficial for soil conservation and in improving soil structure. In paddy fields, blue green algae play a significant role in nitrogen fixation.

The most commonly occurring algae isolated from the soil are the members of cyanophyceae and chlorophyceae e.g. *Nostoc, Cylindrospermum, Anabaena* and *Chlorella, Chlorococcus* and *Scytonema*. In addition to these, certain diatoms are also frequently occur in the soil.

E. Protozoa: Protozoans are present in great abundance in the upper layer of the soil and their number has a direct effect on bacterial population, because they ingest bacteria. Depending upon the condition of the soil, protozoans may exist in vegetative or cystform. Protozoans present in the soil belong to the class Mastigophora(species of *Bodo, Cercobodo, Cercomonas Monas, Spiromonas*etc) class sarcodina(*Amoeba, Biomyxa, Nuclearia, Trinema)* and class ciliata are (*Colpoda, Gastrostyla, oxytricha*etc).

F. Viruses: Viruses are present in very small number in the soil. Bacteriophages ingest bacteria and actinomycetes and some viruses infect the fungi present in the soil.

2. Microorganisms in aquatic environment

Water is unique environment for micro-organisms. A large number of micro-organisms are carried from air and soil through various sources before water reaches reservoirs like rivers, lakes, ocean etc. Aquatic habitats are only marginally aerobic. Microorganisms occurs in all

depths. Drifting microbial life of aquatic environment is called Plankton. There are various types of microorganisms in the aquatic environment .Some major examples are as follows:

I. **Bacteria:** Bacteria are heterotrophic organisms of water, which may live in close association with the algal flora of water. They develop well both in fresh and marine water, near the submerged vegetation and just above the mud layer. Anaerobic bacteria and few fungi also grow at the bottom sediments where are algae are absent. Pigmented and non-pigmented bacteria like *Pseudomonas, Chromobacterium, Achromobacter, Flavobacterium* and *Micrococus* occur frequently in unpolluted water *Escherichia coli, Streptococcus faecal is, Proteus vulgaris* and *Clostridium perfringens*, the characteristic intestinal tract inhabiting bacteria are also found in polluted water. Species of certain bacteria like *Pseudomonas vibrio, Favobacterium* and *Achromobacter* may be present in surface layers of sea water. These marine bacteria have important roles in nitrogen, sulphur, Phosphorus and carbon cycles within the sea.

II. **Fungi:** Certain fungi like *Saprolegnia, Manoblepharis* and Chytrids occur in well aerated waters. These are saprophytes that grow on dead algae and small animals mainly in fresh water habitats. They are important decomposers in aquatic environments. Some water moulds are parasitic on the gills of fish. The fungi which occur in sea water include the species of *Chytridium, Patersonia* and *Ophiobolus.*

III. **Algae:** Many planktonic and benthic species of algae occur in different habitats of water both fresh and marine. On the basis of their habitat in fresh and marine water, algae are divided into following three categories:

(a) **Epipelic Algae:** Algae growing on deposit sediment in water. e.g.:- *Oscillatoria, Navicula*.

(b) **Epipsammic Algae:** Algae attached among the coatings of bacteria on sand grains.

e.g. – *Fragilaria, Chaetophora*, etc. (Fresh water) and *Raphoneis, Amphora, Rivularia*

etc. (Marine forms)

(c) **Plankotonic Algae:** Free floating algae. e.g., *Anabaena, Pandorina, Chlamydomonas* etc. (Fresh water) and *Rhizosolenia, Coratium, Chaetoceros, Peridium*etc (Marine forms).

IV. **Protozoa:** The Protozoans are generally found in water films surrounding the soil particles. Protozoans like *Uroglenopsis*, along with algae *Eudorina* and *Volvox* produce an undesirable odour to water and pollute the fresh water ecosystem. Aquatic protozoans, in planktonic forms are very commonly distributed in fresh and marine water. These Protozoans are Ciliates, Flagellates and heliozoans etc.

3. Micro-organism in Association with plants

Plant parts leaves, stems, flowers, fruits, seeds and roots are literally covered with micro-organisms of various kinds. Some of the common associations of soil-micro organisms with plant are –

(A) Rhizosphere – Microbes influencing root and its surrounding. These are

(a) Legume Root Nodule e.g., Rhizobium

(b) Associative Nitrogen fixative. e.g., *Azotobacter* and *Azospirillum.*

(B) Mycorrhizae- These are fungus- root association.

The roots of about 80% of all kinds of vascular plants are normally involved in symbiotic association with mycorrhizae. Generally three types of mycorrhizal association have been found:

I **Ectomycorhizal:** In this fungi grow as external sheath around the tip of the root with limited inter cellular penetration of the fungus into the cortical region of root. This type of association is common in oak, birch, beech and coniferous trees.

II **Endomycorrhizal**: In this, fungal hyphae penetrate the outer cortical cells of the plant root where they grow intracellularly and form coils, swellings, or minute branches. These are characterized by two intracellular structures called Vesicles and arbuscules. That's why these are called vesicular – arbuscular mycorrhizae (VAM).

This is found in wheat, corn, beans, tomatoes, apples, oranges and many commercial crops and grasses.

III **Ectendomycorrhizal Association:** This type of association has more persistant Intracellular infections of cortical cells found predominantly in the family Orchidaceae.

(C) Actinorrhizae: Actinomycete association with plant roots is called actinorrhizae. They are formed by the association of frankia strains. Frankia strains are capable of nitrogen fixation and are important in the life of plants.

(D) Tripartile Association: When a plant develops relationship with two different types of micro-organisms it is called Tripartile association. Following types of associations are examples of tripartite association:

(i) Endomycorrhizae plus rhizobia including *Rhizobium* and *Bradyrhizobium.*

(ii) Endomycorrhizae and actinorrhizae.

(iii) Ectomycorrhizae and actinorrhizae.

(iv) Ectendomycorrhizae and actinorrhizae.

4. Microbes in Air

Air as a matter of fact, it not a suitable medium for the growth of the micro-organisms and studies indicate that higher the altitude, one might expect to find fewer micro-organisms. Air itself does not support growth of the microorganisms but they are either borne on dust particles, in moisture droplets expelled by men during talking, coughing and sneezing. Micro-organisms are more numerous in the air during dry weather than in wet weather because rain washes them out of the air.

A variety of microorganisms are found in air over populated land areas. These include spores of *Bacillus* and *Clostridium*, ascospores of Yeasts, Conidia of moulds, cysts of protozoans, unicellular algae, Non spore forming bacteria (*Micrococcus luteus*), non-pathogenic bacteria, gram negative rods (*Chromobacterium*) etc. The spores of many pathogenic fungi (e.g., rusts) causing crop diseases, plant pollens, seeds (minute) are transmitted from one place of another through air currents.

A number of human diseases are air borne and are transmitted by infectious dust e.g. Diphtheria(*Corynebacteriumdiphtheria)*;Tuberculosis(*Mycobacteriumtuberculosis)*, whooping cough (*Bordetella pertussis)*, children's influenza (*Haemophilies influenzale)* etc.

5. Microorganisms in Food

Microbes are in direct competition with the human for the nutrients present in food. So foods are ideal culture media for microbes, and different food items become contaminated with microbes, which are present in soil, the bodies of plants and animals, water, air, equipments during processing or preparation. Microorganisms that occur in foods may be divided into following categories:-

I- Beneficial organisms which bring about desirable fermentations e.g., those which are used in the preparation of cheese, vinegar etc.

II- Harmful organisms which are responsible for decay of substances rich in organic matter, and undesirable fermentations.

III- Pathogenic organisms causing dreadful diseases and food poisoning by their toxic secretions.

IV- Microorganisms themselves form food e.g., single cell proteins and Mushrooms.

The effect depends upon the type and numbers of microbes and also on the nature of food i.e., whether cooked, preserved or processed. Sometimes specific microbes are added to food to get a desired effect. e.g., Poipickled cabbage (*Lactobacillus plantarum*).

Food rich in proteins (meat, eggs, etc.), those with carbohydrates (vegetables and fruits) are spoiled by different types of micro-organisms by the process of putrefaction and decomposition (e.g. *Pseudomonas, Micrococcus* and *Bacillus).*

6. Microbes in Milk

A number of micro-organisms thrive in milk (which is one of the nature's most preferred food), and its products. Milk inside the udders is free from bacterial contamination, but as it comes through the teat of the udder, it is contaminated as bacteria are always present at the teat canals of the udder. *Micrococci* and *streptococci* constitute the teat microflora.

Milk can be easily contaminated by pathogenic organisms to the milk in various ways. Since bacteria are present everywhere including hay, feeds and ground. The important direct possible ways of contamination are –

(i) Milking utensils (ii) Hay and other feeds, (iii) Hands of milkers, (iv) The udder of cow and buffalo and (v) the skin of animal. Various types of micro-organisms are found in milk. The presence and absence of these organisms depend on sanitary quality and conditions of production. The important microorganisms in milk are:

I. **Bacteria:** Bacteria form the major section of microbes that grow well in milk. They may produce beneficial or desirable effects and detrimental or undesirable effects. The study of these bacteria in relation to milk and milk products is known as dairy bacteriology.

(a) Beneficial Effects

Microorganisms are deliberately added to milk to produce fermented milk products, to create new pleasing food flavours and odours. Yogurt is produced by adding two bacteria: *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in the ratio of 1:1. *Streptococcus* produces acid and *Lactobacillus* produce aroma components. Cheese, which has been thought to be developed 5000 B.C. is one of the oldest human foods. About 2000 varieties of cheese are produced all over the world. Cheeses are classified on the basis of texture or hardness as:-

Cheese		**Bacteria Used**
I. Softcheese (ripened)	-	*Streptococcus lactis* and *S.cremoris*
II. Soft cheese (unripened)	-	*Streptococcus lactis* *S. cremoris* *S. thermophilus* *Lactobacillus bulgaricus*
III Semi soft	-	*Streptococcus lactis* and *Brevibacteriumlineus* *S. cremoris*
IV. Hard	-	*S. lactis, S.cremoris* and *Lactobacilluscasei* *S.durans, S. thermophilus*
V. Very Hard	-	*S. lactis, S. cremoris, S. tshermophilus*and *Lactobacillus bulgaricus*

(b) Detrimental or undesirable Effect

Spoilage occurs when microorganisms degrade the carbohydrates, proteins and fats of milk. Examples of common types of spoilage in dairy products and associated bacteria are following:-

Spoilage type		**Bacteria involved**
Souring	-	*Lactobacillus* sp. and *Streptococcus* sp.
Sweet curdling	-	*Bacillus* sp., *Proteus* sp., *Micrococcus* sp.
Gas production	-	*Clostridium* sp. and coliform bacteira
Red rot	-	*Serratia*sp.
Grey rot	-	*Clostridium*sp.

II **Yeasts:** They are found in milk and milk products. They act on lactose and produce acid and carbon dioxide. Some produce gassy fermentation and some are lipolytic in action. These contaminate milk through feed and soil. e.g.,*Torulacremoris, Torulalactis.*

III. **Moulds:** They contaminate and grow in large number on the surface of butter, cream, khoa and cheese. The colour may be black, grey, blue or white. They produce undesirable odour also. Some are lipolytic and some are proteolytic e.g. *Penicillium* sp, *Cladosporium* and *Gleotrichum.*

IV **Bacteriophages:** Bacteriophages present in milk kill the bacteria in the starters and interfere in the process of fermentation which is essential to produce certain milk products such as butter and cheese.

V **Viruses and Protozoa:** These are not common organisms present in milk products but they occur in some rare conditions.

7. Microbes of Human body

The human foetus in uterus is free from bacteria and other microorganisms. Within hours after birth, it begins to acquire a normal microbiota within the first week or two. After that a variety of microorganisms become associated with the human body. Thousands of microbes are present around us and many inhabit human body in natural course. Most of the microbes of the human body are commensals, as they do not harm the host. They obtain their nourishment from secretion and excretory wastes of the human body. Some microbes act as scavengers by ingesting excretory wastes or are beneficial to the host. e.g., – certain intestinal bacteria synthesize vitamin E and K, whereas others protect the host from the pathogenic microbes.

I. **Microbes of skin**

The human skin always remains in contact with bacteria present in the air, but most of them are unable to grow since the skin secretes some bactericidal substances.*Staphylococcus, Streptococcus, Propioni bacterium,* moulds, yeasts and some pathogenic bacteria live on the surface of the skin. Some dermatophytic fungi viz. *Epidermophyton, Microsporum* and *Trichophyton* may colonize in the skin and produce athlete's foot and ringworm diseases.

II. **Microbes of the mouth cavity**

Continuous presence of soluble nutrients and abundance of moisture in the mouth cavity provides a suitable environment for the growth of microorganisms. Some common microbes are *Staphylococcus aureus, S. epidermidis, S. mitis, Lactobacilli, Actinomycetes, Bacteroidesoralis, Candida albicans, Treponemadenticala, Mycobacteria, Entamoeba* sp. and *Trichomonas*etc.

III. **Microbes of Gastro – Intestinal tract**

Several micro-organisms such as *staphylococcus aureus, S. epidermidis, Haemophilus influenzae* and *Neisseria* inhabit the pharynx. Stomach contains very few microorganisms because of its acidic pH. When the stomach functions normally, it is devoid of microbes due to the presence of gastric juices. Many gram + ve facultative bacteria and fungus *Candida albicans*are found in the duodenum. Similarly a large number of micro-organisms are found in the large intestine (colon). They include gram (-)ve *bacilli* gram (+)ve*bacilli, Enterobacter, Escherichia coli, Proteus* and *Candida albicans*. In addition to these certain Protozoans *Trichomonashominis, Entamoeba hartmsanni*, are also present.

IV. **Microbes of the mucous membrane of the eye**

Staphylococcus albus, Comybacterium xerosis and mycoplasmas are usually associated with the mucous membrane of the eye.

V. **Microbes in Respiratory tract**

We inhale a large number of adsorbed micro-organisms along with dust- particles. Most of them are trapped in the nasal cavity. Some Staphylococci, aerobic Corynebacteria, besides other cocciand bacilli inhabit the nasal cavity.

VI. **Microbes of Genito-urinarytract**

The upper genitourinary tract consisting of kidneys, ureters and urinary bladder is usually free of microorganisms. In both male and female, a few bacteria as *Staphylococcus epidermidis, Streptococcus faecalis* and *Corynebacterium* sp. are usually present in the distal portion of urethra. In the adult female genital tract, the major microorganisms are the acid tolerant *Lactobacillus* sp., *Bacteroides* sp., aerobic *corynebacteria, Peptostreptococus* sp. and *Enterococci, Mycobacterium smegmatis* and mycoplasmas.

1.5 CLASSIFICATION

In the early 19th century the traditional classification of living organisms was given by Aristotle. He classified the living organisms into two kingdoms Plantae and Animalia. Plantae includes algae, fungi and Bacteria & other plants. Other kingdom Animalia includes all animals including protozoa.

A three kingdom classification was given by E. Haeckel in 1866. Heincludeda the third kingdom Protista and classified living organisms as follows:

1- Protista (Algae, fungi, bacteria and Protozoa)
2- Plantae (excluding unicellular algae and fungi)
3- Animalia (excluding protozoa.)

Later, Copeland (1956) suggested a four kingdom system of classification of living organisms. He placed bacteria and blue-green algae in the fourth Kingdom Monera and fungi in the third kingdom Protista.

Whittaker in 1969 proposed a five kingdom system of classification of living organisms. His classification is as follows:

1- Monera (Bacteria and Cyanobacteria)
2- Protista (unicellular algae, slime molds and protozoa)
3- Fungi
4- Plantae (Eukaryotic multicellular plants)
5- Animalia (excluding protozoa)

Hawker and Linton in 1971 classified microorganisms into 3 heads:

I. **Viruses:** Specific group of sub-cellular obligate parasites.

II. **Prokaryote:** organisms with prokaryotic organization. This included-
   (a) Bacteria (unicellular forms without a definite nucleus).
   (b) Higher Bacteria (filamentous actinomycetes to filterable mycoplasmas).
   (c) Rickettsiae (Parasitic bacteria having the appearance of tiny rod shaped or spherical resembling bacteria and viruses).
   (d) Cyanobacteria (photosynthetic forms of prokaryotic organization)

III. **Eukaryote :** organisms with eukaryotic organization which includes-
   (a) Algae (unicellular eukaryotic algae belonging to Chlorophyceae, Chrysophyceae and Euglenophyceae).
   (b) Fungi (moulds) (fungi with unicellular to multicellular hyphae with cottony growth belongs to Phycomycetes, Ascomycetes, Basidiomycetes, and deuteromycetes).
   (c) Slime moulds (organisms with slimy mass of naked motile protoplasm called plasmodium).
   (d) Protozoa (unicellular animals without chlorophyll. and are divided into sarcodina, sporozoa, ciliophora and mastigophora.
   (e) Nematodes (multicellular thread like cylindrical worms).

Recently microorganisms are classified into two major groups:

(A) Acellular Infectious agents
(B) Cellular Infectious agents

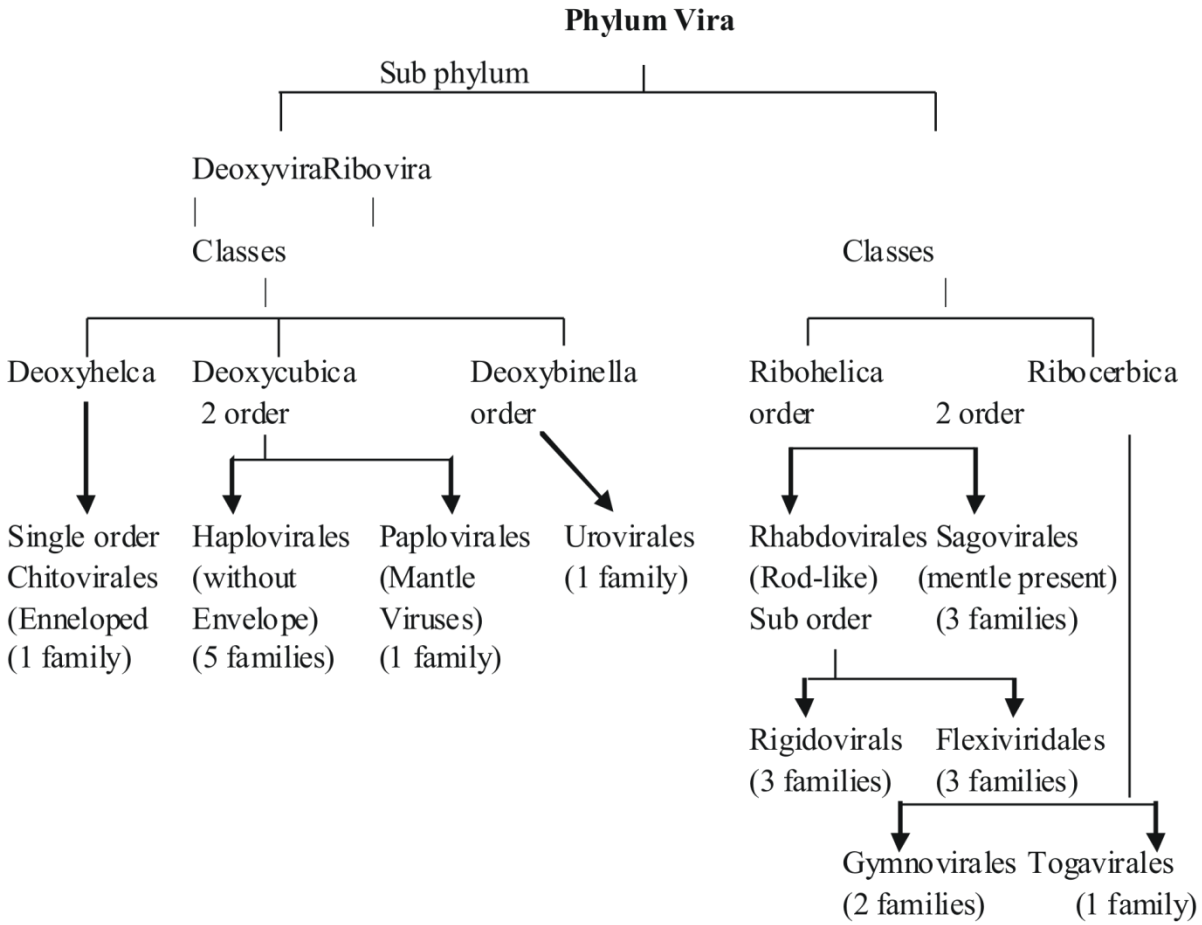
(A) Acellular Microorganisms: These are called acellular organisms as they do not possess three minimum characteristics of a cell- Presence of a membrane, genetic material and metabolic machinery.

These are of following types:

I. **Viruses:** Ultramicroscopic obligate parasites made up of nucleic acid, lack cytoplasm and cell organelles and multiply only within the living host cell.

A Provisional committee or Nomenclature of viruses (P.C.N.V.), headed by system proposed by Lwoff, Horne and Tournier (1962). According this system all viruses are grouped into a single phylum –Vira which is divided into subphyla, classes, orders, suborders and families. On the basis of following characters:

   (a) Type of nucleic acid DNA or RNA.
   (b) Symmetric helical Cuboidal or biral.
   (c) Presence or absence of envelope around capsid.
   (d) Diameter of helical nucleo capsids and no. of capsomeres present in cuboidal viruses.



II **Viroids:**

These are small naked RNA molecules. These were first discovered by Diener (1971). They replicate autonomously. Viroid RNA is usually single stranded, circular and is of very low molecular weight viroids cause various plant disease such as potato spindle tuber (by PSTV), citrus exocortis, chrysanthemum stunt, cucumber pale fruit etc.

III **Prions:** These are rod-shaped proteinaceous infectious particles without nucleic acid; Prions were named by S.B. Prusiner (1984) who was awarded Nobel Prize in Medicine in 1997. Prions cause a variety of mammalian neurodegenerative diseases. These are generally fatal and are referred to as transmissible spongiform encephalopathies (TSES).These include diseases like scraple, disease of sheep; CreutzfeldtJakob disease (CJD) and Kuru – Human diseases. Prions also cause madcow disease of bovino spongiform encephalopathy (BSE).

IV **Virusoides:** They are also called satellite RNA. They are encapsulated by plant viruses packaged together with a viral genome. They cannot replicate independently and do so with the help of an associated virus.

(B) Cellular Micro-organisms: These microorganisms are cellular as their cells possess a membrane, genetic material and metabolic machinery. These are two types on the basis of presence or absence of nuclear membrane.

1. Prokaryotic microbes & 2. Eukaryotic microbes.

1. Prokaryotic microbes

These are unicellular microorganisms. These show prokaryotic cell organization. Prokaryotic micro-organisms are again subdivided into two-

(A) Archebacteria & (B) Eubacteria

(A) Archebacteria: These are the primitive type of bacteria which lack muramic acid in their cell walls; the membrane lipids have ether linked aliphatic branched chains. These possess distinctive RNA polymerase enzymes. Their ribosome is also of different composition in shape. These bacteria are categorized into (a) methanogenic bacteria (b) extreme halophiles and (c) thermoacidophiles.

(B) Eubacteria (True bacteria): This comprises a vast majority of bacteria. The Peptidoglycan cell wall contains muramic acid. The membrane lipids have ester linked straight chained fatty acids.

These are divided into following groups –

(i) Spirochetes: These are gram-negative, chemohetrotrophic bacteria, and are distinguished by their structure and motility. They lack external rotating flagella but still can move through very viscous solutions, exhibiting creeping or crawling movement when in contact with a solid surface. Their unique style of motility is due to the presence of an axial filament. Two or more than a hundred prokaryotic flagella called periplasmic flagella, extend from both ends of the cyinder and often overlap one another. Spirochetes are anaerobic, facultatively anaerobic or aerobic. Some pathogenic forms are: *Treponemapallidum*, *Borrelia burgdorferi*, *Leptospira* and causing syphilis, lyme disease and Leptospirosis respectively.

(ii) Rickettsias: These belong to order Rickettsiales. These are a group of Gram-negative intracellular parasitic bacteria. These resemble viruses in their very small size and intracellular existence. They differ from viruses in having both DNA and RNA, a plasma membrane, ribosome, enzymes etc. They are intermediate between bacteria and viruses. Some of the important pathogenic forms are:

Rickettsia prowazeki causes typhus fever;
Rickettsia rickettsii causes rockey mountain spotted fever;
Rickettsia orientalis causes scrub typhus; and
Rickettsia burnetti causes Q fever

(iii) *Mycoplasma* (Mollecutes)

Mycoplasmas are prokaryotes without cell wall and are placed in the class molecules (Mollis = Soft; cutis = skin). They are pleomorphic and occur in any shape such as spherical, pear shaped, branched and helical filaments. They are non-motile but can glide along liquid covered surfaces. They are usually facultative anaerobes but a few are obligate anaerobes. Their genome is one of the smallest (about 5-10 x 10^8daltons) found in prokaryotes. They live

as saprophytes or parasites or pathogens of plants, animals, man or insects. The common examples of mycoplasmaare:

Mycoplasma pneumoniae (causing mycoplasmal pneumonia); *Mycoplasmamycoides* and *gallisepticum* causing contagious bovine pleuropneumonid in cattles, and in chicken respectively. *Mycoplasma urealyticum* causes genital infection.

(iv)Cyanobacteria (Blue green algae)

Cyanobacteria are blue green algae or blue green bacteria. They form a connecting link between bacteria and green plants. They are prokaryotic. They are photoautotrophs as they contain chlorophyll which is located in thyllakoids. Their photosynthetic system closely resembles that of eukaryotes, because they possess phycobili proteins as accessory pigments like red algae. Cyanobacteria vary greatly in shape and appearance. They may be unicellular and exists in colonies of various shapes or multicellular and form filaments called trichomes.

Cyanobacteria are not toxic to man. The cytoplasm contains phycobillins and carboxysomes. They have heterocysts which are specialized cells for nitrogen fixation and akinetes for spore formation. Common examples are: *Anabaena, Nostoc, Chlorococcus, Oscillatoria, Stigonema*etc.

(V) Actinomycetes:

Actinomycetes include fungus like bacteria. (Actis = rays; mykes = fungus)

These are aerobic, gram positive bacteria that form branching usually non-fragmenting hyphae and bear asexual spores. Most actinomycetes are non-motile. Motility is confined to flagellated spores only. They are mainly soil inhabitants and can degrade a variety of organic compounds.

Actinomycetes are chemoorganotrophs, as they rely on organic compound for their energy. These organisms are of great economic importance as they produce about 85% of know antibiotics.

The important actinomycetes are the following: **Streptomyces**(produces streptomycin): Erythromycine & Chloramphenical, tetracycline; **Micromonospora**(produces gentamycin) .Some actinomycetes fix atmospheric nitrogen and live symbiotically (e.g. *Frankia*sp). A few actinomycets are pathogens of human, animals and plants e.g. *Thermoactinomyces vulgaris* whichcauses a respiratory disease called Farmer's lung in humans.

2. Eukaryotic Microbes

These are they microorganism with eukaryotic organization. Such organisms are divided into three parts: I Unicellular Eukaryotic Microbes, II Multicellular Eukaryotic Microbes (Fungi) & III Helminthes.

I. Unicellular Eukaryotic Microbes

These are solitary or colonial unicellular microbes with eukaryotic organization. They are usually aerobic forms, and can be motile or non- motile. Motility is due to cilia, flagella or pseudopodia. They are subdivided into following types:

(a) Photosynthetic Protists (Unicellular algae) :

It includes unicellular photo synthetic organisms belonging to different groups Chlorophyceae, Euglenophyceae, Xanthophyceae, Pyrrophyceae, Bacillariophyceae and Chrysophyceae. Some major group of this are

1- **Dianoflagellates:** These belong to Chrysophyceae. They are unicellular with cellulose cell wall. They possess two flagella. They reproduce only asexually. Sexual reproduction is unknown. Some of them e.g. *Gonyaulax*secretes a toxin which kills marine animals like fish known as 'red tide'. Some Dianoflagellates are phosphorescent and make the sea Scarface glow in the dark.
2- **Diatoms:** These belong to bacillariophyceae. They lack flagella. Diatoms are diploid and reproduce asexually as well as sexually. Diatoms are nearly indestructible because of deposition of silica in their cell walls. They leave behind large amounts of cell wall deposits called diatomaceous earth.
3- **Euglenoids:** These are Euglena like protists. They lead plant like and animal like lives. These are free living forms found in fresh water ponds and ditches on damp soil. They do not possess a cell wall. The flagellum is duplicated before the cell divides. e.g. *Euglena.*

(b)Consumer – Decomposer protists:

These resemble fungi in appearance and life style but are more closely related to protists in cellular organization, reproduction and life cycle. These include:

I. Acellular Slime moulds (Myxomycota)

They are wall less mass of multinucleate protoplasm e.g. *Physarum.* It slowly streams or glides over decaying leaves or logs. This strand of protoplasm is called plasmodium. Feeding is by phagocytosis.

II. Cellular Slime moulds(Acrasiomycota)

These are numerous individual amoeboid cells which aggregate together and move like a mass of protoplasm. The individual cells are not fused. This is called pseudo plasmodium. e.g.*Dictyostelium.*

III. Water mould (Oomycota):

They consist of finely branched filaments called hyphae. Their cell walls are made up of glucanc and cellulose. Very small amount of chitin is found in rare cases They reproduce sexually by a large egg cell and a small antheridium, asexual reproduction by biflagellate zoospores. They are saprophytes (e.g. Saprolegnia) or parasites (e.g. *Phytophthora infestans).* Another significant feature of this group is presence of hydroxyproline amino acid in the wall protein.

(c) Protozoan Protists:

Protozoa are animal like protists that can be defined as motile eukaryotic unicellular microorganisms. They resemble multicellular animals in their morphology and physiology.

Some of them secrete a resistant Covering and form cyst which protects organisms against adverse environments. These organisms move by pseudopodium, flagella or cilia, and reproduce asexually and sexually. They play an important role in food chains and food webs. Many of them are parasitic in humans and animals.

I. Multicellular Eukaryotic Fungi:

This group includes the organisms which are placed in the kingdom fungi by Whittaker. They exist primarily in the form of filamentous hyphae. They lack chlorophyll, and reproduce asexually, sexually or by both methods. They are important decomposers and play an important role in recycling of minerals, thus are of great importance to humans in both beneficial and harmful ways. They are further divided into four groups (1) Phycomycetes (2) Ascomycetes (3) Basidiomycetes and (4) Deuteromycetes.

(3). Helminthes: The kingdom Animalia has only one group of microscopic organisms the helminthes. Tape worm, flukes and round worms are collectively called helminthes. There are two types of parasitic helminthes based upon morphological form. They are the flatworms, belonging to phylum Platyhelminthes with a very thin segmented body and round worms belonging to phylum Aschelminthes with an elongate, cylindrical, unsegmented body plan.

All helminthes are multicellular animals. They absorb nutrients through their body wall while living in the host intestine; most worms complete their life cycle on two hosts, and have the capacity to regenerate. Some pathogenic helminthes are: *Ascarislumbricoides* (Round worm); *Necatoramericanus* (Hook worm); *Fasciola hepatica* (Sheep liver fluke); *Taeniasolium* (Pork tape worm) and *Taeniasaginata* (Beef tap worm) etc.

1.6 MAJOR MICROBES

1.6.1 Major Microbes of food

Food is an indispensable item for all living organisms. All food items are associated with microbes in one form or other. Even naturally occurring food such as fruits and vegetables contain microorganisms. Major microbial flora of common food are listed below:

Types of food	Microbial flora

1. Milk	I. Biochemical Types - *Streptococcus lactis, S. cremoris* II. Acid producers –*Lactobacilli, Microbacteria, Coliform, Micrococcus* III. Gas produces –*Coliforms, Clostridium, Torulacremoris* IV. Proteolytic –*Bacillus, Pseudomonas, Proteus, Streptococcus* V. Lipolytic –*Pseudomonas, Achromobacter, Candida, PenicssSSillium* VI. Mesophilic- *Bacillus coagulans* VII. Thermophilic – *Bacillus stearothermophilus*
2. Dairy Products	*Lactobacillus, Leuconostoc, Micrococcus, Streptococcus, Geotrichum*
3. Raw milk	*Alcaligene, Bacillus, E.coil, Lactobacillus, Leuconostoc* and *Streptococcus*
4. Fruits and vegetables	*Bacillus, Pseudomonas, Salmonella, Corynebacterium, Erwinia, E.coli, Aspergillus. Botrytis, fusarium, Trichothecium, saccharomyces, monilia*and*Rhizopus*
5. Egg & Egg Products	*Pseudomonas fluorescens, P.ovalis, Salmonella, Proteus thamnidium,* moulds and yeasts.
6. Meat	*Clostridium, Enterobacteria, Micrococcus, Streptococcus faecalis, Proteus, Pseudomonas, Staphylococcus, Aspergillus, Candida.*
7. Fish	*Pseudomonas, Chromobacterium, Micrococcus, Flavobacterium, Corynebacterium, Sarcina, Serratia, Bacillus, E.coli, Clostridium.*
8. Bread	*Saccharomyces cerevisiae, Enterobacter. Lactobacillus brevis, Clostridium, Bacillus polymyxa, Serratiamarceseens* (red or bloody bread), *Rhizopusnigricans, Penicillium, Aspergillums, Mucor* (Bread mould).
9. Poultry	*Pseudomonas, Proteus, Chromobacter. E.coli, Salmonella, Bacillus.*
10. Shellfish	*Bacillus, Alcaligenes, Proteus, Coliforms.*
11. Fermented Food	*Streptococcus actis, lactobacillus, Clostidium, Leuconostoc, Acetobacter, Saccharomyces,Pediococcus*
12. Beef	*Cladosporium, Sporotrichum*
13. Seafood	*Pseudomonas* and *Vibrio*
14. Pickles	*Brettanomyces, Debaryomyces, Leuconostocmesenteroides.*

Besides these, some microorganisms are foods intoxicating. The food intoxication results due to ingestion of exotoxins secreted by microbes in food. The intoxication symptoms appear immediately after consuming contaminated food because it does not require growth of disease causing microorganisms. Some of the major food intoxicating diseases, their causal organisms and foods involved are listed below:

(A) Major Food intoxications

Disease	Microbe	Food involved
Food poisoning	*Bacillus cereus*	Meats, potatoes, Cereals. Rice products, pudding.
Botulism	*Clostridium botulinum*	Fish, meats and low acid canned food.
Perfringens food poisoning	*Clostridium perfringens*	Animal and Poultry Products.
Staphylococcal food Poisoning	*Staphylococuus aureus*	Meat, dairy Products, Poultry, custard and starch containing food.

(B) Major Food inflations

Campylobacteriosis	*Campylobacter jejuni*	Raw milk, raw chicken and poultry products.
Listeriosis	*Listeria monocytogenes*	Poorly processed dairy products.
Salmonellosis	*Salmonella typhimurium*	Poultry, eggs, dairy products, meat.
Shigellosis	*Shigellasonnei, S. flexneri*	Insanitary cooked food, fish, potatoes and salads.
Yersiniosis	*Yersinia enterocalitica*	Milk and meat product.
E.coli enteritis	Cheese and raw vegetable	

1.6.2 Major Microbes of Water

Water is unique physical environment, andfavours the existence of many types of micro organisms that are not common in soil. Micro organisms occur in all depths. The surface film and bottom sediments have a high concentration of microorganisms. Drifting microbial life of aquatic environment is called **plankton**, composed of phytoplankton and Zooplankton. The bottom region of the body of water harbours largest number and kinds of microorganisms called **benthic** microorganisms. The movement of water by wind, tide and currents affect the distribution of microorganisms.

(a) Major microbes of Ponds and Lakes

Lakes and ponds of temperate region show thermal stratification which influences the microbial population in different seasons. Common fresh water micro organisms are *Pseudomonas*, *flavobacterium*, *Aeromonas*, and *Acaligenes*, *Clostridium, Thiothrix* and *Thiobacillus.* Besides this both, Cyanobacteria and many algae contribute to massive water blooms.

(b) Major Microbes of sea

Diatoms, Cyanobacteria, Silicoflaellates, Dinoflagellates,etc.Chlamydomonas are major phytoplanktons. Many microorganisms, particularly algae and cyanobacteria cause a condition called Red sea and Red tides.Brown, amber or greenish yellow colourationis also due to abundance of micro organisms.

Common marine forms are *vibrio, Actinobacter, Pseudomonas, Flavobacterium, Staphylococcus*, several sps of Phycomycetes, Deuteromycetes and Myxomycetes and a number of protozoa and species of fungi also occur in sea water.

(c) Microbes of Domestic water

The domestic water is obtained from rivers, streams, ponds, dams, lakes, wells and bore wells. The micro organisms of domestic water include viruses, bacteria, algae, protozoa and fungi. Some of the microbes are listed below:

Bacteria :	*Streptococcus faecalis, S. bovis, S. equines, Pseudomonas, Alginomonas, Xanthomonas, Escheriachia coli, Enterobacter, Aerobacter, Salmonella, Bacillus, Micrococcus, Shigella, Proteus, KlebsiellaSerratia*
Viruses :	*Poliovirus*
Protozoa :	*Entamaebahistolytica* and *Giardia*
Fungi :	*Achlyaamericana, Dictyuchuspisci, Pythiumundulatum, Saprolegnia*
Algae :	*Anabaena, Microcyrtis, Nostoc, Oscillatoria, Oedogonium, Spirulina*

(d) Microbes of Sewage or Waste Water

Major microbes include coliform bacteria and micro organisms other than coliform bacteria. Major Microbes other than Coliform bacteria are:-

Bacteria:*Streptococcusfaecalis, S. faecium, S. bovis* and *S. equines*; some slime forming bacteria; *Sphaerotilus* and *Gallionella* (Iron bacteria); *Thiobacillus* (Sulphur bacteria).

Algae: *Microcystis, Spirulina* etc. produce nuisance characteristics and produce toxic substance also.

Viruses:Polio virus (enter through the human and animal intestinal tracts).

(e) Pathogenic water borne microbes

Some of the bacterial viruses and protozoan pathogens are able to survive in water and infect humans. Some of the water borne diseases is listed below:

Microorganism		**Disease**
Vibrio cholera	-	Cholera
Camphylobacter	-	Gastroenteritis and Diarrohea
Salmonella typhi	-	Typhoid
Leptospira	-	Jaundice, Haemorrhagic effects.
Giardia lamblia	-	Diarrohea
Cryptosporidium	-	Acute enteroicolitis
Naegleriafowleri	-	Primary amoebic meningo encephalitis (PAM)

1.6.3 Major Microbes of Soil

There are five major groups of micro organisms in the soil. They are bacteria, fungi, Algae, Protozoa and Viruses.

I. **Bacteria:** These are the largest group of soil microbes. Practically all kind of bacteria may be found in Soil. Major bacteria of soil are categorized as :

1. True bacteria: Of all the micro organisms' true bacteria are the most abundant in soils. Most commonly isolated bacteria from soils are –

(a) Gram negative bacilli:*Pseudomonas, Agrobacterium,Achrobacter, Rhizobium, Flavobacterium.*

(b) Gram positive non Sporing bacilli: *Corynebacterium, Arthrobacter, cellulomonas* :

(c) Gram positive cocci : *Micrococcus, Sarcina*

(d) Gram Positive Spore formingbacilli : *Bacillus* (aerobic) *Clostridium* (anaerobic)

2. Nitrogen fixing bacteria:

(a) Symbiotic bacteria: Live in the roots of legumes e.g.*Rhizobium*sps., (*Rhizobium leguminosarum* on peas, lentils etc, *R. japonicum* on soyabeans, *R. phaseoli* on beans, *R. trifoli* on Red, White and other clovers etc).

(b) Non- symbiotic bacteria live freely in soils-Azobacter (Aerobic), Clostridium (Anaerobic)

(c) Sulphuroxidizer:*Thiobacillusthiooxidans, Desulfovibriodesulfuricans*

(d) ProteolyticBacteria:*Clostridiumhystolyticum, Proteus vulgais, Pseudomonas fluorescens, Bacillus cereus.*

(e) Bacteria involved in nitrification:Nitrosomonas, Nitrosococcus, Nitrosospira, and Nitrosocystis.

Besides above mentioned bacteria, some soil inhabiting bacteria are pathogenic and cause plant diseases. e.g. Agrobacterium causes galls, Erwinoa sp. causes soft rot as well as dry necrosis in carrots, potato and cucumber.

II. **Actinomycetes**: Many actinimycetes are present in the soil, e.g. Streptomyces, Nocardia, Micromonospora. The filamentous actinomycete Streptomyces produces an odour causing compound called geosmin. Streptomyces species are responsible for potato scab disease.

III. **Cyanobacteria and other Algae** :

A number of cyanobacteria are abundant lyoccurin moist soils, e.g. Anabaena, Nostoc, Cylindrospermum microcystis, Oscillatoria etc. They fix molecular nitrogen present in the air and improve the fertility of soil. These are used as biofertilizers. Besides cyanophyceae, other algal genera are also present in soil. These are *Chlorella, Chlorococcum, Cladophora, Botrydiopsis, Bumilleria. Navicula, Pinnularia, Fragilaria* etc.

IV. **Fungi**: *Mucor, Rhizopus, Pythium,Penicillum, Aspergillus, Alternaria, Hormodendron, cladosporium* and yeasts are common in vineyard and orchard soils. Some soil fungi are good soil binders. e.g., *Aspergillus,Cladosporium, Rhizopus* and *Penicillium.* Some are pathogenic and cause diseases. e.g., *Alternariasolani* causing early blight of potato.

V. **Protozoa**: Many types of Protozoa are found in the soil. Some major soil protozoans are :

Mastigophora-	*Bodo, Cercomonas, Monas, Euglena, Spiromonas, Spongomonas, oikomonas*
Sarcodina-	*Amoeba, Biomyxa, Harmanella, Lecythium, Nucbaria, Trinema, Naegleria*
Cilliata-	*Colpidium, Calpoda, Gastrostyla, Halteria, Oxytricha, Pleutotricha, Vorticella, Uroleptus*

Protozoa do not serve any major function in the soil. They engulf bacteria and maintain some equilibrium of the bacterial flora of soil.

VI. **Viruses**: Bacterial Viruses (Bacteriophages), as well as plant and animal viruses, are present in soil. Viruses transmit genetic material from one bacterium to another through transduction. The bacteriophages have some effects on the ecology of the bacteria also.

The Rhizosphere microbes:

Rhizosphere is the area of soil around the root system. Based on the intimacy of microbial association with root system, the rhizosphere is divided into two regions, namely endorhizosphere and exorhizosphere. Microorganisms colonize the rhizosphere to utilize them as food. Certain fungi form symbiotic association with root to form mycorrhiza. Some examples of rhizospheremicroorganisms are as follows:-

Fungi –*Aspergillus flavus, A. niger, A. fumigates, A. terreus; Cladosporium herbarum; Fusarium oxysporum, F. solani.*

Bacteria –*Pseudomonas, Rhizobium, Bacillus, Agrobacterium, Micrococcus, Azobacter, Mycobacterium*

Microorganisms as Bio fertilizers

The nutrients of biological origin added to the soil to enrich the soil fertility are called biofertilizers or microbial fertilizers. The organisms used as biofertlizers include:

Rhizobium, Azospirillum, Azotobacter, Azotococcus, Anabaena, Nostoc, Plectonema and *Tolypothrix.*

Phosphate Solubilizing microbes: *Bacillusmegaterium, Xantnomonas, Pseudomonas, Aspergillus,* and *Pencilliumdigitatum.*

The spores of VAM fungi like *Glomus, Gigaspora, A.caulospora, Sclerocystis* and *Endogone*are used as VAM biofertilizers.

1.7 SUMMARY

In this unit you have learnt that–

- Microbiology is the study of microorganisms.
- Microorganisms are invisible creatures, too small to beseenwiththe naked eye.

- Microbes are widely distributed and are omnipresent. Theycan be isolated from air, water, soil, in living plants and animals and dead organic substanses.
- Microbes areeitherUnicellularormulticellularornon cellular forms, and are prokaryotes or eukaryotes.
- Microorganisms include viruses, Bacteria, Algae, Fungi and Protozoa. Non cellular microbes are viruses. Unicellular microbes have single cells e.g., Protozoa, Bacteria, someAlgae and some fungi. Multicellular microbes have many cells e.g., FungiandAlgae.
- Microbes have the capacity for rapid growth. They do not depend on the availability of specific micronutrients in theenvironment.
- Some of the microbes (bacteria and cyanobacteria) can fixatmosphericnitrogen.
- Soil is an excellent natural medium formicroorganisms. Man depends upon the soil for his food. The soil depends uponthemicro organisms for its fertility.
- Food is an indispensable item for alllivingmicro organisms. All food items are associatedwithmicroorganismsinoneform or other.
- In aquatic environment micro organisms occur from water surface to greater depths. The various water sources as ponds, pools, Lakes, rivers and oceans show a great diversity in theirmicroflora.

1.8 GLOSSARY

Actinomycetes: Gram Positive Bacteria that are characterized by the formation of branching filaments.

Adenoviruses: A group of DNA viruses, causing infection of the upper respiratory tract.

Antibiotic: A substance of microbial origin that has antimicrobial activity and it kills the other micrograms.

Archaebacteria: A group of bacteria that includes primitive type of bacteria in which cell wall lack muramic acid.

Bacillus: A rod shaped bacterium.

Bacteriophage: A virus whose host is a bacterium and replicates within bacterial cell.

Biofertilizers: The nutrients of biological origin added to the soil to enrich the soil fertility are called biofertilizers.

Botulism: A neuroparalytic disease due to an exotoxin produced by the bacterium *clostridium botulinum* in improperly canned or preserved food.

Chemoorganotraphs: organisms relying on organic compounds for their energy source.

Ectomycorrhiza: Amycorrhiza in which the fungal hyphae grow only intercellularly, never entering the cell wall of the host cell.

Endomycorrhiza: Amycorrhiza in which the fungal hyphae penetrate the cell wall of the host cell.

Epipelic Algae: Algae growing on deposit sediment in water.

Epipsammic Algae: Algae attached among the coatings of bacteria on sand.

Eubacteria: True bacteria in which cell wall contain muramic acid.

Mycorrhiza: A symbiotic association between fungus and plant roots.

Prions: Proteinaceous rod shaped infectious particles without nucleic acid.

Symbionts: Two organisms living together with mutual benefits.
VAM: Vesicular Arbuscular Mycorrhizal fungi. These are also called endomycorrhiza.
Virioids: Small naked RNA molecule.
Yoghurt: Fermented liquor made from milk.

1.9 SELF ASSESSMENT QUESTIONS

1.9.1 Choose the correct answer from the given below:

(a) Which one of the following isarchaebacteria?
(i) Blue– green (ii) Rickettsias
(iii) green sulphar (iv) Methanogens

(b)Which of the following water is free from bacteria?
(i) deep well water (ii) sea water
(iii) Water of hot spring (iv) rain water as it falls down

(c) Bacteria which in association with legume roots fix atmospheric nitrogen are called:

(i)*Azobacter* (ii) *Paseudomonas*
(iii)*Rhizobium* (iv) *E.coli*

1.9.2 Fill in the blanks:

a) The Characteristic earthy smell of freshly ploughed field is due to a compound called ..... produced by *streptomyces.*

b) Actinomycete association with plant root is called.....

c) Botulism is caused by.....

1.9.3 Answer the following in one word or in one sentence.

a) Name the microorganism which causes red rot of milk.

b) Name a symbiotic bacterium.

c) What name is given to a virus which infects bacteria?

1.9.4 Write true or false (T/F).

a) Prions are single stranded RNA containing cellular infections agents.

b) Flagellated cells are absent in cyanobacteria.

c) Viruses contain both RNA and DNA.

1.9.5 Explain the following –

i) Associative nitrogen fixation.

ii) How do microorganisms contribute to body odour?

iii) Compare prokaryotic and eukaryotic microorganisms.

Answers Keys:

1.9.1- (a)-(iv), (b)-(iv), (c)-(iii)

1.9.2- (a)-Geosmin, (b)-Actinorrhizae, (c)- *Clostridium botulinum*

1.9.3-(a)-*Serratiamarcescens*, (b)-Rhizobium, (c)- Bacteriophage

1.9.4-(a)-False, (b)- True, (c)- False

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1.12 TERMINAL QUESTIONS

1. Give a brief account of Salient features of main groups of microorganisms.
2. Write short notes on the following:

   (i) Archebacteria (ii) Viroids and Prions(iii) Mycoplasma

   (iv) Cyanobacteria (v) Actinomycetes (vi) Spirochete.
3. Discuss the different approaches to classification of microorganisms.
4. What are the applications of the following microorganisms?

   (a) *Rhizobium* (b) *Bacillusthuringiensis*
5. List various types of microbial food spoilage and name the organisms responsible in each instance.
6. Discuss the distribution of microorganisms in soil.
7. Differentiate the following:

   (i) Prokaryotes and Eukaryotes

   (ii) Ectomycorrhiza and Endomycorrhiza

   (iii) Autotrophs and Heterotrophs

   (iv) Archaebacteria and Eubacteria

UNIT 2- ISOLATION AND CULTIVATION OF MICROORGANISMS, INSTRUMENTS USED IN MICROBIOLOGICAL STUDIES

2.1 OBJECTIVES

It is well known that microorganisms occur in natural environments; and are contaminated and mixed with several other forms of life. To know more about them and to study them individually, they have to be separated from mixed forms and need to be cultured under artificial conditions.This unit deals with Isolation of microorganisms and their growth in the laboratoryconditions and also various culture techniques and instruments used formicrobiological studies. After reading this unit,one will be ableto:

- Get an idea of the cultivation of microorganisms.
- Know about different culture media and their preparation.
- Understand the methods of isolationof microorganisms.
- Obtain pure cultures.
- Know about thecultivation and culture techniques ofviruses.
- Study the different microbiological instruments.

2.2 INTRODUCTION

You know that microorganisms occur innatural environment; they arecontaminated and mixed with several other forms of life. In order to understandmore about them we have to separate and study them individually for this purpose we have to isolate microorganisms and culture them under artificial conditions. The technique of growing microorganisms in an artificial medium is known ascultivation.

Cultivation of microorganisms is done in the laboratory and requires favorable environmental conditions such as nutrient sources of energy, appropriate temperature, oxygen and pH etc. Since various types of microorganisms grow together in a suitable environment, a number of isolation techniques are used to obtain pure culture of just one species of a microorganism.

2.3 STERILIZATION

In microbiology Sterilization is an important term which needs making anything free of any form of life. For detail study of a microorganism, one needs a pure culture of an organism. It ia obtained by taking utmost care to avoid contamination through the atmosphere, glassware, media or other instruments used in the culture technique. The growth of unwanted microbes in the culture is called contamination and these unwanted microbes are called contaminants.

A number of precautionary measures are taken to prevent contamination during sterilization and creating a germ free condition is called aseptic condition. There are following three methods of sterilization:

(A) Physical

(B) Chemical

(C) Gaseous

(A) Physical Methods :

The frequently used physical methods are

I. Sterilization by heat-

(a) Dry heat sterilization

(b) Wet heat sterilization

II. Sterilization by filtration

III. Sterilization by ultra violet radiations

IV. Sterilization by ionizing radiations.

I. Sterilization by heat-Heat is most commonly applied in the microbiological laboratories for sterilization.

(a) Dry heat sterilization: Direct heating of the instruments in a flame is an easy way of sterilization. Inoculating needles, scissors, forceps, scalpels etc. are commonly sterilized by direct heat while the neck and mouth of specimen tubes, flasks and culture tubes are also passed through flame till they become sterilized. The process of sterilizing the articles with flame is called flaming.

Another method of dry heat sterilization is to keep thoroughly washed and dried glass wares such as Petri dishes, beakers, flasks, culture tubes etc. inside thermostatically controlled electric oven. Complete sterilization is accomplished by maintaining a temperature of 160^0C for not less than 4 hrs. inside the oven.

(b) Wet heat sterilization: Wet heat(steam) is more efficient method and is preferred in sterilizing the media used for culturing micro-organisms. The common ways by which Wet heat is employed in the laboratory are: Boiling, Pasteurization, Tyndallization and autoclaving.

i. Boiling: It is a common method of sterilization. All the instruments used for cultivation are kept in a container filled with distilled water and allowed to boil for at least 15 minutes. If the articles are not to be used immediately, these should be stored in a sterile container.

ii. Pasteurization: Many substances such as milk are treated with uncontrolled heating at temperatures well below boiling. This process is called pasteurization. Milk, beer and many other beverages are usually pasteurized. This process does not sterilize a beverage, but it does kill any pathogen present. Milk can be pasteurized in two ways (i) in the older method; the milk is heated at 63^0C for 30 minute. (ii) Flash pasteurization consists of quick heating to about 72^0C for 15 seconds followed by rapid cooling.

iii. Tyndallization: Sometimes a heat sensitive material is sterilized by fractional steam sterilization, called tyndallization. The container with the material to be sterilized is heated at 90-100^0C for 30minutes on each of three consecutive days and incubated at 37^0C in between. The first heating will destroy all microbes except bacterial endospores. Most of these germinate when incubated at 37^0C and are killed by the

second heating. Any remaining spores are destroyed by the second incubation and third heat treatments, many materials specially the liquid media for microbial cultures.

iv. **Autoclaving**: Steam under pressure is more efficient method of sterilization this technique and is known as autoclaving. Autoclave is a cylindrical metallic vessel with double walls. There are various types of autoclaves in use.

1) **Simple autoclave**: In which the body is made up of gun metal and it is cylindrical in appearance and closed at one end by hinged door. A gasket seal is provided between the door and cylinder. It can withstand high temperature. A perforated metal tray is provided within the barrel which is used for keeping those articles which are to be sterilized. The water present below the perforated tray is boiled by an electric heater to produce the steam.

2) **Steam jacketed autoclave:** It is a modified form of simple autoclave. In simple autoclave much of heat is wasted from the surface of barrel. To check this, a steam jacket is provided around the barrel in large autoclave. Inside the autoclave steam pressure is increased and the temperature increases proportionately. Usually autoclaving is done at 15 lb. pressure for 15 minutes. The autoclave is used to sterilize most of the solid and liquid media required for microbial cultures.

II. Sterilization by filtration :

It is a best way to sterilize the solutions of heat sensitive materials. This method simply removes the microbes instead of directly destroying them. There are two types of filters.

(a) Depth filters: These consist of fibrous or granular materials that have been bonded into a thick layer filled with twisting channels of small diameter. The solution containing microorganisms is sucked through this layer under vacuum and microbial cells are removed by physical screening, Depth filters are made of diatomaceous earth, unglazed porcelain (chamberlain filters), asbestos filters etc.

(b) Membrane filters: These filters have replaced depth filters. Such filters are circular porous membranes, made of cellulose acetate, cellulose nitrate, polycarbonate, polyvinylidene fluoride or other synthetic materials. Membranes with pores about 0.2, μm in diameter are used to remove vegetative cells from solutions. The solution is forced through the filler with a vacuum or with pressure from a syringe or nitrogen gasbottle and collected in previously sterilized containers. Membrane filters remove microorganisms like a sieve with minute pores.

(c) Air sterilization by filtration: Air can also sterilize by filtration. Surgical masks and cotton plugs on culture vessels are two common examples that let air in but keep microorganisms out. Laminar flaw biological safety cabinets fitted with high efficiency particulate air (HEPA) filters remove 99.97% particles from the air.

III. Radiation:

Sunlight is the major source of radiation on the earth. It includes visible light, ultraviolet radiation (UV), Infrared rays and radio waves. At the surface of the earth, very little UV

radiation is found. The ozone layer presents between 25 and 30 miles above the earth's surface absorbs somewhat larger UV rays. This elimination of UV is crucial because it is quite damaging to living system. UV rays kill all kinds of microorganisms due to its shortwave length and high energy does not penetrate glass, dirt films, water and other substances very effectively.

Many forms of electromagnetic radiation are very harmful to microorganisms. As the wavelength of electromagnetic radiation decreases, the energy of radiations increases e.g. gamma rays and x-rays are much more energetic than the visible light of infrared rays. Electromagnetic radiation acts like a stream of energy packets called photons. Each photon possesses a quantum of energy whose value depends upon the wavelength of the radiation.

IV. The ionizing radiations, because of very short wavelength or high energy cause atoms to lose electrons ionize. Two major ionizing radiations are:

(i) **X-rays** produced artificially

(ii) **Gamma rays**– Which are emitted during ionizing radioisotope decay. Low levels of ionizing radiations cause mutations and may indirectly results in death while higher levels are directly lethal. Ionizing radiation is an excellent sterilizing agent and penetrates deep into objects. Gamma radiation has also been used to pasteurize meat and other food.

(B) Chemical Methods:

It is a quick method of sterilizing instruments, glass apparatus or any other article used in culture technique. The chemicals usually act as disinfectants because they cannot readily destroy bacterial endopores. There are a number of chemicals known for their property as:

(a) Disinfectant or germicidal (germ killing)
e.g. Lysol, Cresol, etc.
(b) Antiseptic (microbial growth stopping)
e.g. Ethyl andisopropyl alcohols.
(c) Sanitizer (reducing microbial population to sap limit)
e.g. Silver nitrate, mercuric chloride and some other forms of mercury.

(C) Gaseous Methods:

Many heat sensitive materials such as disposable plastic syringes, petridishes, catheters, heart lung machine components etc. are now a days sterilized with ethylene oxide gas. It is both microbicidal and sporicidal. It kills by combining with cell proteins. It is very effective sterilizing agent as it rapidly penetrates packing materials, even plastic wraps.

Betapropiolactone (BPL) is occasionally used to sterilize vaccines and sera. It also destroys microorganisms more rapidly than ethylene oxide but does not penetrate materials well and may be carcinogenic. Because of this, BPL has not been used extensively.

2.4 PREPARATION OF CULTURE MEDIA

The organisms are grown on suitable culture media. A culture medium is a nutrient preparation which provides a balanced mixture of the required nutrients at concentrations that will permit good growth of microorganisms.

The culture media are generally of following types:

I. Living culture media.
II. Natural culture medium
III. Synthetic culture medium.
IV. Complex media or non-Synthetic.

Culture media are variously classified as:

(A) On the basis of composition :

1- Living culture media :

Such media require living cells, tissues or callus to be parasitized by the microorganisms to be cultured. Chick embryo is commonly used for cultivation of certain viruses.

2- Natural or Empirical culture Media :

The empirical or natural media mostly contain either one or many in gradient. In such medium, the exact composition is not defined. Natural media are convenient and inexpensive. However, these are not ideal media for many organisms. Milk, Skim milk, wine diluted blood, vegetable juices are some of the natural media.

3- Synthetic medium:

Synthetic medium is prepared by mixing many components in a particular ratio. In this the exact chemical composition of the medium is known. Such media contain highly pure organic and inorganic compounds. Nutrient Agar is synthetic medium.

4. Complex Media:

Complex media are those, in which chemical composition is not well defined. These media are non- synthetic. These media are useful for culturing a variety of microorganisms. Peptone, yeast extract, meat extract, Beef extract etc. used in complex media.

(B) On the basis of Physical state :

1- Liquid media: These are defined as water based solutions that do not solidity at temperature above freezing and flow freely when the Container is tilted. These media are made by dissolving various solutes in distilled water. The liquid media are termed broths, milks or infusions.

2- Semisolid Media: The media which exhibit a clot-like consistency at room temperature are called semi soiled media. They do not flow freely. They contain a solidifying agent agar or gelatin that thickens them but does not produce a firm substrate. These are used to determine the motility of bacteria and to localize a reaction at a specific site.

3- Solid Media: The media which provides firm surface on which cells can form discrete colonies are called solid media. These are useful in isolating and sub culturing bacteria and fungi. They are of two types.

(i) Liquefiable solid media: These are also called reversible solid media, They contain a solidifying thermoplastic agent. The most widely used agent is agar-agar. Agar is solid at room temperature and is flexible and mouldable. It has the property to hold moisture and nutrients. It is a non-digestible nutrient for the vas- majority of microorganisms.

(ii) Non-liquefiable solid media: These are not thermoplastic. They includes materials such as rice grains (used to grow fungi), cooked meat and potato slices. These media start out solid and remain solid after heat sterilization.

Potato dextrose agar (PDA)

This is used for growing fungi and is prepared in the laboratory. The ingredients are :

Potatoes peeled and sliced – 200gm.
Dextrose – 20 gm.
Agar – 15 gm.
Distilled water– 1000 ml.

Potatoes sliced are first steam cooked in 500 ml water and agar is mixed in other 500 ml. water. Now both are mixed together; filtered and dextrose is added.

(C) On the basis of function (Functional Types)

1. **General Purpose media:** Those media which support the growth of many microorganisms, are called general purpose media.

These are non- synthetic and contain a number of nutrients that could support the growth of both pathogens and non-pathogens.

2. **Enriched Media:** The specially fortified media are called enriched media. These contain complex organic substances such as blood, serum, hemoglobin or special growth factors like vitamins, amino acids which are the requirments of some microorganisms to grow. Bacteria that require growth factors and complex nutrients are called as fastidious (e.g.: *Streptococcus pneumonia*).
3. **Selective and Differential Media:** These media are meant for special microbial groups. These help in the preliminary identification of a genus or even a species, in a single step.

(a) Selective Media: These contain one or more substances that inhibits the growth of certain microbe/ microbes but not others e.g. Use of dyes like basic fuchsin and crystal violet favours the growth of gram negative bacteria. Some selective media contain strong inhibitory substances.

e.g.: Tellurie is used to isolate oral streptococci from saliva.

Some nutrients are used in the media specifically, e.g. cellulose for cellulose digesting bacteria.

(b) Differential Media: These media are used for the growth of several types of microorganisms but are used to differentiate different groups of microbes. These are also used for in tentative identification of microorganisms on the basis of their biological variations. Variations maybe in colony size and the colour, the colour changes of media and the formation of bubbles and precipitation properties. These

variations are due to the type of agents added and the way the cells react to them. Blood agar is a differential as well as enriched medium. It distinguishes between hemolytic and non- hemolytic bacteria. Hemolytic bacteria produce clear zones around their colonies as a result of red blood cell destruction.

(D) Some Miscellaneous Media:

1- **Reducing Medium**: A reducing medium contains a substance cystine that absorbs oxygen reducing its availability. These media are useful in growing anaerobic bacteria and also in determining oxygen requirement.

2- **Carbohydrate fermentation Medium:** These contain sugars that can be fermented.

3- **Transport Media:** These are used to maintain and preserve specimens for a period of time prior to clinical examination. These are also used to sustain delicate species that die rapidly if not kept under stable condition.

4. **Assay Media:** These are used to test the effectiveness of antimicrobial drugs and also to assess the effect of antiseptics, cosmetics and preservatives on the growth of microorganisms.

5. **Enumeration Media:** These are used by industrial and environmental microbiologists to count the numbers of organisms in milk, water, food, soil and other samples.

2.5 DISPENSING THE MEDIUM

Dispensing is the process in which the medium is poured into the sterilized flasks, culture tubes and Petri dishes. The unsterilized medium is usually poured into the flasks and culture tube by semiautomatic syringe, funnel and automatic filter. The liquid medium (Broth) is dispensed into culture tube or flasks which are plugged with nonabsorbent cotton wool plugs. The pouring of sterilized medium is usually carried out in Petri dishes which are already sterilized in a special sterilized inoculation chambers.

2.6 SOME IMPORTANT CULTURE MEDIUM

Some important media which are generally used for culturing the variousmicro-organisms are given below.

(A) For Bacteria

I. Nutrientagar: Beef extract - 3.0 gm.
Peptone -5.0 gm.
Agar - 15.0 gm.
Distilled Water - 1,000 ml.

Heat unit/ agar and peptone dissolve. Adjust PH to 6.6 to 7.0.

II. Asparagin Mannitol agar

K_2HPO_4----------1.0 gm.
KNO_3----------0.5 gm.

$MgSo_4$ $7H_2O$----------0.2 gm.
Fe cl_3 $6H_2O$----------in traces.
Nacl----------0.1 gm.
Asparagine----------0.5 gm.
Mannitol----------1.0 gm.
Agar----------15.0 gm.
distilled water----------1,000ml.

After the agar and salts have been dissolved add the mannital and adjust the PH to 7.4.

Mac Conkey's agar medium is a typical selective medium. It made up of the following components –

Peptone	-	20 gm.
Lactose	-	5gm.
Neutralredsolution (1%)	-	10 ml.
Nacl	-	5gm.
Bile salt	-	1.5 gm.
Agar	-	13.5 gm.
Crystal violet	-	0.001gm.
Distilled water	-	1.000 ml.

It is used for the culture and isolation of gram negative lactose fermenting bacteria.

- Blood Agar is the most commonly used differential medium. The blood agar medium consists of the following components ;

Infusion from beef heart	-	500gm.
Tryptose	-	10gm.
Nacl	-	5gm.
Agar	-	15gm.
Distilled water	-	1000ml.

If mixtures of bacteria are inoculated into this medium, the hemolytic and non-hemolytic bacteria can be identified.

(B) For fungi:

I. Potato dextrose agar (PDA)

Potato and sliced potato ----------200gm.
Dextrose----------20 gm.
Agar----------15 gm.
Distilled water ----------1,000ml.

II. Czapek- Dox agar :

Sucrose ----------30.0 gm.

Sodium nitrate ----------2.0 gm.
Potassium chloride ----------0.5gm
Magnesiumsulphate--------- 0.5 gm.
Ferrous sulphate ----------0.01 gm.
Dipotassium phosphate--------1.0 gm.
Agar ----------15gm.
Distilled water ----------1,000ml.

III. Rose- Bengal agar (Cooke's medium)

Glucose ----------10.00 gm
Peptone ----------5.00 gm.
Dipotassium phosphate ---------- 1.00 gm.
Magneciumsulphate ----------0.50gm.
Streptomycin ----------30.00gm.
Agar ----------15.00 gm.
Rose- Bengal---------- 0.035 gm.
Distilled water ---------- 1,000ml.

The antibiotic is sterilized separately and added aseptically to the sterilized medium. The medium is specially recommended for isolation of fungi in the presence of large numbers of bacteria,

IV. Smith and Dawson's medium :

Glucose ---------- 10.0 gm.
$NaNo_2$----------1.0 gm.
$KH_2\ Po_4$----------1.0 gm.
Agar ----------15.0gm.
Rose Bengal ----------0.067 gm.
Soil extract---------- 1,000 ml.

The soil extract is prepared by autoclaving500 gmof loam soil in 1200 ml. water for one hour. The extract is then filtered through paper.

(C) For Actinomycetes:

Ken knight and Munaier's medium
Dextrose ----------1.00 gm.
$KH_2\ Po_4$----------0.10 gm.
$NaNo_3$ ----------0.10 gm.
Kcl----------0.10gm.
MgSo4 $7H_2O$----------0.10 gm.
Agar ----------15.0gm.
Distilled water----------1,000 ml.

(D) For specialized fungus :

(i) For Yeasts

Malt extract agar

Malt extract----------30.0 gm.
Peptone----------5.0 gm.
Agar----------20.0 gm.
Distilled water----------1,000 ml.

(ii) For *Aspergillus niger* :
Raulins Medium
Sugar ---------- 70.0 gm
Tartaric Acid---------- 4.0 gm.
Ammonium nitrate ------4.0 gm.
Potassium carbonate ------- 0.6 gm.
Ammonium phosphate-----0.6 gm.
Magnesium carbonate-------o.4 gm.
Ammonium sulphate--------- 0.25 gm.
Zinc sulphate (crystal)-------- 0.07 gm.
Ferrous sulphate(crystal)------0.07 gm.
Potassium silicate ----------0.07 gm.
Distilled water ---------- upto1,000ml.

2.7 METHODS OF OBTAINING PURE CULTURE

Pure Culture: A culture containing only one type of microorganism is called a pure culture. The microbes are found distributed everywhere in nature and hence they occur only as a mixed form. When culture media are inoculated with substances such as soil, water or excreta, many kind of organisms develop simultaneously, and it results the growth of a mixed culture of microbes. For the study of a single microorganism a pure culture of that organism is required.

Any technical procedure for obtaining pure culture is dependent upon the isolation of a single viable microbe which is allowed to multiply in a suitable culture medium. The first reliable method of isolation of pure culture was devised by Robert Koch in 1881 and his theory is known as 'Koch's Postulated'

Koch's Postulates

1- The pathogen or organism must be constantly associated with the symptoms of the disease in all diseased plants examined.
2- The pathogen must be isolated and grown in pure culture on nutrient media.
3- The pathogen from pure culture must be isolated and inoculated on healthy plants of some species on which the disease appears. andit must produce the same symptom of disease on inoculated plants.
4- The pathogenmust be again isolated in pure culture and its culture and characteristics must be resemble with previous culture. So that the isolated pathogen is identified and confirmed for the disease.

If nutrient agar isinoculated with fluid and is then solidified and kept underfavorable temperature, many of the microbes that have been introduced are able to multiply and formdistinct colonies. If the colonies are not closely crowded, a pure culture may be obtained by touching a colony with the tip of a sterile needle and inoculating it in a fresh culture medium.

2.8 METHODS OF ISOLATION

The isolation of one kind of microorganisms from a mixture is called pure culture technique. The petridishes or flasks with sterilized needles are inoculated with an organism and are placed in culture chamber for its growth. Before inoculation both the hand and inoculation instruments etc. are sterilized by wiping them with cotton wool soaked in alcohol.

Some important methods used for obtaining pure culture are as follows:

I. Pour Plate Method :

In this method the mixed culture is diluted in sterile medium and the diluted mixture is added to culture tubes containing melted agar medium. The contents of the tubes are then poured into a sterile petridish and allowed to solidify. The plates are then incubated. The cells of different of microorganisms develop into colonies and cells from individual colony are picked up for further culture.

II. Streak Plate method :

Streaking is the most widely used method of isolation. This method is most suitable for bacterial and fungal cultures. In streak plate method, the mixed culture is taken on a sterile wire loop (inoculum) and is drawn back and forth on a solid agar medium in a petridish. The successive streaks thin out the culture sufficiently. In this method, isolated individual cells are deposited on some region of the plate. The needle is flamed and allowed to cool after each streaking. Several such streaks are made on the medium. The streaking is done in some definite pattern (**Fig.2.1)**.

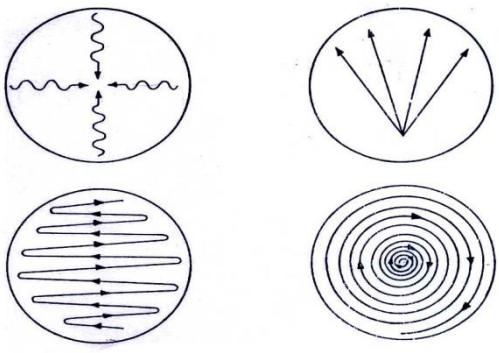


Fig.2.1 Different ways of streaking on agar plate

Streaking method needs a lot of care not to break the surface of the medium during streaking incubation each cell grows into a colony.

III. Serial Dilution Method :

The dilution of sample in successive stages is called serial dilution. This method is most suitable for the bacteria and fungi which cannot be easily isolated by streaking method. The mixture of microorganisms is diluted serially in culture tubes of sterile medium until the last tube contains only a single organism. In this method, the dilution factor increases in a regular fashion e.g.: 1/10, 1/100, 1/1000 etc.

In this method 1ml. of sample is mixed to 9ml. of sterile water in culture tube. This gives a tenfold dilution and this dilution factor is represented as 1/10 or 10^{-1}, Now from this 10 fold dilution, 1 ml. of sample is taken and is added to 9 ml. of sterile water taken in a second culture tubes Now the second tube contains a 100 fold dilution and the dilution factor is represented as 1/100 or 10^{-2}

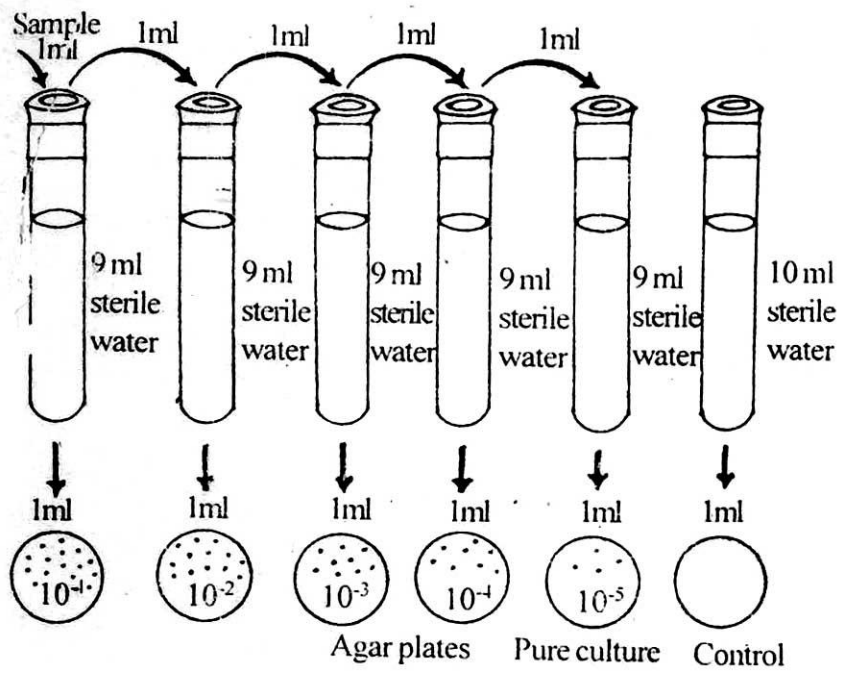


Fig.2.2 Serial dilution technique

Similarly from tube two, 1ml. of sample is taken and is added to 9 ml. of sterile water taken in a third culture tube. Now the third tube contain a 1000 fold dilution factor is represented as 1/1000 or 10^{-3}. In same way, culture tubes 4 and 5 are prepared. Tube 5 provides 10^{-4} dilution and tube 5 provides 10^{-5} dilution. A 6^{th}Tube is prepared as a control containing 10 ml. of sterile water only.

Then from each tube, 1 ml. of diluted sample is taken and is added to an agar plate (a petridish containing 10 to 15 ml. of melted agar medium). The 6 agar plates are incubated at 25-30^0C for 24 hours. A luxuriant growth of most of the bacteria is obtained. By the serial dilution, the chance of dominant organism in pure condition in the culture is increased. Ultimately, a little amount of the suspension is pipette out and spread over the medium of petridish(**Fig.2.2)**.

IV- Spread Plate Method:

It is a modification pour plate method. In this method, the mixed culture is serially diluted in sterile distilled water. A small amount of diluted mixture is then poured on the surface of the agar plate and it is spread evenly using a sterile bent glass rod. The isolated cells grow into colonies.

V- Single cell method:

In this method, a suspension of microbes is placed on the cavity slide. Thereafter, a single cell is removed with the help of sterile micropipette with the aid of a microscope. The cell is then transferred to sterile culture. The colony obtained, originated from single cell.

VI- Enrichment Culture Method:

In this method, a particular nutrient, which favours the growth of the desired bacterium, is added to the medium. When the mixed culture is placed in this enriched medium, the desired bacterium will grow dominantly.

VII- Selective culture method:

In this method a selective medium is taken which contains a chemical, which suppresses undesirable species. e.g.: Crysisal violet inhibits gram positive bacteria, when crystal violet is added to the medium, the medium will select the gram negative bacteria.

VIII- Differential Culture Method:

In this method the specific chemicals are used in the medium and different microorganisms are isolated on the basis of their colour, e.g.: in eosin- methylene blue agar medium, *E. coli* will produce colonies with a brilliant green metallic colour and *Acrobactor acrogens* will produce pink colonies with dark centres.

After obtaining the pure culture of a desired microbe, it may be grown and maintained as a pure culture. This pure culture can be maintained by transferring the organisms from one to another culture tube. This process is called subculturing.

2.9 CULTIVATION OF VIRUSES

Viruses are unable to reproduce independently, So, they cannot be cultured like other microorganisms. These are cultured indifferent ways depending upon the type of living host which they require for multiplication.

I. Cultivation of plant viruses:

Plant viruses can be cultivated in various ways. Plant tissue cultures, cultures of separated cells or cultures of protoplast may be used for cultivating plant viruses, Viruses can also be grown in whole plants. Leaves of a healthy plant are mechanically inoculated when rubbed with a mixture of viruses and an abrasive such as carborudum orcelite. When the cell walls are broken by the abrasive, the viruses come in direct contact with plasma membrane and infect the exposed host cells. Alocalized necrotic lesion often develops

due to the rapid death of cells in the infected area. Even when lesions do not appear, the infected plant may show other symptoms such as change in colour or leaf shape. Some plant Viruses are transmitted only if a diseased part is grafted into a healthy plant.

II. Cultivation of Animal Viruses :

In the past animal viruses were cultivated by inoculating suitable host animals or embryonated eggs, usually six to eight days after laying. Before inoculation the shell surface of egg is disinfected with iodine and penetrated with a small sterile drill. After inoculation, the hole is sealed with gelatin and the egg incubated. Because viruses reproduce only in a certain parts of the embryos, so they must be injected into the proper region. The virus infection produces a local tissue lesion called pock appearance which varies and is characteristic of the virus.

Now a days, animal viruses are grown in tissue culture on monolayer of animal cells. This technique evolved with the development of growth media for animal cells and by the discovery of antibiotics for the preventions of bacterial and fungal contamination.

III. Cultivation of Bacteriophages :

Bacteriophages are cultivated in either broth or agar cultures of young, actively growing bacterial cells. The number of host cells destroyed is so large that turbid bacterial cultures may clear rapidly as a result of cellysis. For preparing agar culture the bacteriophage sample is mixed with cool, liquid agar and a suitable bacterial culture. This mixture is then quickly poured into a petridish containing a bottom layer of sterile layer. Wherever a virion comes to rest in the top agar, the virus infects an adjacent cell and reproduces eventually. Bacteriallysis result in a plaque in the opaque layer. The appearance of plaque is characteristic of the phage being cultivated.

2.10 CULTURE TECHNIQUES

Microbiologists use five basic techniques (also called five I's) to culture, manipulate, examine and characterize microorganisms. These are:

(i) Inoculation
(ii) Isolation
(iii) Incubation
(iv) Inspection
(v) Identification

These techniques are used by all microbiologists, whether a beginner laboratory student or researcher is attempting to isolate a useful bacterium from the soil or a clinical microbiologist trying to find out the cause of patient's infection. These techniques thus, help in handling and maintaining microorganisms as discrete entities.

(i) Inoculation: The process of transfer of inoculum (a sample containing microorganisms) into a container of nutrient medium, which provides an environment in which they grow is called inoculation. Inoculum may be obtained from soil, water sewage, foods, air and inanimate objects. For determining the cause of an infectious

disease, inoculum is obtained from body fluid (blood, cerebrospinal fluid), discharges (Sputum, urine, faeces) or diseased tissue.

The culture vessels (culture tubes, conical flasks, or petridishes) containing appropriate culture media are inoculated using tools such as loops, needles, pipettes, etc. For a properly controlled experiment, sterilization of the glassware, equipment and culture media is necessary. This means that the inoculation must start with a sterile medium. All inoculating tools and culture vessels should be sterile. Measures are also taken to prevent the entry of undesirable microbes while inoculating. This procedure is carried out in special rooms fitted with UV lamps. Now a days for inoculation, special laminar flow (biological safety cabinets), fitted with HEPA fitters are used.

For inoculation in culture tubes agar slants are prepared.

Preparation of Agarslants:

Liquified agar medium is poured into culture tubes. The culture tubes are plugged with cotton wool and sterilized in autoclave. The sterilized tubes are taken out and placed in a slanting position and then allowed to cool. The sloppy surface provides maximum area of the agar medium in the culture tube for the growth of microorganisms (**Fig.2.3)**.

Fig.2.3 Culture tube with agar slant

Transfer of the Inoculum: The inoculation is done in inoculation chamber, completely sterilized with ultraviolet light. The hand should also be cleaned and sterilized with rectified spirit. For culture tube inoculation, the tube containing inoculum with sterilized agar medium slant is held in one hand and the inoculating needle in the other. The cotton plugs of the tubes are taken out with the help of fingers in front of the flame of spirit lamp. The inoculum is picked out with the help of needle and then inserted within the agar surface of the tube. Plugging of the tube is immediately done to avoid contamination. For petridish inoculation, the lid is removed to a minimum and inoculation is done in the centre of the dish. The inoculated tubes or petridishes are finally incubated at desired temperature.

(ii) Isolation: Isolation is separation of the pathogen from host tissue or from mixed culture or its inoculation in culture media. To achieve proper isolation, a small number of cells is inoculated into a relatively large volume or an expansive area of medium. It generally requires (i) a medium with firm surface (ii) a petri plate (a clear flat dish with a cover) and (iii) an inoculating loop. There are several ways of isolation to obtain pure culture: as I. Pour plate method II. Streak plate method III. Serial Dilution method. IV. Spread plate method V. Single cell method. VI. Enrichment culture method VII. Selective Culture method. VIII. Differential culture method.

All these methods of isolation are already discussed in detail in earlier chapter.

(iii) Incubation: Once a culture vessel containing medium is inoculated, it is placed in a temperature controlled chamber or incubator to facilitate multiplication. This is called incubation. Usually they are incubated at 20 to 40^0C in Laboratory. Incubators can also control the content of atmospheric gases like oxygen and carbon dioxide that may be required for the growth of microbes. During the incubation period, the microbe multiplies and produces some visible manifestation of growth.

(iv) Inspection: It is essential to inspect a culture macroscopically during various stages of inoculations. Colonies of microorganisms are readily visible especially the colonies of bacteria and fungi appears. Colonies are actually large masses of clinging cells. The colonies exhibit distinctions in size, shape, colour and texture. Colony development on agar surface helps the microbiologists in identifying microorganisms. Once a microorganism is isolated, it is a standard practice to make a second level. Cultural called a subculture. This is done by removing a small sample from one well isolated colony and transferring it into a separate container of media. Because a colony consists of only one type of microorganism, it yields a pure colour or auxenic culture for further testing and identification.

Cultural inspection is also done by using a microscope. This gives information of many characteristics of cell microbiology including size, shape and details of internal and external structures.

(v) Identification: The microorganisms isolated are identified by a combination of microscopic and macroscopic appearance. These are useful in differentiating simple prokaryotic cells from larger complex eucaryotic cells. Although, appearance is of no use in the identification of bacteria because of similar morphologies. For their identification other techniques that characterize their cellular metabolism are used. These involve biochemical tests that can determine fundamental chemical characteristics such as nutrient requirements, products releasing during growth, temperature and gas requirements and methods of deriving energy.

By compiling macroscopic and microscopic characters and results of biochemical tests, a profile is prepared which is then used in the final identification of a microbe. Thus, microorganisms are identified in terms of their macroscopic morphology, their microscopic characters, and their biochemical reactions and genetic characteristics.

Maintenance of culture:

Culture of microorganism is maintained for further studies. Many research laboratories require a line of stock cultures. The stock cultures are continuously maintained species that represent "living Catalogues". The largest culture collection is 'American Type culture collection located in Rockville, Maryland, U.S.A. It maintains frozen and freeze-dried fungal, bacterial, viral and algal cultures.

Disposal of cultures:

Some cultures are potential health hazard. Therefore, they require immediate and proper disposal. Microbial cultures are disposed off in two ways: (1) Steam sterilization by

autoclave, and (ii) incineration (burning). Both are effective methods for destroying microorganisms.

2.11 INSTRUMENTS USED IN MICROBIOLOGICAL STUDIES

Following are some of the basic requirements and equipments for culturing microorganism under artificial conditions:

I. Culture vessels: Culture media are contained in culture vessels usually culture tubes, conical flasks and petridishes. These are made of high quality corning glass. The petri dishes are special culture dishes named after their inventor Julies Richard Petri. Petri developed these dishes in 1887. They consists of two round halves, top half overlapping the bottom.

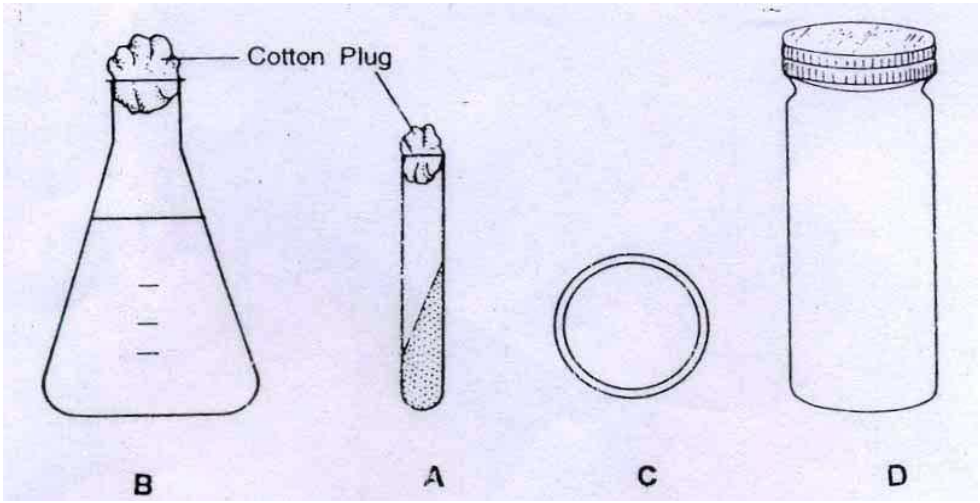


Fig.2.4 Culture vessels; A. Culture tube B. Conical flask. C. Petri disc. D. Culture Bottles

Culture tubes are of two size, the smaller are without rim and Large with rim. Almost all sizes of flasks ranging from 50 ml to 1000 ml are used for microbial culture. These are used to contain culture media before and after the sterilization and also for culture the pathogen in liquid or semisolid medium (**Fig.2.4)**.

II. Plugs: Tubes and flasks containing media or culture are always plugged with cotton wool so that, any air which enters inside is filtered from all contaminating microorganisms. A plug should project inside the tube about an inch and should have tuft outside the tube, by which it can be taken out. The plug should fit accurately and tightly, but not so tightly that it cannot be extracted when gripped between any two fingersof one hand. The plug should also retain its shape, so that after withdrawl, it can readily be a reinstered.

III. Inoculating tools: For inoculation, tools like inoculating needles, inoculating loops, syringes, etc. are used. Inoculating needles/ loops are made up of plantinum wire/ nichrome wire fixed into a metal or glass rod at one end. In inoculating needles the wire is straight whereas in inoculating loops, the free end of the wire is bent in the form of a loop (**Fig.2.5**).

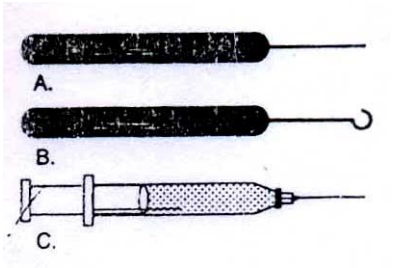


Fig.2. 5 Inoculating tools: A. Inoculation needle, B. Inoculation loop, C. Syringe

IV Sterilization Equipments:

Following equipments are used in sterilization of glass ware and culture media:

(i) Oven for dry sterilization
(ii) Autoclave for steam sterilization
(iii) Filter sterilization Equipment
(iv) Sterile rooms or inoculation chambers
(v) Laminar flow biological softy cabinets

(i) Oven for dry sterilization:

Dry heat is mainly used to sterilize glass ware or other heat stable materials. The objects are wrapped in aluminium foil and exposed to a temperature of 170^0C for 90 minutes in an oven. It is an electrically operated device. It consists of a big chamber with insulated walls and is fitted with electrical hearers to raise the temperature level and a thermostat to maintain temperature at a desired level (**Fig.2.6**).

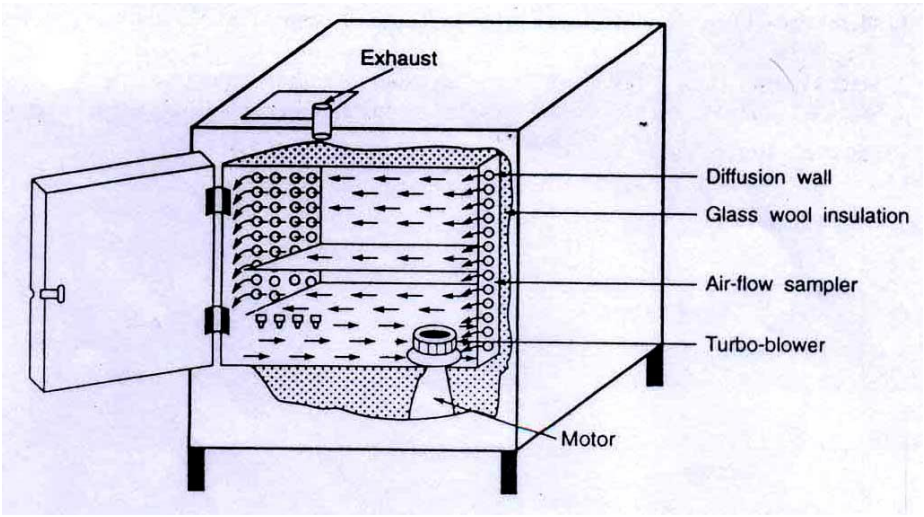


Fig.2.6. Hot air oven

(ii) Autoclave: Autoclave is the instrument used to sterilize culture media, glass ware and other tools by high pressure steam, which developed inside the sterilizing chamber by heating water. Steam pressure increases inside the chamber with increasing heating time. The body of autoclave is made up of a thick double walled cylinder. Inside the

cylinder at its bottom is fitted an electric immersion rood. The mouth of the cylinder is fitted with a heavy tightly fitting lid. A pressure gauge is attached to the lid to monitor pressure inside the cylinder. An outlet valve is also attached to the lid. Laboratory autoclaves are commonly operated at a steam pressure of 15lbm^2 above atmospheric pressure which corresponds to a temperature of 120^{0}C. Even bacterial spores that survive several hours of boiling are rapidly killed at 120^{0}C. The temperature at 15lb pressure for 15minutesis sufficient to kill any organism, and to achieve complete sterility. If an autoclave is not available, pressure cookers can be used for purpose of sterilization (**Fig.2.7**).

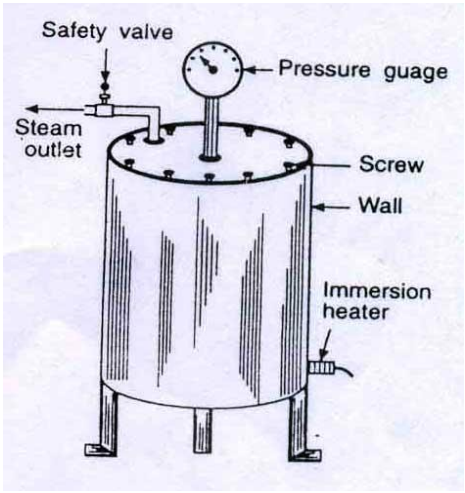
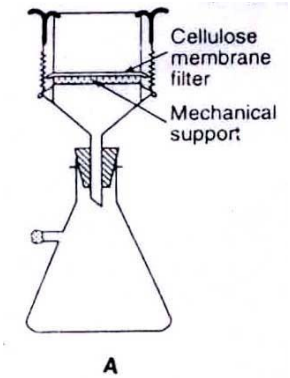


Fig.2.7 Autoclave

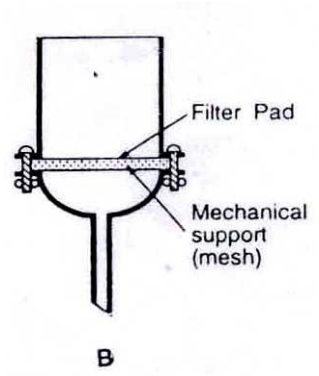
(iii)Filter Sterilization Equipment :

Solutions of heat labile materials are sterilized by filtration through filters capable of retaining microorganisms. The most commonly used filters are:

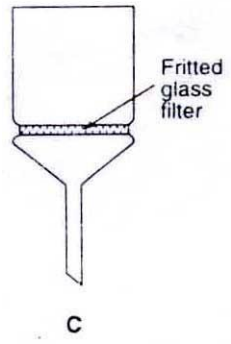
1. **Membrane filters** (Millipore filters): These consist of porous discs of cellulose esters.



2. **Seitz filters**: these consist of discs of asbestos cellulose mixture.



3. **Sintered glass filters:** These are prepared by fusing together fine glass fragments.



4. **Candle filters:** These are made up of unglazed ceramic.

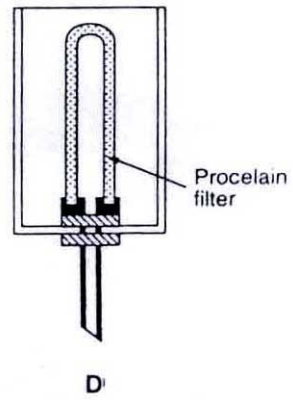


Fig.2.8 A to D Various types of filters

The filter is appropriately mounted on a funnel like structure. The mounting is inserted into a receiving flask. The complete assembly is sterilized by heat. The solution to be sterilized is poured into the filter. Its passage through filter is accelerated either by suction on the receiving flask or by pressure on the unfiltered liquid (**Fig.2.8. A-D**).

(iv) Sterile Rooms (Inoculation chambers) :

Inoculation is done in sterile rooms or inoculation chambers. These are fitted with ultraviolet bulbs / lamps which emit UV light in wave length range 260 to 270 mm. They are useful for killing microbes in air and on object surfaces.

(v) Laminar flaw Biological Safety Cabinets :

The laminar flaw cabinets are available in different sizes. They can be placed where needed and thus, eliminated the necessity for a separate room. Laminar flow biological safety cabinets are filled with high efficiency particulate air (HEPA) fitters which remove 99.97% of 0.3 μmparticles. These are one of the most important air filtration systems. Laminar flaw cabinets force air through HEPA fitters, then project a vertical curtain of sterile air across the cabinet opening to protect a researcher from microorganisms being handled within the cabinet and to prevent room contamination (**Fig.2.9**).

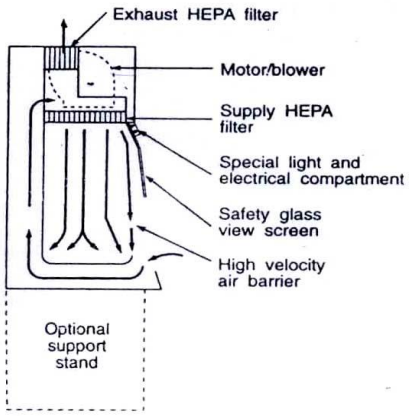


Fig.2.9, A schematic diagram showing the air flow pattern in a biological safety cabinet

V. Incubators: Majority of fungi grow reasonably well at room temperature, however in order to induce maximum rate of growth and in some cases, to promote the formation of certain type of spores and fruiting structures, higher or lower temperature is essential. Incubator is the instrument, used for such purpose. It is an electrical instrument similar to hot air ovens in construction and operation. The range of temperature varies from room temperature generally between 20^0C to 50 or 60^0C. The cultures of microbes are incubated at suitable temperature in these chambers (**Fig.2.10**).

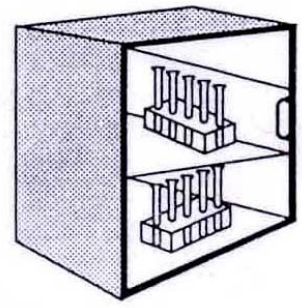


Fig.2.10, An incubator for maintaining cultures

VI. Colony Counters:

This device is used to count the colonies of microorganisms developing on a culture plate. These are generally of 2types -

A. Quebec colony counter :

In quebec colony counter, there is a platform which is marked with cross ruling (small squares). An illuminator is there below the platform to illuminate the colonies and above the platform is present a magnifying lens. This lens magnifies the colonies and thus helps in counting. For counting the colonies, the culture plate is mounted on the platform and illuminated from below. The colonies are counted easily against back ground of small squares (**Fig 2.11. A**).

B. Electrical colony counter :

The electrical colony counters are fined with an electrode for marking the location of each colony, when a colony is touched by the electrode; it is automatically recorded in the counter (**Fig.2.11. B)**.

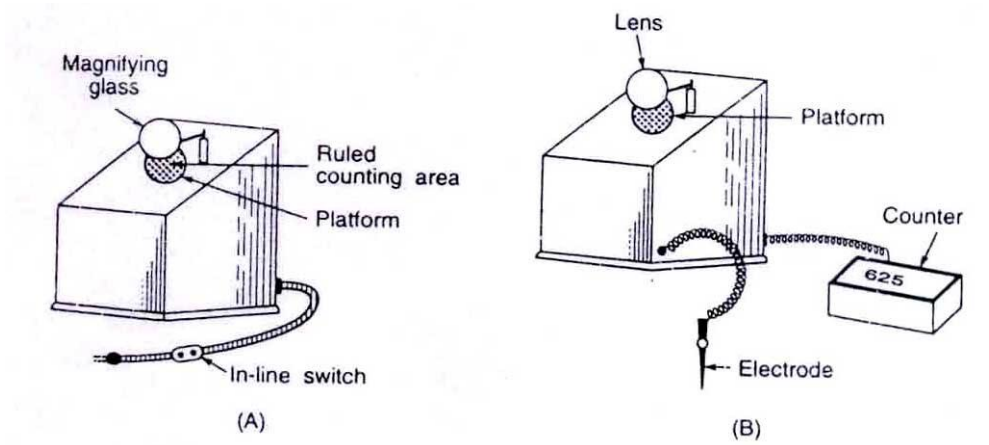


Fig.2.11. Colony counters A. Quebec colony counter B. Electrical colony counter

C. Counting Chambers :

To determine microbial members through direct counting, counting chambers are used. These are easy, inexpensive and relatively quick. It also gives information about the size and morphology of the organisms, being counted. **Petroff Hausser** counting chambers are used for counting bacteria. Haemocytometers are used for counting large eukaryotic microorganisms. Large microorganisms such as protozoa, algae and yeasts (non filamentous forms) can be directly counted with **electric counters like coulter counter.** It gives accurate results with larger cells and is extensively used to count red and white blood cells. It is not useful in counting bacteria because of small debris particles, formation of filaments etc (**Fig.2.12)**.

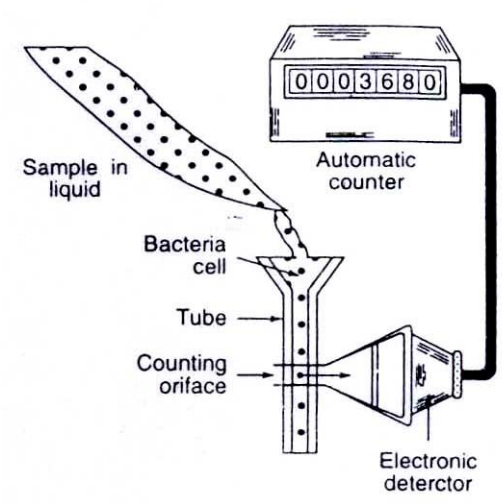


Fig.2.12 Coulter counter

2.12 SUMMARY

In this unit you have learnt that –

- Microorganisms occur in natural environments; they are contaminated and mixed with several other form of life.
- To study microorganism individually we have to separate them from mixed forms and culture or grow them under artificial conditions.
- As they are widely distributed and undesirable microbes may enter into an experiment and cause misleading results. To overcome these problem different methods like sterilization, pure culture etc is done.
- In the isolation of microorganism for detailed study, one must take care to avoid contaminations, for this various sterilization methods are used to produce aseptic condition for microbial culture.
- The microorganisms are grown on suitable culture media. A culture medium is a nutrient preparation which provides a balanced mixture of the required nutrients at concentrations that will permit good growth of microorganism.
- Various types of culture media are prepared for different microorganisms, nutritional requirements of microbes vary from a few simple inorganic compounds to complex organic compounds. So culture media used for culturing microbes are extremely varied in nutrient component and consistency.
- Microorganisms usually grow in complex, mixed, population containing several types. Isolation of single species or single microorganism is done to obtain pure culture or auxenic culture various ways like spread plate method streak method, Dilution methods, pour plate method, etc. are used to get pure culture or single individual.
- For culturing obligate parasites (that cannot grow on artificial media) e.g. viruses, live cell culture or host animals are required.

- For all the culture process, to grow, to examine and to characterize microorganisms five basic techniques are used by all microbiologists. These are: (1) Inoculation (ii) Incubation (iii) Isolation (iv) Inspection and (v) Identification.
- Culture vessels, inoculation tools, sterilization equipments, inoculation chambers, Laminate flow, incubators, colony counters and counting chambers are the basic equipments used in culture techniques.

2.13 GLOSSARY

Acclimated micro-organism: Any microorganism that is able to adapt to environmental changes, such as change in temperature.

Actinomycetes: A group of micro-organisms apparently intermediate between bacteria and fungi, and classified as either.

Agar: A gelatin like material obtained from red algae and used to prepare culture media on which microbes are grown.

Antimicrobial: A chemical that either destroys or inhibits the growth of microscopic and submicroscopic organisms.

Auxenic culture: Pure culture i.e. a microorganism of a single species, growing in a medium free of other microorganisms.

Bacterial filter: A special type of filter through which bacterial cells cannot pass.

Bacteriophage: A virus that infects specific bacteria and usually kills them.

Bacteriostatic: A chemical or physical agent that prevents bacterial growth and their multiplication without killing.

Culture: to grow microorganisms artificially on a prepared food material.

Culture medium: The prepared food material on which micro-organisms are cultured.

Disinfectant: A physical or chemical agent that frees a plant, organ or tissue from infection.

Incubation period: The Period between penetration of a host by a pathogen and its first appearance of symptoms on the host.

Inoculate: To bring a pathogen into contact with ahost plant.

Inoculation: Transfer of a pathogen into a host or culture media.

Inoculum: The pathogen or causal part which causes disease when brought into contact with the host.

Isolate: A single spore or culture derived from a mixture of microbes culture.

Isolation: Separation of a pathogen or microbe from the host and its culture on a nutrient medium.

Mechanical Inoculation: Inoculation of plant with a virus through transfer of sap from a virus infected plant to a healthy plant.

Secondary Inoculum: Inoculum produced by Infections that took place during the same growing season.

Serum: The watery portion of the blood remaining after coagulation.

Sterilization: The elimination of pathogen or microorganisms from any surface, or making free from microbes.

Virus: A submicroscopic obligate parasite consisting of nucleic acid and protein.

2.14 SELF ASSESSMENT QUESTIONS

2.14.1 Choose the correct answer from the given below:

(a) Agar agar is obtained from:

(i) Blue green algae (ii) Green algae

(iii) Brown algae (iv) Red algae

(b) Special culture dishes were invented by:

(i) Robert Kotch (ii) Julius Richard Petri

(iii) Coulter (iv) None of the above

(c) The instrument in which wet heat sterilization is done to sterilize objects is:

(i) Autoclave (ii) Laminar flow

(iii) Hotair oven (iv) Inoculation chamber

2.14.2 Fill in the blanks:

(a) A medium of known composition is called..... medium.

(b) A Pure culture is a population of cells derived from.....

(c) One of the most important air filtration system filled with high efficiency particulate air (HEPA) is.....

2.14.3 Answer the following in one word or in one sentence:

(a) How are heat labile materials sterilized?

(b) Name the electrical counter with the help of which largemicroorganisms such as protozoa, algae and yeasts can be directly counted.

(c) Heat sensitive materials e.g. disposable plastic, syringes, petri dishes, catheters etc. are sterilized with which chemical?

2.14.4 Write True or False (T/F):

(a) Cultivation of microorganism on a solid agar medium in a culture tube kept in a slanting position is called agarslant culture.

(b) The process of transfer of a sample containing micro organism into a container of nutrient medium is called incubation.

(c) Selective and differential media are extensively used in isolation and identification of microbes.

2.14.5 Explain the following –

(a) Types of culture media.

(b) Methods of sterilization for cultivation.

(c) Instruments used in cultivation of microbes.

Answers Keys:

Q.1- (a)-(iv), (b)-(ii), (c)-(i)

Q.2- (a)-Synthetic, (b)-single cell, (c)- Laminar flaw cabinets

Q.3- (a)-Filter sterilization, (b)-Coulter counter, (c)-Ethyline oxide

Q.4-(a)-True, (b)-False, (c)- True

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2.17. TERMINAL QUESTIONS

1. Give an account of the cultivation of micro organisms.
2. Write short notes on the following –

   (i)Synthetic media (ii) Non synthetic media

   (iii)Autoclave (iv) Laminar Flow biological safety chamber

   (v) Koch's postulates (vi) Dry heat sterilization.
3. Describe briefly the Basic requirements and apparatus for culturing microorganism.
4. Discuss the different types of culture media used for successful culturing of microbes.
5. Define sterilization with reference to microbiology Discuss different sterilization methods.
6. Describe in brief the five basic techniques of culturing microorganisms under artificial conditions.
7. Differentiate the following –
   i) Pure culture and Mixed culture
   ii) Selective media and Differential media
   iii) Inoculation and contamination
   iv) Pasteurisation and Tyndallisation

UNIT-3- STRUCTURE, CLASSIFICATION, NUTRITION, REPRODUCTION AND ECONOMIC IMPORTANCE OF BACTERIA

3.1 OBJECTIVES

After reading this unit student will be able:

- To study the structure of bacteria.
- To understand the characteristic features of bacteria.
- To know different shapes of bacteria.
- To classify the bacteria.
- To analyze the nutrition and economic importance of bacteria.

3.2-INTRODUCTION

"Bacteria, the Hidden Heroes of the earth are very much powerful"

Bacteria Bacteria are microscopic unicellular prokaryotic organisms characterized by the lack of a membrane-bound nucleus and membrane-bound organelles. Once considered a part of the plant kingdom, bacteria were eventually placed in a separate kingdom, Monera. Bacteria fall into one of the two groups, Archaebacteria (ancient forms thought to have evolved separately from other bacteria) and Eubacteria. A recently proposed system classifies the Archaebacteria, or Archaea, and the Eubacteria, or Bacteria, as major groupings (sometimes called domains) above the kingdom level.

Bacteria were the only form of life on earth which exist for 2 billion years. They were first observed by Antony van Leeuwenhoek in the 17th century; bacteriology as an applied science began to develop in the late 19th century, as a result of research in medicine and in fermentation processes, especially by Louis Pasteur and Robert Koch.

These microorganisms remarkably adaptable to diverse environmental conditions. They are found in the bodies of all living organisms and on all parts of the earth—in land, terrains and ocean depths, in arctic ice and glaciers, in hot springs, and even in the stratosphere. Our understanding of bacteria and their metabolic processes has been expanded by the discovery of species that can live only deep below the earth's surface and by species that thrive without sunlight in the high temperature and pressure near hydrothermal vents on the ocean floor. There are more bacteria, as separate individuals, than any other type of organism; there can be as many as 2.5 billion bacteria in one gram of fertile soil.

3.3 STRUCTURE OF BACTERIA

Bacteria display a wide diversity of shapes and sizes. Their cells are about one-tenth the size of eukaryotic cells and are typically 0.5–5.0 micrometers in length (**Fig.3.1**). However, a few species- for example, *Thiomargarita namibieniensis* and *Epulopiscium fishelsoni* - are up to half a millimetre long and are visible to the unaided eye; *E. fishelsoni* reaches 0.7 mm. Among the smallest bacteria are members of the genus *Mycoplasma*, which measure

only 0.3 micrometres, as small as the largest viruses. Some bacteria may be even smaller, but these ultra microbacteria are not well-studied.

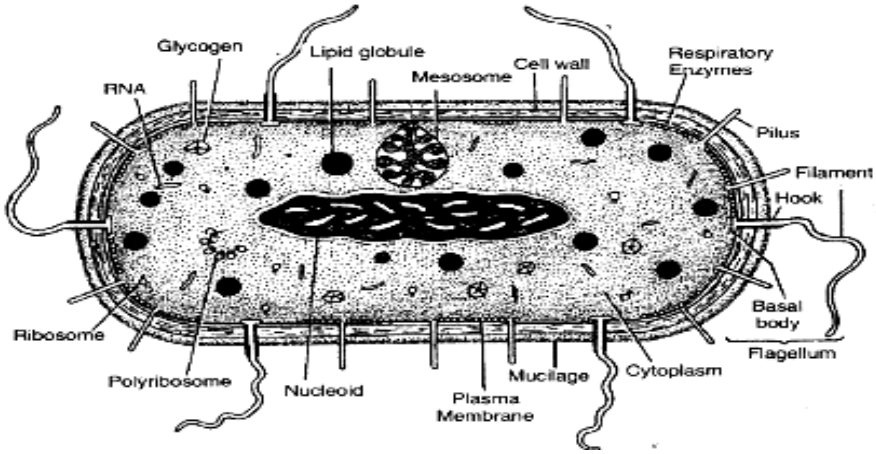

Fig.3.1: The Bacterial cell

Most bacterial species are either spherical, called *cocci* (*sing*. coccus, from Greek *kókkos*, grain, seed), or rod-shaped, called *bacilli* (*sing*. bacillus, from Latin *baculus*, stick). Elongation is associated with swimming. Some bacteria, called *vibrio*, are shaped like slightly curved rods or comma-shaped; others can be spiral-shaped, called *spirilla*, or tightly coiled, called *spirochaetes*. A small number of species even have tetrahedral or cuboidal shapes. More recently, bacteria were discovered deep under Earth's crust that grows as branching filamentous types with a star-shaped cross-section. The large surface area to volume ratio of this morphology may give these bacteria an advantage in nutrient-poor environments. This wide variety of shapes is determined by the bacterial cell wall and cytoskeleton, and is important because it can influence the ability of bacteria to acquire nutrients, attach to surfaces, swim through liquids and escape predators.

Fig.3.2: A biofilm of thermophilic bacteria in the outflow of Mickey hot Springs, Oregon, approximately 20 mm thick

Many bacterial species exist simply as single cells, others associate in characteristic patterns: *Neisseria* form diploids (pairs), *Streptococcus* form chains, and *Staphylococcus* group together in "bunch of grapes" clusters. Bacteria can also be elongated to form filaments, for example the Actinobacteria. Filamentous bacteria are often surrounded by a sheath that contains many individual cells. Certain types, such as species of the genus *Nocardia*, even form complex, branched filaments, similar in appearance to fungal mycelia.

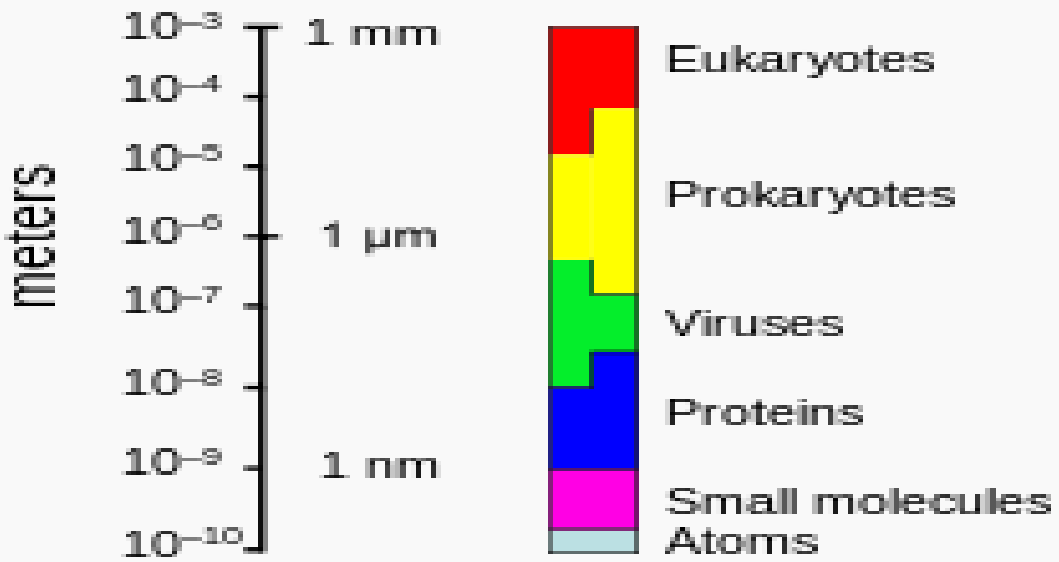

Fig.3.3- The range of sizes shown by prokaryotes, relative to those of other organisms and biomolecules

Bacteria often attach to surfaces and form dense aggregations called biofilms or bacterial mats (**Fig.3.2**). These films can range from a few micrometers in thickness to upto half a meter in depth, and may contain multiple species of bacteria, protists and archae. Bacteria living in biofilms display a complex arrangement of cells and extracellular components, forming secondary structures such as microcolonies, through which there are networks of

channels to enable better diffusion of nutrients. In natural environments, such as soil or the surfaces of plants, the majority of bacteria are bound to surfaces in biofilms. Biofilms are also important in medicine, as these structures are often present during chronic bacterial infections or in infections of implanted medical devices, and bacteria protected within biofilms are much harder to kill than individual isolated bacteria (**Fig.3.3**).

Even more complex morphological changes are sometimes possible. For example, when starved of amino acids, Myxobacteria detect surrounding cells in a process known as quorum sensing, migrate toward each other, and aggregate to form fruiting bodies up to 500 micrometres long and containing approximately 100,000 bacterial cells. In these fruiting bodies, the bacteria perform separate tasks; this type of cooperation is a simple type of multicellular organisation. For example, many cells migrate to the top of these fruiting bodies and differentiate into a specialised dormant state called myxospores, which are more resistant to drying and other adverse environmental conditions than are ordinary cells.

The bacterial surface

Cell Wall: The cell envelop is composed of the plasma membrane and cell wall. As in other organisms, the bacterial cell wall provides structural integrity to the cell. In prokaryotes, the primary function of the cell wall is to protect the cell from internal turgor pressure caused by the much higher concentrations of proteins and other molecules inside the cell compared to its external environment. The bacterial cell wall differs from that of all other organisms by the presence of peptidoglycans which is located immediately outside of the cytoplasmic membrane. Peptidoglycan is made up of a polysaccharide backbone consisting of alternating N-Acetylmuramic acid (NAM) and N-acetylglucosamine (NAG) residues in equal amounts. Peptidoglycan is responsible for the rigidity of the bacterial cell wall and for the determination of cell shape. It is relatively porous and is not considered to be a permeability barrier for small substrates. While all bacterial cell walls (with a few exceptions e.g. extracellular parasites such as *Mycoplasma*) contain peptidoglycan, not all cell walls have the same structures. The cell wall is required for bacterial survival, but several antibiotics stop bacterial infections by interfering with cell wall synthesis. There are two main types of bacterial cell walls, those of gram-positive bacteria and those of gram-negative bacteria, which are differentiated by their Gram-staining characteristics. For both these types of bacteria, particles of approximately 2 nm can pass through the peptidoglycan. If the bacterial cell wall is entirely removed, it is called a protoplast but when it is partially removed, it is called a spheroplast. *β-Lactam antibiotics* such as penicillin inhibit the formation of peptidoglycan cross-links in the bacterial cell wall. The enzyme lysozyme, found in human tears, also digests the cell wall of bacteria and is the body's main defence against eye infections in human beings.

The gram-positive cell wall

Gram-positive cell walls are thick and the peptidoglycan layer constitutes almost 95% of the cell wall in some gram-positive bacteria and as little as 5-10% of the cell wall in gram-negative bacteria. The cell wall of some gram-positive bacteria can be completely dissolved by lysozyme. In other gram-positive bacteria, such as *Staphylococcus aureus*, the walls are

resistant to the action of lysozyme. The matrix substances in the walls of gram-positive bacteria may be polysaccharides or teichoic acids. The latter are very widespread, and found only in gram-positive bacteria. There are two main types of teichoic acid: ribitol teichoic acids and glycerol teichoic acids. The latter one is more commonly found. These acids are polymers of ribitol phosphate and glycerol phosphate, respectively, and only located on the surface of many gram-positive bacteria. However, the exact function of teichoic acid is not fully understood. A major component of the gram-positive cell wall is lipoteichoic acid. One of its purposes is providing an antigenic function. The lipid element is to be found in the membrane where its adhesive properties assist in its anchoring to the membrane (**Fig.3.4**).

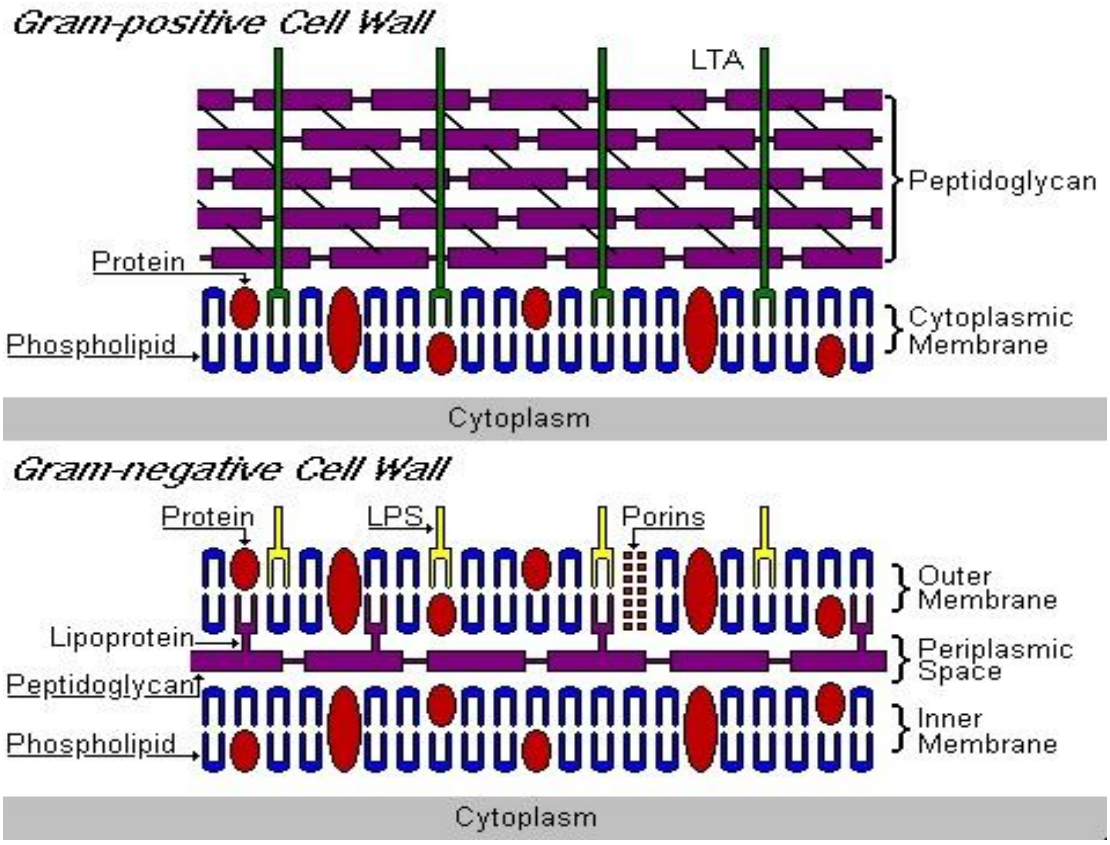

Fig.3.4: Cell Wall composition of Bacteria

The Gram-negative cell wall

Gram-negative cell walls are thin and unlike the gram-positive cell walls, they contain a thin peptidoglycan layer adjacent to the cytoplasmic membrane. The chemical structure of the outer membrane's lipopolysaccharides is often unique to specific bacterial sub-species and is responsible for many of the antigenic properties of these strains. Lipopolysaccharides, also called endotoxins, are composed of polysaccharides and lipid A which are responsible for much of the toxicity of gram-negative bacteria. It consists of characteristic lipopolysaccarides embedded in the membrane (**Fig.3.4**).

Plasma Membrane: The plasma membrane or bacterial cytoplasmic membrane is composed of a phospholipid bilayer and thus has all of the general functions of a cell

membrane such as acting as a permeability barrier for most molecules and serving as the location for the transport of molecules into the cell. In addition to these functions, prokaryotic membranes also function in energy conservation as the location about which a proton motive force is generated. Unlike eukaryotes, bacterial membranes (with some exceptions e.g. *Mycoplasma* and methanotrophs) generally do not contain sterols. However, many microbes do contain structurally related compounds called hopanoids which likely fulfil the same function. Unlike eukaryotes, bacteria can have a wide variety of fatty acids within their membranes. Along with typical saturated and unsaturated fatty acids, bacteria can contain fatty acids with additional methyl, hydroxy or even cyclic groups. The relative proportions of these fatty acids can be modulated by the bacterium to maintain the optimum fluidity of the membrane.

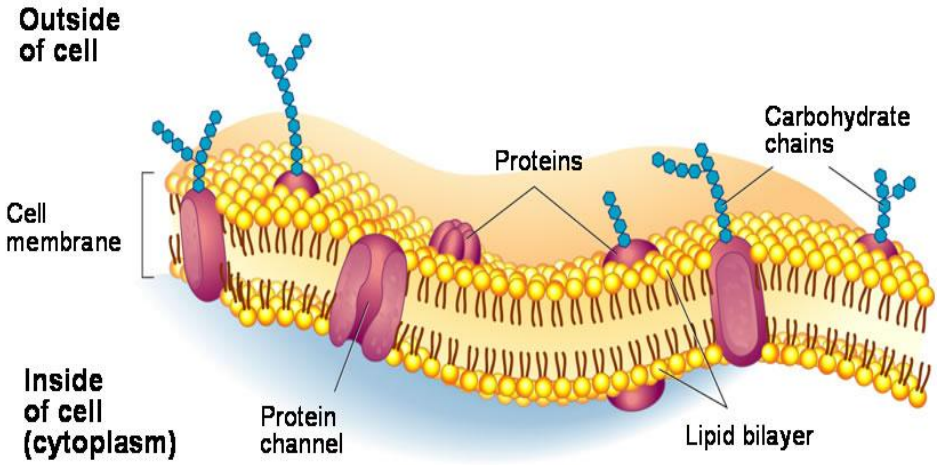

Fig.3.5. - Structure of bacterial plasma membrane

As a phoshpholipid bilayer, the lipid portion of the outer membrane is impermeable to charged molecules. However, channels called porins are present in the outer membrane that allow for passive transport of many ions, sugars and amino acids across the outer membrane. These molecules are therefore present in the periplasm, the region between the cytoplasmic and outer membranes. The periplasm contains the peptidoglycan layer and many proteins responsible for substrate binding or hydrolysis and reception of extracellular signals. The periplasm is thought to exist in a gel-like state rather than a liquid due to the high concentration of proteins and peptidoglycan found within it. Because of its location between the cytoplasmic and outer membranes, signals received and substrates bound are available and transported across the cytoplasmic membrane using transport and signalling proteins imbedded there (**Fig.3.5**).

Flagella: Many kinds of bacteria have slender, rigid, helical flagella (singular, flagellum) composed of the protein flagellin. These flagella range from 3 to 12 micrometers in length and are very thin—only 10 to 20 nanometers thick. They are anchored in the cell wall and help to spin or pulling the bacteria through the water like a propeller. The number and position of flagella vary in bacteria. The arrangement may be monotrichous (a single polar flagellum), lophotrichous (a cluster of polar flagella), amphitrichous (flagella at both the ends either singly or in cluster), cephalotrichous (two or more flagella at one end of bacterial cell),

peritrichous (cell surface evenly surrounded by several lateral flagella) or atrichous (cells devoid of flagella).

A flagellum consists of three basic parts – the basal body, hook and filament. The basal body attaches the flagellum to the cell wall and plasma membrane. It is composed of a small central rod inserted into a series of rings. In gram positive bacteria, only a distal (inner) pair of rings is present while in gram negative bacteria two pairs of rings (the proximal and distal) are connected by a central rod. The hook of the flagellum is present outside the cell wall and connects filament to the basal body. It consists of different proteins. The hook of gram positive bacteria is slightly longer than the gram negative bacteria. The outermost long part of flagellum is called filament. It is made up of globular proteins called flagellin which are arranged in several chains and form a helix around a hollow core (**Fig.3.6**).

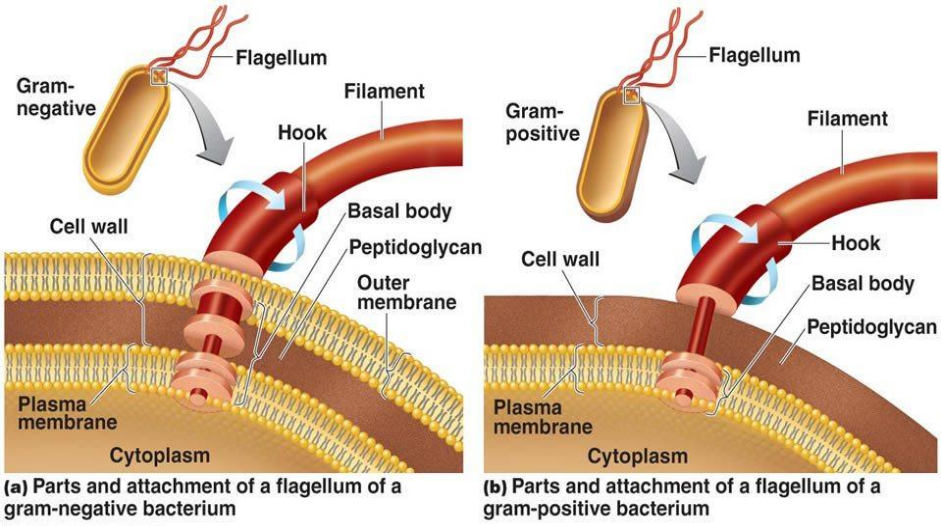

Fig.3.6- Structure of Bacterial flagella

Pili (Fimbriae): These are other hair like structures that occur on the cells of some bacteria. They are shorter than bacterial flagella. They are .2 – 20 μm long and about 7.5 to 10 nanometers thick (**Fig.3.7)**. Pili help the bacterial cells attach to appropriate substrates and exchange

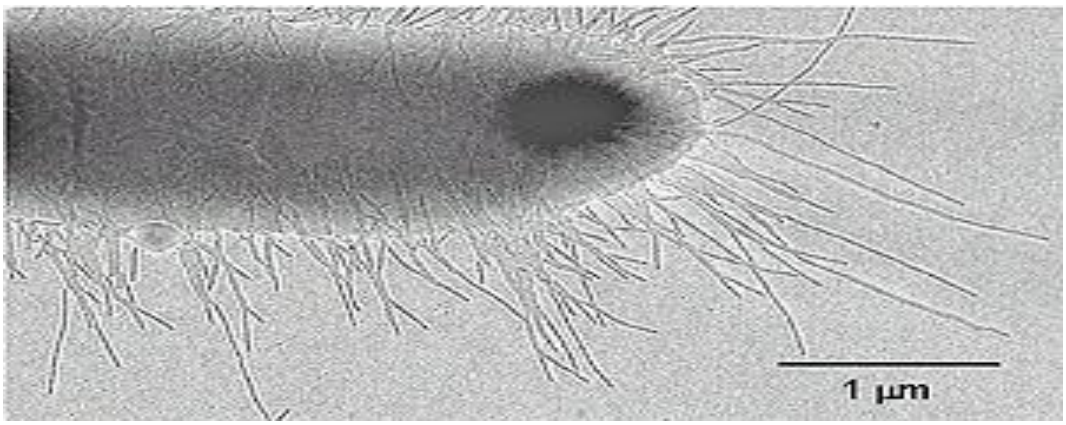

Fig.3.7- Bacteria showing structure of pili

genetic information. Pili originate from cytoplasm. According to the function, pili are of two types, (a) common pili which act to adhere the cell to the surface, (b) sex pili which join the other bacterial cell for transfer of genome. These structures also affect the metabolic activity of the bacterial cell. They are also equipped with antigen properties as they act as thermolabile non-specific agglutinogen.

The Cell Interior

The most fundamental characteristic of bacterial cells is their prokaryotic organization. Bacterial cells lack the extensive functional compartmentalization seen within eukaryotic cells.

Internal membranes: Many bacteria possess invaginated regions of the plasma membrane that function in respiration or photosynthesis. These are called mesosomes. These structures form the site for respiratory activity. The cytoplasmic membrane is also the site of many metabolic activities, e.g. the organic and inorganic nutrients are transported by permeases through plasma membrane. It consists of enzymes of biosynthetic pathways that synthesize different components of cell wall, such as peptidoglycogen, techoic acid, phospholipids and polysaccharides.

The cytoplasm of bacterial cell is thick and semi-transparent. It lacks cytoskeleton and cytoplasmic streaming compartmentation of cell organelles is absent in bacterial cell. Concentrated deposition of certain substances is dectectable in the cytoplasm of some bacteria. The volutin granules are found in some bacteria which serve as reserve source of phosphate. Another reserve carbon and energy source called polybeta-hydroxybutyrate is also found in aerobic bacteria. In some bacteria that live in aqatic habitats, bright refractile bodies which are hallow and have regular shape with more or less conical ends are observed by electron microscopy. These are gas vacuoles which provide buoyancy.

Nucleoid region: Bacteria lack nuclei and do not possess the complex chromosomes characteristic of eukaryotes. Instead, their genes are encoded within a single double-stranded ring of DNA that is crammed into one region of the cell known as the nucleoid region. Many bacterial cells also possess small, independently replicating circles of DNA called plasmids. Plasmids contain only a few genes, usually not essential for the cell's survival. They are best thought of as an excised portion of the bacterial chromosome.

Ribosomes: Bacterial ribosomes are smaller than those of eukaryotes and differ in protein and RNA content. Antibiotics such as tetracycline and chloramphenicol can be used to observe the difference—they bind to the bacterial ribosomes and block protein synthesis, but they do not bind to eukaryotic ribosomes. The ribosomes have two sub units, a larger 50 s sub unit and a smaller 30 s sub unit. Each is composed of proteins and ribosomal RNA.

3.4- CLASSIFICATION

Classification is done to describe the diversity of bacterial species by naming and grouping organisms based on similarities. Bacteria can be classified on the basis of cell structure, cellular metabolism or on differences in cell components such as DNA, fatty acids, pigments, antigens and quinones. While these schemes allowed the identification and

classification of bacterial strains, it was unclear whether these differences represented variation between distinct species or between strains of the same species. This uncertainty was due to the lack of distinctive structures in most bacteria, as well as lateral gene transfer between unrelated species. Due to lateral gene transfer, some closely related bacteria can have very different morphologies and metabolisms. To overcome this uncertainty, modern bacterial classification emphasizes molecular systematics, using genetic techniques such as guanine/cytosine ratio determination, genome-genome hybridization, as well as sequencing genes that have not undergone extensive lateral gene transfer, such as the rRNA gene. Classification of bacteria is determined by publication in the International Journal of Systematic Bacteriology, and Bergey's Manual of Systematic Bacteriology. The International Committee on Systematics Bacteriology (ICSB) maintains international rules for the naming of bacteria and taxonomic categories and for the ranking of them in the International Code of Nomenclature of Bacteria.

The term "bacteria" was traditionally applied to all microscopic, single-cell prokaryotes. However, molecular systematic showed prokaryotic life to consist of two separate domains, originally called *Eubacteria* and *Archaebacteria*, (now called *Bacteria* and *Archaea)* that evolved independently from an ancient common ancestor. These two domains, along with Eukarya, are the basis of the three domain system, which is currently the most widely used classification system in microbiology. However, due to the relatively recent introduction of molecular systematic and a rapid increase in the number of genome sequences that are available, bacterial classification remains a changing and expanding field. For example, a few biologists argue that the Archaea and Eukaryotes evolved from Gram-positive bacteria.

Identification of bacteria in the laboratory is particularly relevant in medicine, where the correct treatment is determined by the bacterial species causing an infection. Consequently, the need to identify human pathogens was a major impetus for the development of techniques to identify bacteria.

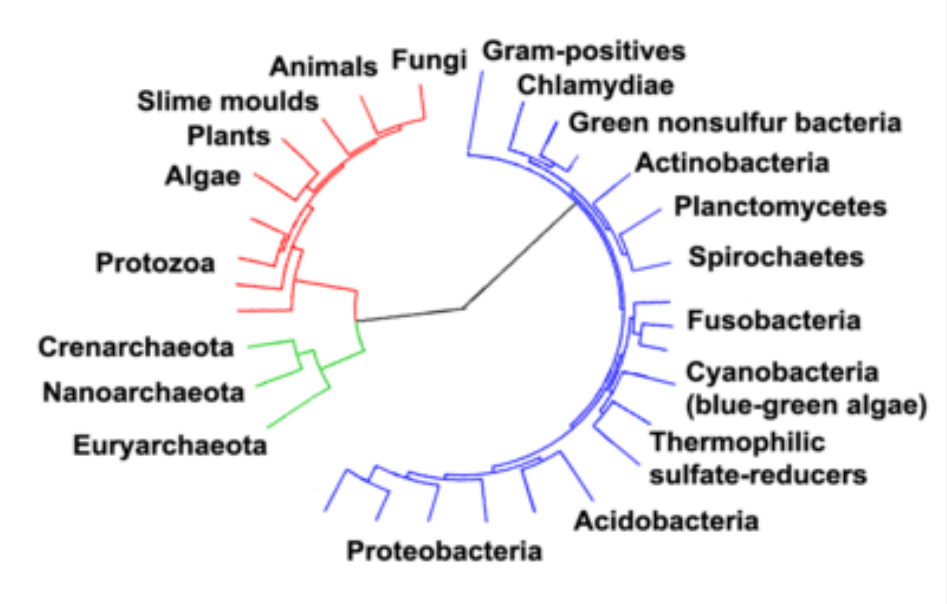

Fig.3.8- Phylogenetic tree showing the diversity of bacteria, compared to other organisms. Eukaryotes are colored red, archae green and bacteria blue

The **Gram stain**, developed in 1884 by Hans Christian Gram, characterises bacteria based on the structural characteristics of their cell walls. The thick layers of peptidoglycan in the "Gram-positive" cell wall stain purple, while the thin "Gram-negative" cell wall appears pink. By combining morphology and Gram-staining, most bacteria can be classified as belonging to one of four groups (Gram-positive cocci, Gram-positive bacilli, Gram-negative cocci and Gram-negative bacilli). Some organisms are best identified by stains other than the Gram stain, particularly mycobacteria or *Nocardia*, which show acid-fastness on Ziehl-Neelsen or similar stains. Other organisms may need to be identified by their growth in special media, or by other techniques, such as serology.

Culture techniques are designed to promote the growth and identify particular bacteria, while restricting the growth of the other bacteria in the sample. Often these techniques are designed for specific specimens; for example, a sputum sample will be treated to identify organisms that cause pneumonia, while stool specimens are cultured on selective media to identify organisms that cause diarrhoea, while preventing growth of non-pathogenic bacteria. Specimens that are normally sterile, such as blood, urine or spinal fluid, are cultured under conditions designed to grow all possible organisms. Once a pathogenic organism has been isolated, it can be further characterised by its morphology, growth patterns, pattern of hemolysis, and staining.

As with bacterial classification, identification of bacteria is increasingly using molecular methods. Diagnostics using such DNA-based tools, such as polymerase chain reaction, are increasingly popular due to their specificity and speed, compared to culture-based methods. These methods also allow the detection and identification of "viable but nonculturable" cells that are metabolically active but non-dividing. However, even using these improved methods, the total number of bacterial species is not known and cannot even be estimated with any certainty. Following present classification, there are a little less than 9,300 known species of prokaryotes, which includes bacteria and archaea; but attempts to estimate the true number of bacterial diversity have ranged from 10^7 to 10^9 total species – and even these diverse estimates may be off by many orders of magnitude (**Fig.3.8**).

3.5- NUTRITION

Bacteria exhibit an extremely wide variety of metabolic types. The distribution of metabolic traits within a group of bacteria has traditionally been used to define their taxonomy, but these traits often do not correspond with modern genetic classifications. Bacterial metabolism is classified into nutritional group on the basis of three major criteria: the kind of energy used for growth, the source of carbon, and the electron donors used for growth. An additional criterion of respiratory microorganisms is the electron acceptors used for aerobic or anaerobic respiration. Carbon metabolism in bacteria is either heterotrophic, where organic carbon compounds are used as carbon sources, or autotrophic, meaning that cellular carbon is obtained by fixing carbon dioxide.

Nutritional types in bacterial metabolism

Nutritional type	Source of energy	Source of carbon	Examples
Phototrophs	Sunlight	Organic compounds (photoheterotrophs) or carbon fixation (photoautotrophs)	Cyanobacteria , Green sulfur bacteria, Chloroflexi,
Lithotrophs	Inorganic compounds	Organic compounds (lithoheterotrophs) or carbon fixation (lithoautotrophs)	Thermodesulfobacteria and Nitrospirae
Organotrophs	Organic compounds	Organic compounds (chemoheterotrophs) or carbon fixation (chemoautotrophs)	*Bacillus* and *Clostridium*

Heterotrophic bacteria include parasitic types. Typical autotrophic bacteria are phototrophic cyanobacteria (**Fig.3.9**), green sulfur-bacteria and some purple bacteria, many chemolithotrophic species, such as nitrifying or sulfur-oxidising bacteria. Energy metabolism of bacteria is either based on phototrophy, (the use of light through photosynthesis), or based on chemotrophy, (the use of chemical substances for energy), which are mostly oxidised at the expense of oxygen or alternative electron acceptors (aerobic/anaerobic respiration).

Fig.3.9- Filaments of photosynthetic cyanobacteria

Bacteria are further divided into lithotrophs that use inorganic electron donors and organotrophs that use organic compounds as electron donors. Chemotrophic organisms use the respective electron donors for energy conservation (by aerobic/anaerobic respiration or fermentation) and biosynthetic reactions (e.g., carbon dioxide fixation), whereas phototrophic organisms use them only for biosynthetic purposes. Respiratory organisms use chemical compounds as a source of energy by taking electrons from the reduced substrate and transferring them to a terminal electron acceptor in a redox reaction. This reaction releases energy that can be used to synthesise ATP and drive metabolism. In aerobic organisms, oxygen is used as the electron acceptor. In anaerobic organisms other inorganic compounds, such as nitrate, sulfate or carbon dioxide are used as electron acceptors. This leads to the ecologically important processes of denitrification, sulfate reduction, and acetogenesis, respectively.

Another way of life of chemotrophs in the absence of possible electron acceptors is fermentation, wherein the electrons taken from the reduced substrates are transferred to oxidised intermediates to generate reduced fermentation products. Fermentation is possible, because the energy content of the substrates is higher than that of the products, which allows the organisms to synthesise ATP and drive their metabolism.

These processes are also important in biological responses to pollution; for example, sulfate reducing bacteria are largely responsible for the production of the highly toxic forms of mercury in the environment. Non-respiratory anaerobes use fermentation to generate energy and reducing power, secreting metabolic by-products as waste. Facultative anaerobes can switch between fermentation and different terminal electron acceptors depending on the environmental conditions in which they find themselves.

Lithotrophic bacteria can use inorganic compounds as a source of energy. Common inorganic electron donors are hydrogen, carbon monoxide, ammonia, ferrous iron and other reduced metal ions, and several reduced sulfur compounds. In unusual circumstances, methane gas can be used by methanotrophs bacteria as a source of electrons and a substrate for carbon anabolism. In both aerobic phototrophy and chemolithotrophy, oxygen is used as a terminal electron acceptor, whereas under anaerobic conditions, inorganic compounds are used instead. Most lithotrophic organisms are autotrophic, whereas organotrophic organisms are heterotrophic.

In addition to fixing carbon dioxide in photosynthesis, some bacteria also fix nitogen gas using the enzyme nitrogenase. This environmentally important trait can be found in bacteria of nearly all the metabolic types listed above, but is not universal.

Regardless of the type of metabolic process they employ, the majority of bacteria are able to take in raw materials only in the form of relatively small molecules, which enter the cell by diffusion or through molecular channels in cell membranes. It has recently been shown that *Gemmata obscuriglobus* is able to take in large molecules via a process that in some ways resembles endocytosis, the process used by eukaryotic cells to engulf external items.

Bacterial growth follows four phases. When a population of bacteria first enters a high-nutrient environment that allows growth, the cells need to adapt to their new environment.

The first phase of growth is the **lag phase**, a period of slow growth when the cells are adapting to the high-nutrient environment and preparing for fast growth. The lag phase has high biosynthesis rates, as proteins necessary for rapid growth are produced. The second phase of growth is the **log phase**, also known as the *logarithmic or exponential phase*. The log phase is marked by rapid **exponential phase**. The rate at which cells grow during this phase is known as the *growth rate* (*k*), and the time it takes the cells to double is known as the *generation time* (*g*). During log phase, nutrients are metabolised at maximum speed until one of the nutrients is depleted and starts limiting growth. The third phase of growth is the **stationary phase** and is caused by depleted nutrients. The cells reduce their metabolic activity and consume non-essential cellular proteins. The stationary phase is a transition from rapid growth to a stress response state and there is increased expression of genes involved in DNA repair, antioxidant metabolism and nutrient transport. The final phase is the **death phase** where the bacteria run out of nutrients and die.

3.6- REPRODUCTION

Unlike in multicellular organisms, increases in cell size and reproduction by cell division are tightly linked in unicellular organisms. Bacteria grow to a fixed size and then reproduce through binary fission, a form of asexual reproduction. Under optimal conditions, bacteria can grow and divide extremely rapidly, and bacterial populations can double as quickly as every 9.8 minutes. In cell division, two identical clone daughter cells are produced. Some bacteria, while still reproducing asexually, form more complex reproductive structures that help disperse the newly formed daughter cells (**Fig.3.10**).

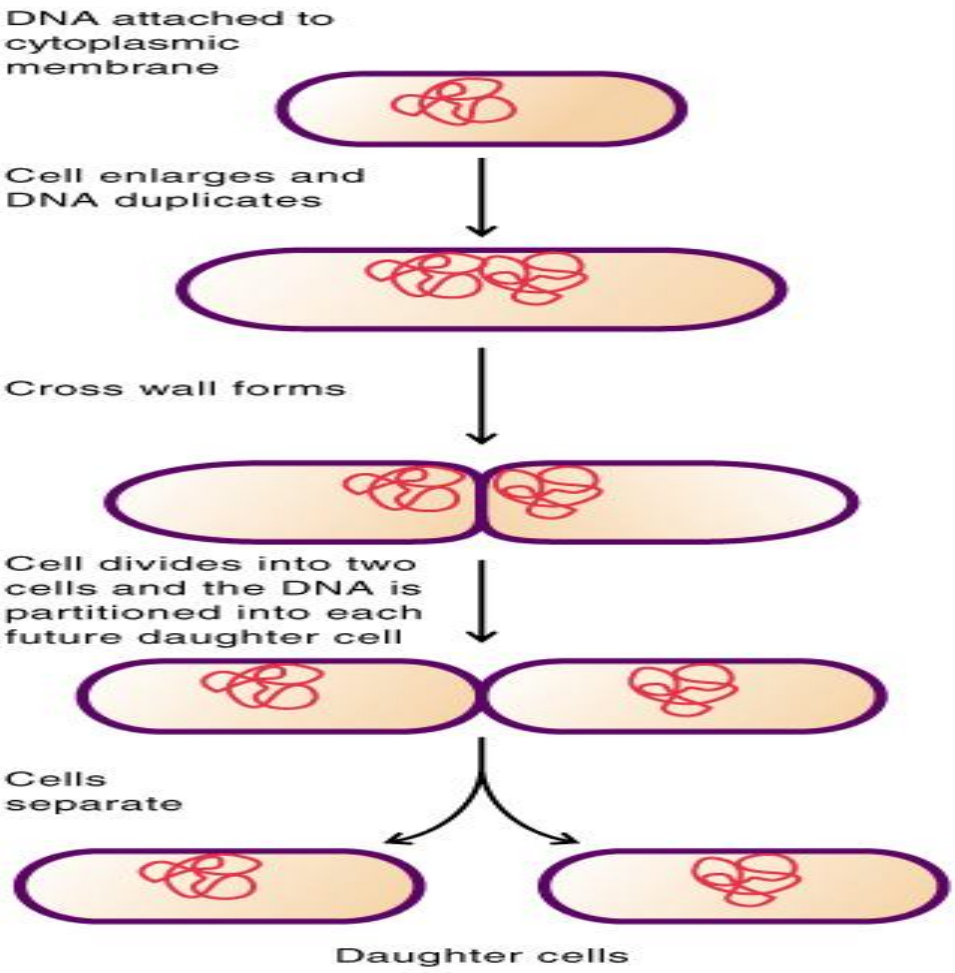

Fig.3.10- Binary fission in bacteria

DNA Transfer

Some bacteria transfer genetic material between cells. This can occur in three main ways. First, bacteria can take up exogenous DNA from their environment, in a process called transformation. Genes can also be transferred by the process of transduction, when the

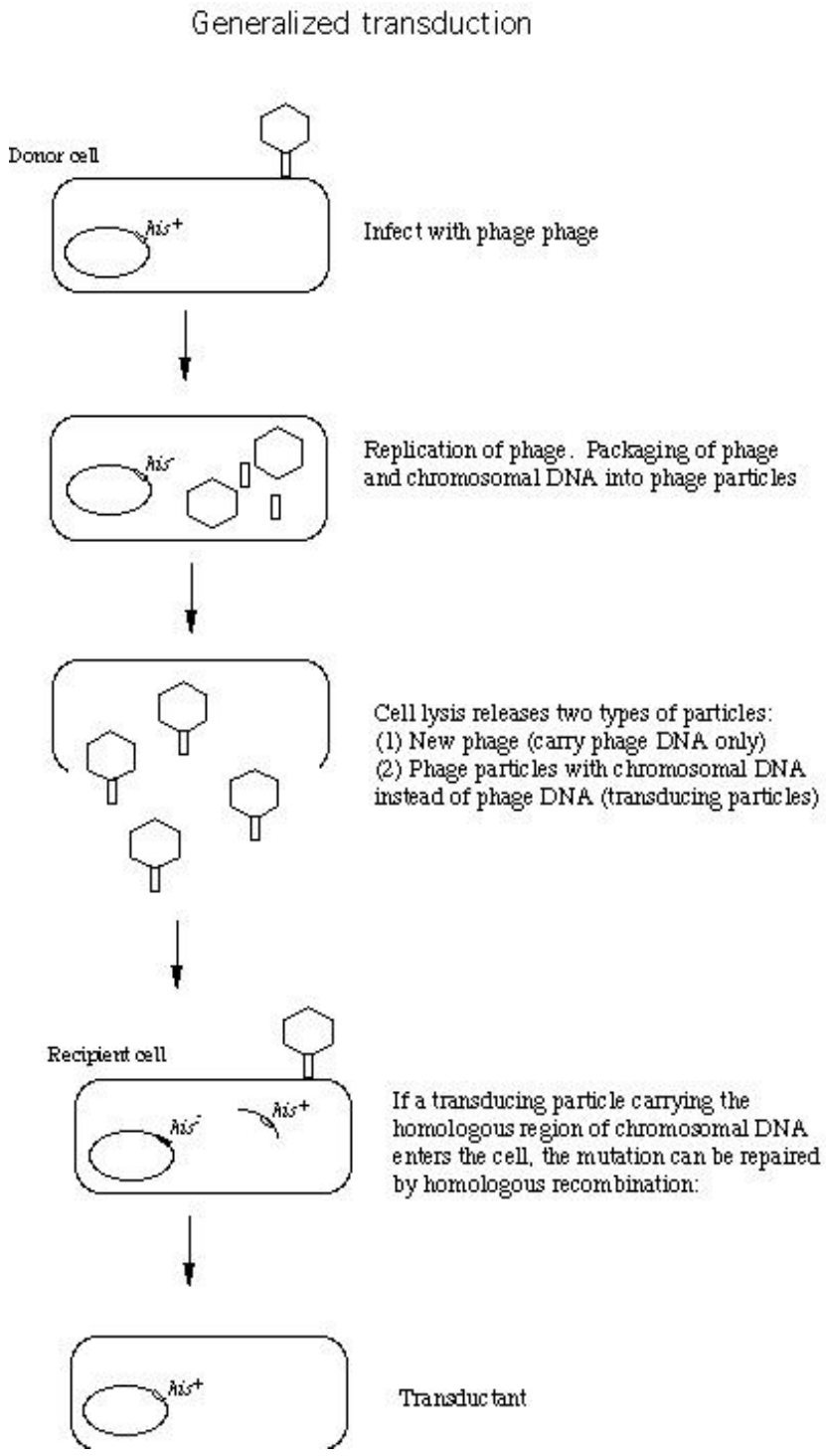

Fig.3.11: Bacterial Transduction

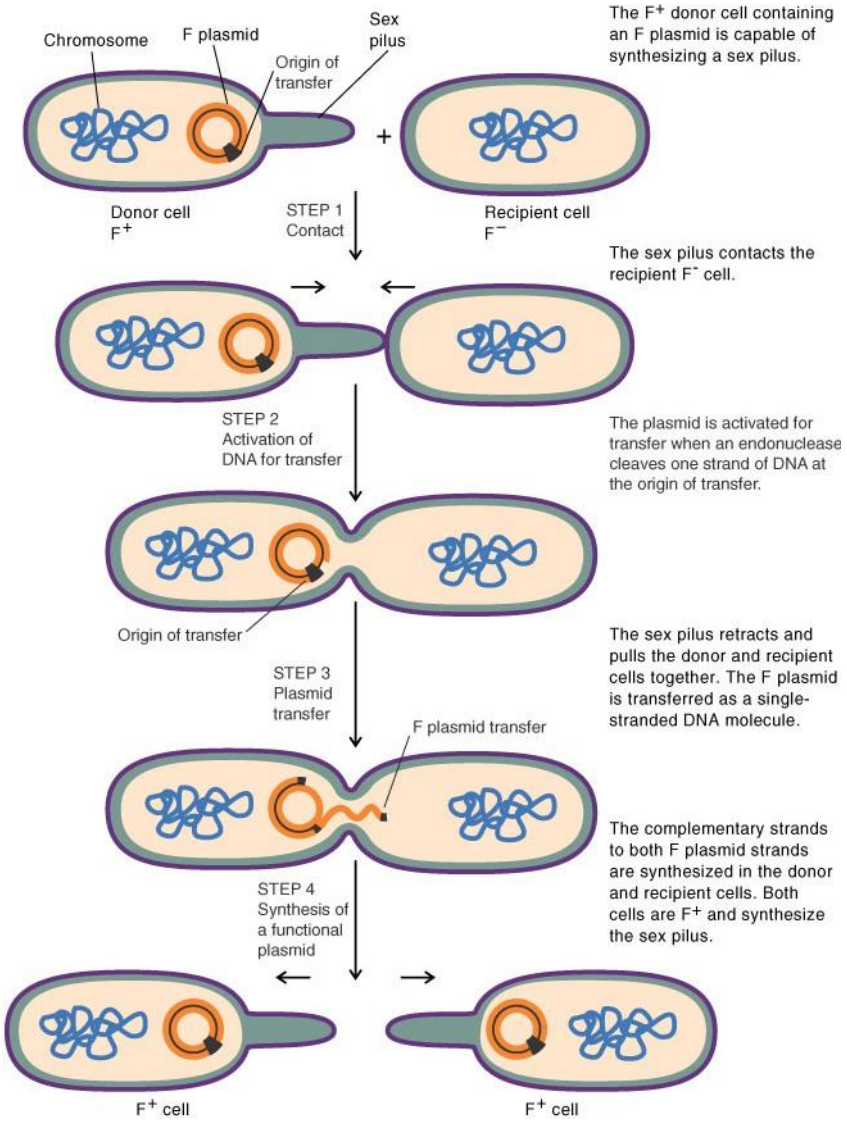

Fig.3.12: Bacterial conjugation in *Bacillus*

integration of a bacteriophage introduces foreign DNA into the chromosome (**Fig.3.11**). The third method of gene transfer is conjugation, whereby DNA is transferred through direct cell contact (**Fig.3.12**).

Transduction of bacterial genes by bacteriophage appears to be a consequence of infrequent errors during intracellular assembly of virus particles, rather than a bacterial adaptation. Conjugation, in the much-studied E. coli system is determined by plasmid genes, and is an adaptation for transferring copies of the plasmid from one bacterial host to another. It is seldom that a conjugative plasmid integrates into the host bacterial chromosome, and subsequently transfers part of the host bacterial DNA to another bacterium. Plasmid-mediated transfer of host bacterial DNA also appears to be an accidental process rather than a bacterial adaptation.

Transformation, unlike transduction or conjugation, depends on numerous bacterial gene products that specifically interact to perform this complex process, and thus transformation is clearly a bacterial adaptation for DNA transfer. In order for a bacterium to bind, take up and recombine donor DNA into its own chromosome, it must first enter a special physiological

state termed competence. In *Bacillus subtilis* about 40 genes are required for the development of competence. The length of DNA transferred during *B. subtilis* transformation can be between a third of a chromosome up to the whole chromosome. Transformation appears to be common among bacterial species, and thus far at least 60 species are known to have the natural ability to become competent for transformation. The development of competence in nature is usually associated with stressful environmental conditions, and seems to be an adaptation for facilitating repair of DNA damage in recipient cells.

In ordinary circumstances, transduction, conjugation, and transformation involve transfer of DNA between individual bacteria of the same species, but occasionally transfer may occur between individuals of different bacterial species and this may have significant consequences, such as the transfer of antibiotic resistance. In such cases, gene acquisition from other bacteria or the environment is called horizontal gene transfer and may be common under natural conditions Gene transfer is particularly important in antibiotic resistance as it allows the rapid transfer of resistance genes between different pathogens.

3.7-ECONOMIC IMPORTANCE

Bacteria play important roles in different fields such as agriculture, industry etc. Some of them are mentioned below:

3.7.1 Role in agriculture

a) **Scavenging Role**: Saprophytic bacteria obtain food from organic remains such as animal excreta, fallen leaves, meat etc. They decompose these substances by action of digestive enzymes aerobically or anaerobically (known as fermentation). Thus they help in sanitation of nature, therefore also known as scavengers. e.g. *Pseudomonas*

b) **Nitrification:** *Rhizobium* bacteria, living in root nodules of leguminous plant symbiotically, helps in fixing atmospheric nitrogen. Similarly, *Nitrosomanas* and *Nitrococcus* convert ammonium salt to nitrites. Nitrites are further changed to nitrates by *Nitrobacter* and *Nitrocystis*. It enables plants to uptake nitrogen.

c) **Production of Organic Manure:** As stated above, saprophytic bacteria help in breaking of complex organic substance to simpler forms. Thus, in this process, they help to convert farm refuse, dung and other wastes to manure.

d) **Preparation of Ensilage:** Ensilage is preserved cattle fodder prepared by packing fresh chopped fodder sprinkled with molasses. Fermentation activity of bacteria produces lactic acid that acts as preservative in ensilage.

e) **Production of fuel:** Bacteria, while converting animal dung and other organic wastes to manure, help in production of fuel. Gober gas plant is an example of this process.

f) **Disposal of sewage:** Bacteria help in disposal of sewage by decomposing it and thus, help in environmental sanitation.

3.7.2 Role in industry

a) **Dairy Industry:** Bacteria such as *Streptococcus lactis* convert milk sugar lactose into lactic acid that coagulates casein (milk protein). Then, milk is converted into curd, yoghurt, cheese etc needed for the industry.

b) **Production of Organic Compounds:** Fermentation (breakdown of carbohydrate in absence of oxygen) process of various bacteria produces organic compounds like lactic acid (by *Lactobacillus*), acetic acid (by *Acetobacter aceti*), acetone (by *Clostridium acetabutylicum*) etc.

c) **Fibre Retting:** The action of some bacteria like *Clostridium, Pseudomonas* etc. help in fibre retting i.e. separation of stem and leaf fibre of plants from other softer tissue.

d) **Curing:** The leaves of tea and tobacco, beans of coffee and coca are cured off their bitterness with the help of action of certain bacteria such as *Bacillus megatherium.*

e) **Production of Antibiotics:** Number of anti-bacterial and anti-fungal antibiotics such as Hamycin, Polymyxin, Trichomycin etc are obtained from mycelia bacteria (like *Streptomyces*). Similarly, Bacillus is used for production of antibiotics such as Bacitracin, Gramicidin etc

f) **Production of Vitamins:** Different kinds of vitamins are produced from bacteria like Riboflavin from *Clostridium butylicum*, Vitamin B12 from *Bacillus megatherium* and Vitamin K and B-complex from *Escherichia coli*.

Harmful effects of bacteria

Though bacteria plays important role in agriculture, industries and natural sanitation etc, they have some harmful effects also.

a) **Food Spoiling:** Saprophytic bacteria always not only help in decomposition of dead matters, but they also cause the rotting of vegetables, fruits, meat, bread etc.

b) **Food Poisoning:** Bacteria like *Staphylococcus aureus* cause food poisoning and cause diarrhoea and vomiting.

c)**Damaging of domestic articles:** *Spirochete cytophaga* deteriorates cotton, leather and wooden articles.

d) **Denitrification:** Bacteria such as *Thiobacillus* and *Microbacillus*convert nitrate of the soil to the gaseous nitrogen. This hampers plant growth.

e) **Desulphurication:** Bacteria such as *Desulfovibrio* convert soil sulphates into hydrogen sulphide.

f) **Cause of Diseases:** It is known that over 90% of human diseases and over 10% of plant diseases are caused by bacteria.

3.8- SUMMARY

Bacteria are microscopic unicellular prokaryotic organisms characterized by the lack of a membrane-bound nucleus and membrane-bound organelles. Bacteria were the only form of

life on earth for 2 billion years. There are more bacteria, as separate individuals, than any other type of organism; there can be as many as 2.5 billion bacteria are estimated in one gram of fertile soil. Bacteria exhibit an extremely wide variety of metabolic types. The distribution of metabolic traits within a group of bacteria has traditionally been used to define their taxonomy, but these traits often do not correspond with modern genetic classifications. Unlike in multicellular organisms, increases in cell size and reproduction by cell division are tightly linked in unicellular organisms. Bacteria grow to a fixed size and then reproduce through binary fission, a form of asexual reproduction. In bacteria, DNA transfer occurs through *Conjugation, Transduction* and *Transformation.* Bacteria are harmful as well as economically important.

3.9-GLOSSARY

Aerobe – An organism capable of living only in the presence of oxygen.
Anaerobe – An organism capable of living in the absence of free oxygen.
Chemotrophs- An organism that obtains the energy required for the synthesis of organic molecules from the oxidation of organic compounds.
Denitrification – The decomposition of nitrate from the surrounding through bacteria during anaerobic respiration.
Double Helix- A molecule composed of two complimentary polymeric chains coiled in the same direction about in the same axis, as DNA.
Growth – The sum total of the various physiological processes that combine to cause an increase in size and dry weight.
Metabolism – The net result of the biochemical processes of a living organism or cells.
Pathogens – An organism able to cause disease. The term restricted to living organisms.
Systematic – The scientific study of the classification of living things, with emphasis on their evolutionary relationship.
Taxonomy – The study of the principles and practices of classification of living things.

3.10- SELF ASSESSMENT QUESTIONS

3.10.1 Short answer questions

Q1 What are bacteria?

Ans. Bacteria are prokaryotic and unicellular beings. Bacteria have simple organization; they have an external cell wall, plasma membrane, circular DNA within the cytoplasm and ribosome for protein synthesis. Some bacteria are encapsulated, i.e., they have a polysaccharide capsule outside the cell wall.

Q2 Are bacteria the only prokaryotic beings?

Ans. Prokaryotic beings are classified into two big groups: archaebacteria and bacteria (also known as eubacteria). Compared to bacteria, archaebacteria have basic differences, like the chemical compositions of their plasma membrane and cell wall and different enzymes related to DNA and RNA metabolism.

Q3 What are halophile, thermoacidophile and methanogen archaebacteria?

Ans. There are three peculiar types of archaebacteria. The halophile archaebacteria only survive in salt-rich environments (even salinity of the sea is not enough for them). Thermoacidophile archaebacteria are characterized by living under high temperatures and low pH. The methanogen archaebacteria are those that liberate methane gas (CH4), they are found in swamps.

Q4 What are examples of human diseases caused by bacteria?

Ans. Some human diseases caused by bacteria are tuberculosis, pertussis, diphtheria, bacterial meningitis, gonorrhea, syphilis, bubonic plague, leptospirosis, cholera, typhoid fever, Hansen's disease, trachoma, tetanus, anthrax.

Q5 How are bacteria classified according to their need for oxygen?

Ans. According to their necessity of oxygen bacteria are classified into anaerobic (those that survive without oxygen) and aerobic (those that do not survive without oxygen).

Q6 According to their morphology how are bacteria classified?

Ans. Bacteria present different morphological patterns. A bacterium can be classified into coccus, bacillus, vibrio or spirochete.

3.10.2 Multiple choice questions

1. The name bacteria was first given by:

(a) Pasteur (b) Alexander
(c) Ehrenberg (d) Robert Koch

2. Bacteria are placed under:

(a) Ascomycetes (b) Schizomycetes
(c) Phycomycetes (d) Myxomycetes

3. Bacteria are found everywhere except:

(a) Cold water (b) Soil
(c) Boiled water (d) Body of man

4. The cell-wall of bacteria is made-up of:

(a) Polysaccharides (b) Lipids
(c) Proteins (d) All of the above

5. The nucleus of bacteria is:

(a) Incipient (b) Absent
(c) Well defined (d) None of the above

6. Bacteria reproduce by:

(a) Fission (b) Conjugation
(c)Transduction (d) All of the above

7. Fertility of soil is increased by:

(a) Nitrogen fixing bacteria (b) Denitrifying bacteria
(c) Heliminthosporium (d) Saprolegnia

8. Sometimes the bacterial cell is enclosed in a:

(a) Cell wall of cellulose (b) Capsule
(c) Plasmalemma (d) Cell membrane

9. Cocci type of bacteria is:

(a) Flagellate (b) Non-flagellate
(c) Both types (d) None of the above

10. Pneumonia is caused by:

(a) Virus (b) Bacteria
(c) Fungi (d) Algae

11. Doctors usually boil their syringe and other surgical instruments before use to:

(a) Remove dust from them (b) Clean them
(c) Sterilize them (d) It is customary

Answer key: 1(c), 2(b), 3(c), 4(d), 5(a), 6(d), 7(a), 8(b), 9(b), 10(b), 11(c)

3.10.3- Short answer questions

Q1 What are Bacteria? In how many classes we divide bacteria on the basis of morphology? Describe with diagram.
Q2 Describe the electron microscopic structure of bacterial cell.
Q3 Discuss the economic importance of bacteria.
Q4 Discuss the various mode of nutrition in the bacteria.
Q5 Do bacteria affect the life of man? How?

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3.12- SUGGESTED READINGS

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- *Text Book of Biotechnology:* H.K. Dass (2004).
- *Microbiology:* L.M. Prescott (2002).

3.13-TERMINAL QUESTIONS

Q1- What are bacteria? Describe in detail the economic importance of Bacteria.

Q2- Explain in detail the reproduction in Bacteria.

Q3- Give a detail note on bacterial cell with a well labelled diagram.

Q4-Differentiate between Gram positive and Gram negative bacteria.

Q5-Write a detailed note on Bacterial conjugation.

UNIT-4- GENERAL ACCOUNT, CLASSIFICATION, STRUCTURE, REPRODUCTION AND ECONOMIC IMPORTANCE OF VIRUSES

4.1- OBJECTIVES

After reading this unit student will be able:

- To study the structure of viruses.
- To learn the characteristic features of viruses.
- To know different shapes and types of viruses.
- To know economic importance of viruses.

4.2-INTRODUCTION

"NEVER UNDERESTIMATE THE POWER OF A MICROBE"

Viruses are no longer considered the simplest form of life. **Viruses** are a unique group of infectious agents whose distinctiveness resides in their simple, acellular organization and pattern of reproduction. Viruses are simple, acellular entities consisting of one or more molecules of either DNA or RNA enclosed in a coat of protein (and sometimes, in additional, substances such as lipids and carbohydrates). They can reproduce only within living cells and are obligately intracellular parasites. In simple words, we can say that these are simple obligate parasites comprising of nucleic acid (DNA or RNA) and protein coat. Despite their simplicity in comparison to cellular organisms, viruses are extremely important and deserve close attention. The study of viruses has contributed significantly to the discipline of molecular biology.

Although in ancients times, people did not understand the nature of their illnesses, they were acquainted with diseases, (such as rabies), which are now known to be viral in origin.

- In fact, there is some evidence that the great epidemics of 165 to 180 A.D. and 251 to 266 A.D., (which severely weakened the Roman Empire and aided its decline), may have been caused by measles and smallpox viruses. Hernán Cortés's conquest of the Aztec Empire in Mexico was made possible by an epidemic that ravaged Mexico City. The virus was probably brought to Mexico in 1520 by the relief expedition sent to join Cortés.
- The term virus was derived from a latin word *virus* means *venom of poisonous fluid.*
- All the causative agents of infectious diseases were put together under this category by L. Pasteur.
- The discovery of **Chamberland-Pasteur filter in 1884** by Charles Chamberland (a collaborator of L. Pasteur) made possible the discovery of viruses. In 1885 Adolph Mayer (a Dutch scientist) and in 1892 D.J. Ivanowsky (a Russian scientist) recognized some microbes responsible for the occurrence of tobacco mosaic disease. The basic criterion of their distinction from other familiar microbial agents was their ability to pass through bacteria-proof filters and the agents were named as filterable viruses.
- The distinction of viruses from other cellular organisms was demonstrated by M.W. Beijerinck (a Dutch bacteriologist) in 1898. During his experiment he discovered that TMV could be precipitated from an alcohol suspension without losing its infectious ability and the fluid was capable of diffusing through agarose gel. These characteristics were not possessed by bacteria or any other living entity. On the basis of these findings

Beijerinck led to the conclusion that fluid itself was living and put forward the principle of*"Contagium vivum fluidum"*(infectious living fluid).

- In 1898 Friedrich Loeffer and Paul Frosch concluded that foot and mouth disease of cattle was caused by a filterable virus rather than a toxin.
- In 1935 the structure of virus was studied by Wendelll M. Stanley. According to Him, A virus is not simply a protein but a nucleoprotein and its infectious principle is the nucleic acid rather than protein. For this discovery **Stanley was awarded Nobel Prize in 1946.**
- F.W.Twort in 1915 and Felix d'Herelle in 1917 discovered that a virus was capable of lysing bacterial cells. such viruses were designated as **bacteriophage**.
- In 1953 Luria described viruses as submicroscopic entities capable of being introduced into specific living cells and reproducing inside such cells only.

4.3-GENERAL ACCOUNT

- Viruses cause many diseases of international importance. Amongst the human viruses, smallpox, polio, influenza, hepatitis, human immunodeficiency virus (HIV-AIDS), measles and the SARS corona virus are particularly well known.
- While antibiotics can be very effective against diseases caused by bacteria, these treatments are ineffective against viruses.
- Since ancient times, there have been documented reports of river waters having the ability to cure infectious diseases, such as leprosy. In 1896, Ernest Hanbury Hankin reported that something in the waters of the Ganges and Yamuna rivers in India had marked antibacterial action against cholera and could pass through a very fine porcelain filter.
- In 1915, British bacteriologist Frederick Twort, superintendent of the Brown Institution of London, discovered a small agent that infected and killed bacteria. He believed that the agent must be one of the following:
- a stage in the life cycle of the bacteria;
- an enzyme produced by the bacteria themselves; or
- a virus that grew on and destroyed the bacteria.
- Control measures rely on vaccines (antibodies raised against some component of the virus) or relief of the symptoms to encourage the body's own defense system.
- Viruses also cause many important plant diseases and are responsible for huge losses in crop production and quality in all parts of the world.
- Infected plants may show a range of symptoms depending on the disease but often there is leaf yellowing (either of the whole leaf or in a pattern of stripes or blotches), leaf distortion (e.g. curling) and/or other growth distortions (e.g. stunting of the whole plant, abnormalities in flower or fruit formation).

4.4-CLASSIFICATION

Due to the ultramicroscopic size, presence of both living and non-living features and absence of fossil records it is very difficult to classify viruses. On the basis of their host range, clinical, epidemiological and pathological symptoms viruses are classified into following four groups:

1. **Plant viruses:** These viruses infect only plants and depending on host they have been subdivided into bacterial viruses, algal viruses, fungal viruses etc.

2. Invertebrate viruses: These infect invertebrates.

3. Vertebrate viruses: These infect vertebrate animals.

4. Dual-host viruses: These viruses infect two different hosts mentioned above.

Holmes in 1948 included all viruses in a single order **Virales** having three suborders:

1. **Phagineae:** This suborder includes bacteriophages.
2. **Phytophagineae:** This suborder includes viruses infecting plants.
3. **Zoophaginea:** This suborder includes viruses infecting animals.

4.5 STRUCTURE

Due to the realization of importance of viruses and their simple structure of viruses, the morphology has been studied over decades and progress has been achieved from the use of several modern techniques- X-ray diffraction, immunology, SEM, TEM, biochemical analysis and electron microscopy.A simple virus particle is often termed as **virion,** comprising of nucleic acid core of genetic material, enclosed within protein coat.The amount of protein in viruses varies from 60-95% (**Fig.4.1**).

4.5.1 Size:

In recent times the size of virus was determined by using filtration technique through collodion membrane of known porosity but now with the advancement of technology ultracentrifugation and electron microscopy are employed. The size varies from 10 to 300 or 400 nm. The smallest viruses are entero viruses, which are less than 30nm in diameter. The largest are orthopox viruses, measuring about 240nm×300nm, i.e. approximately 1/10 the size of red blood cell. The bacteriophages are about 65nm×200nm.

4.5.2 Nucleic acid:

The viruses contain a nucleic acid core of genetic material which may b either DNA or RNA. The viruse containing DNA are termed as Deoxyviruses and those having RNA are termed as Riboviruses. Generally,

I) All plant viruses have single stranded RNA.

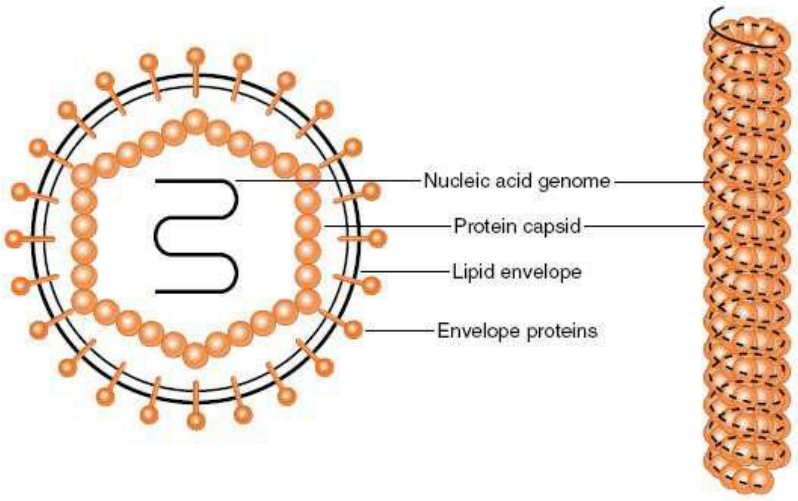
II) Animal viruses have either single or double stranded (rarely) RNA or double stranded DNA.

III) Bacterial viruses contain mostly double stranded DNA but can also have single stranded DNA or RNA.
IV) Most of the insect viruses contain RNA but only a few have DNA.
The number of nucleotide pairs in a molecule varies from 1,000 - 250,000 pairs but the number of pairs in a specific virion is always constant.

4.5.3- The protein coat:

The nucleic acid core is enclosed by a protective protin sheath called the **capsid.** Each capsid consists of several identical protein subunits, known as capsomeres. The proteins may be of single or several types. The number of proteins and the arrangement of capsomeres are characteristic feature of viruses and thus can be useful in their identification and classification. The capsomeres may be in the form of pentamer or hexamer.

In some complex forms (e.g., influenza and herpes virus) the capsid is covered by an envelope. It usually consists of some combination of lipids, protein and carbohydrates. Envelope of many viruses has projections called **spikes,** responsible for attachment with host.



COMPLEX STRUCTURE **SIMPLE STRUCTURE**

ENVELOPED VIRUS

Fig.4.1 Structure of Virus

4.5.4. Bacteriophage

Viruses which infect bacterial cells are known as bacteriophage or viruses of bacteria. Bacteriophages were first observed in 1915 by F. Twort in England and in 1917 by F.d'Herelle in France. D' Herelle used the term bacteriophage (eaters of bacteria). Bacteriophages are found in all habitats where bacteria can survive and live as obligate parasites. They are found in soil, fruits, sewage water, milk, vegetables and root nodules of legumes. Some phages have also been found in the intestine of birds and animals. In human beings, phages can be found intestine, urine, blood, saliva, pus and nasal exudate (**Fig.4.2**).

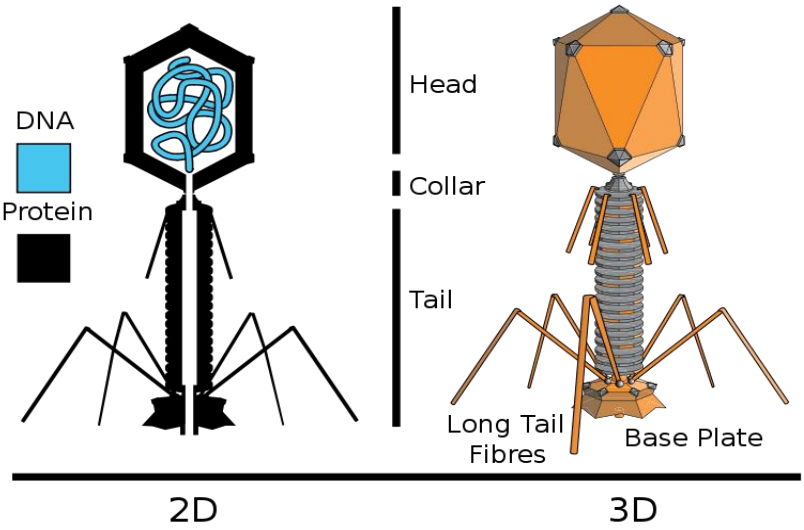


Fig.4.2 Diagram of a typical tailed bacteriophage structure

- A bacteriophage is a virus that infects bacteria. The term is commonly shortened to phage.
- Bacteriophages are similar to viruses that infect eukaryotes (plants, animals, and fungi) in that there are many different kinds of structures and functions. These are typically made of an outer protein hull that has genetic material inside it. The genetic material can be ssRNA, dsRNA, ssDNA, or double-stranded DNA between 5 and 500 kilo base pairs long with either circular or linear arrangement.
- Bacteriophages are usually between 20 and 200 nanometers in size.
- Phages are ubiquitous in the environment, and humans are routinely exposed to them at high levels through food and water without adverse effect.

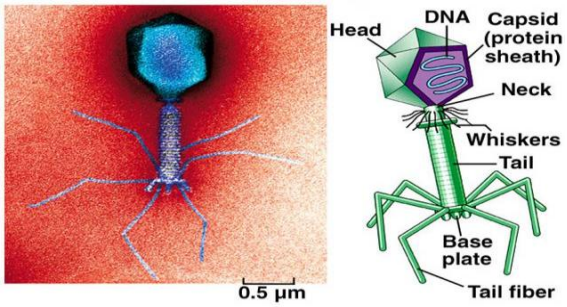


Fig.4.3: T4 Bacteriophage

4.5.5-Types of phages

1. T-phages (T1-T7; T standing for type):

These are characterized by the presence of a tail and exhibit the largest groups of phages having ds DNA.T-phages have been divided into following three sub-groups -

i) T-even phages (T2, T4 and T6).

ii) T-odd phages (T1, T3 and T7).

2. Virulent and temperate phages: As we know, viruses for microorganisms are called phages and those for bacteria are bacteriophages. A virus can be thought of as genetic elements within a protein coating. When injected into a cell, this genetic material may alter the host so that its energy and metabolism are directed entirely toward making more virus particles, and ultimately the host cell dies. Some phages are always actively seeking cells in which to reproduce. Other phages can attach to the bacterial chromosome and remain passive for a long time. **Active phages are called virulent**. **The passive ones are called temperate or lysogenic;** they may become virulent if the system is shocked by an event such as a temperature or pH change, but some host cells survive and continue to multiply. When not in their virulent stage, temperate phages have no discernable effect on their hosts.

T4 is one of many virulent phages that infect *E. coli*. Its sheath fastens to the cell wall, and constriction forces some phage DNA (some phages are RNA) into the cell to make it into a factory for making more phage particles (**Fig.4.3**).

4.5.6 Structure of Bacteriophage

As the bacteriophages cannot be separated by bacterial filters, their structural details are known through electron microscopic studies of some larger particles (T-even group) which infect bacterium E. coli. The appearance of phage resembles a tadpole or spermatozoid; it is differentiated into a head and a tail. The head may be prismoid (T, T2, T6) or hexagonal (T3 and T7) The phages which are filamentous do not show differentiation into head and tail. The size of head is approximately 950Å× 650Å. The extended part between head and the tail is called collar. The tail is equal to the length of the head and has a diameter of 80Å. At the proximal end of tail a hexagonal end plate is present which is approximately 200Å thick having 6 tail pins (fibreson) in its under surface (the size of each is about 1500Å). The tail pins help in adsorption of phage particle on the surface of the bacterium and the enzymes secreted by tail pins are responsible for the lysis of bacterial cell wall.

Chemical composition

Bacteriophages contain protein -50 to 60 % ; nucleic acids (either DNA or RNA) 40 to 50 % some lipids ; Head wall - 2,000 similar subunits of proteins ; molecular wt of phage DNA - 2,500,000.

4.6 REPRODUCTION

Replication

Although the basic mechanism of penetration and multiplication is similar in all the viruses, the process is best studied in bacteriophages. These multiply by two alternate methods-

I. Lytic cycle.

II. Lysogenic cycle.

4.6.2: Lytic cycle

T-even bacteriophages multiply by lytic cycle. This cycle involves following four steps -

i. Infection

ii. Synthesis of phage components in the host cells.
iii. Assembly of new phage particle.
iv. Liberation of phage particles from the host cells.

i. Infection:

The infection starts with adsorption of the phage on the host bacterium with the help of its tail fibres and transfer of phage nucleic acid into host cell. The adsorption depends on the mutual affinity of the phage and bacterium. Some phages infect a particular bacterium and others adsorb themselves at a specific site only (receptor site).

ii. Synthesis of phage components in the host cell:

Inside the bacterial cell, the phage nucleic acid takes over the protein synthesis machinery of the cell. It suppresses the bacterial protein and directs the metabolism of the cell to synthesize the proteins of phage particle. This is accomplished by the synthesis of viral specific m-RNA which later directs the host cell to synthesize proteins which are used as subunits of the protein coat of the phage particle. These proteins are called late proteins. Towards the end of replication of phage nucleic acid, a late protein, called phage lysozyme is synthesized.

iii. Assembly of new phage particle:

Assembly of nucleic acids and proteins (late proteins) into new phage particles is called maturation. This process is controlled by viral genome. It includes condensation of nucleic acid in crystalline form, aggregation of protein subunits around DNA to form head, attachment of core tube with tail plate, attachment of tail with head and attachment of tail fibres to the end plate.

iv. Liberation of phage particles from the host cells:

Lysis of the host cell is essential for the liberation of phage particles. It is facilitated by the lysozyme secreted by the phage DNA in the host celll. The host cell ruptures as a result of lysis and the phage particles are liberated.

The entire cycle of phage development is completed in 30-90 minutes. In an infected bacterium 7-8 phage particles are formed per minute and a total of about 200 phages are formed in a bacterium (**Fig.4.4**).

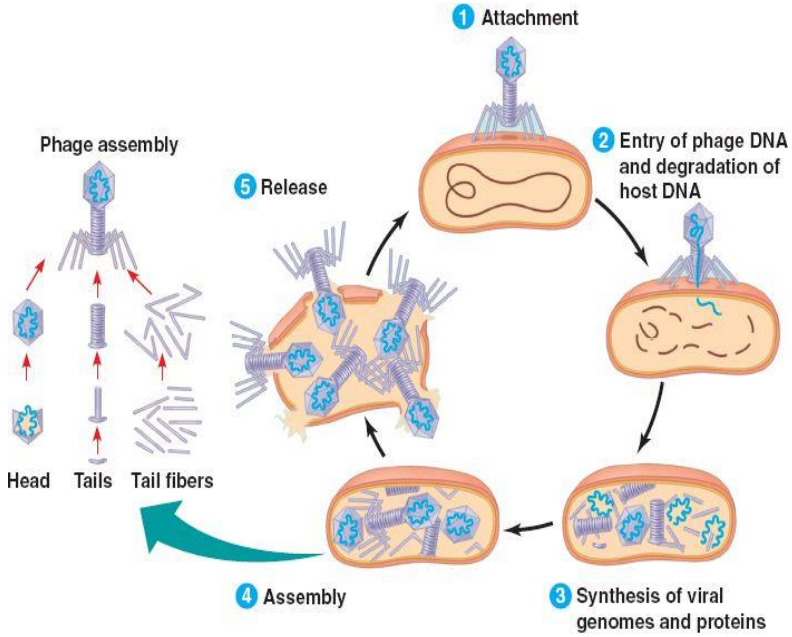


Fig.4.4: Lytic Cycle of T4 Bacteriophage

4.6.2. Lysogenic cycle:

Some phages do not cause lysis of host cell and exhibit lysogenic cycle. Phages multiplying by this method are known as lysogenic phages or temperate phages and participating host cells are called lysogenic cells. The phage injects its DNA into host cell. The linear phage DNA becomes circular and integrates within the bacterial chromosome by recombination. The inserted phage DNA is now called prophage. The activity of prophage genes is repressed by two repressor proteins which are synthesized by phage genes. This checks the synthesis of new phages within the host cell. Each time bacterial cell divide, the prophage multiplies along with bacterial chromosome. The prophage remains latent within the progeny cells (**Fig. 4.5**).

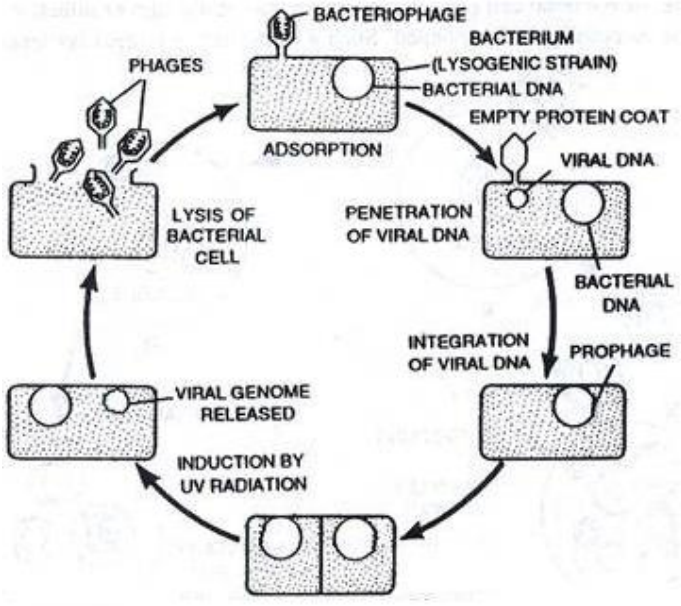


Fig.4.5: Lysogenic life cycle of Lamda (ƛ) phage

4.7 ECONOMIC IMPORTANCE

Viruses are entities which infect all cellular forms eukaryotes (vertebrate animals, invertebrate animals, plants, fungi) and prokaryotes (bacteria and archaea). They are obligate parasites and reproduce only in living organisms. Therefore these organisms are very important and we need to understand the nature of viruses, how they replicate and how they cause disease. This knowledge permits the development of effective means for prevention, diagnosis and treatment of virus diseases through the production of vaccines, diagnostic reagents and techniques, and antiviral drugs. These medical applications constitute major aspects of the science of virology.

4.7.1 Negative impacts of viruses:

Veterinary virology and plant virology are also important because of the economic impact of many viruses that cause disease in domestic animals and crop plants: foot and mouth disease virus and rice yellow mottle virus are just two examples. Another area where viruses can cause economic damage is in the dairy industry, where phages can infect the lactic acid bacteria that are responsible for the fermentations that produce cheese, yogurt and other milk products. Viruses are important agents of many human diseases, ranging from the trivial (e.g. common colds) to the lethal (e.g. rabies), and viruses also play roles in the development of several types of cancer. Virus diseases can also affect the well-being of societies. Smallpox had a great impact in the past and AIDS is having a great impact today.

Different viral diseases attack different parts of the body

S.NO.	Viruses	Target parts/organisms	Source of spread
1	**Chicken virus and measles virus**	Cells of skin causing watery blisters and red rashes.	Direct or indirect contact
2	**Influenza virus**	Lining of nose and throat	Spread through direct contact, Spitting and coughing
3	**Polio virus**	Muscles	Infected faeces and flies
4	**HIV virus**	Immune system	Direct and sexual contact with an infected person.
5	**Tobacco mosaic virus**	Tobacco plant	Direct contact

4.7.2-Positive impact of viruses:

- **Phage typing of bacteria:** Some groups of bacteria, such as certain Salmonella species, are classified into strains on the basis of the spectrum of phages to which they are susceptible. Identification of the phage types of bacterial isolates can provide useful epidemiological information during outbreaks of disease caused by these bacteria.

- **Sources of enzymes:** A number of enzymes used in molecular biology are virus enzymes. Examples include reverse transcriptases from retroviruses and RNA polymerases from phages.
- **Pesticides:** Some insect pests are controlled with baculo viruses and myxoma virus is used to control rabbits.
- **Anti-bacterial agents:** In the mid-20th century phages were used to treat some bacterial infections of humans. Interest declined with the discovery of antibiotics, but has been renewed with the emergence of antibiotic-resistant strains of bacteria
- **Anti-cancer agents:** Genetically modified strains of viruses, such as herpes simplex virus and vaccinia virus, are being investigated for treatment of cancers. These strains have been modified so that they are able to infect and destroy specific tumor cells, and unable to infect normal cells.
- **Gene vectors for protein production:** Viruses such as certain baculo viruses and adenoviruses are used as vectors to take genes into animal cells growing in culture. This technology can be used to insert into the cells genes encoding useful proteins, such as vaccine components, and the cells can then be used for mass production of the proteins.
- **Gene vectors for treatment of genetic diseases:** Children with severe combined immunodeficiency (baby in the bubble syndrome) have been successfully treated using retroviruses as vectors to introduce into their stem cells a non-mutated copy of the mutated gene responsible for the disease.

4.7.3- Viroids

- These are pathogenic agents in plants.
- They show unencapsidated naked small circular RNA molecules, and are a few hundred nucleotides long, with high degree of secondary structure.
- They lack the protein coat of viruses.
- These organisms do not code for any protein.
- They replicate in the nuclei of infected cells.
- Viroids rapidly move from cell to cell and through the phloem.
- They can be readily transmitted mechanically in the field, by vegetative propagation, and some by pollens and seeds.

4.8 SUMMARY

- Viruses are simple, acellular entities consisting of one or more molecules of either DNA or RNA enclosed in a coat of protein (and sometimes, additional, substances such as lipids and carbohydrates).
- A simple virus particle is often termed as **virion,** comprising of nucleic acid core of genetic material, enclosed within protein coat. The amount of protein in viruses varies from 60-95%.

- The viruses contain a nucleic acid core of genetic material which may b either DNA or RNA. The viruses containing DNA are termed as Deoxyviruses and those having RNA are termed as Riboviruses.
- The nucleic acid core is enclosed by a protective protein sheath called the **capsid.** Each capsid consists of several identical protein subunits, known as capsomeres. The proteins may be of single or several types. The number of proteins and the arrangement of capsomeres are characterstic feature of viruses and thus can be useful in their identification and classification.
- These multiply by two alternate methods-
- Lytic cycle
- Lysogenic cycle.

Viruses are on the borderline between living and non-living things. They show a varied range of shapes. They may be spherical - (such as the adenovirus and influenza virus), Rod like – (such as the tobacco mosaic virus) or many – sided (such as bacteriophages)

The structure of Virus is very simple. There is just a core of DNA surrounded by a sheath of Protein. There is no cytoplasm, nucleus or cell membrane. Viruses are not free living. They grow and multiply only in living cells.

The reproduction of Virus is one characteristic which they share with living organisms, but they reproduce only within another living cell. Outside a living system the viruses neither reproduce nor respire.

Viruses cause various diseases in a specific host like plant, animal or in bacteria. Viruses which attack bacteria and infect bacterial cells are known as bacteriophage. They destroy the host cells by reproducing within them.

4.9 - GLOSSARY

Nucleic acids: Nucleotides + phosphate + sugar.

Obligate parasite: An obligate parasite, or holoparasite, is a parasitic organism that cannot complete its life-cycle without exploiting a suitable host.

SEM: Scanning electron microscope.

TEM: Transmission electron microscopy.

Unencapsidated: Not encapsulated.

4.10 SELF ASSESSMENT QUESTIONS

4.10.1 Very short answer type questions

Q1. Which genetic material is found in all plant viruses?

Q2. Who discovered bacteriophages?

Q3. Define viroids?

Q4. What is the name of protein coat in viruses?

Q5. Mention the type of genetic material in most of the animal viruses?

Q6. What is the name of the subunit of the protein coat in viruses?

Q6. Name the two explicative cycles of bacteriophages?

Q7. What are bacteriophages?

4.10.2 Multiple choice questions

1. The fact which supports the idea that viruses are living is that they:
(a) duplicate themselves (b) penetrate plasma membrane
(c) can be crystallized (d) are made of protein and DNA

2. Bacteriophages were discovered by:
(a) Griffith (b) Subramanian
(c) Twort (d) none of the above

3. Which one of the following is bacterium eater?
(a) Coliphage (b)bacteriophage
(c) Cyanophage (d) TMV

4. Outer layer of viruses is composed of:
(a) Fats (b) Protein
(c) Carbohydrate (d) Nucleic acid

5. Who discovered TMV?
(a) Bawden (b) Iwanowski
(c) Stanley (d) Twort and d'Herelle

6. Genetic material in a bacteriophage is :
(a) RNA (b) DNA
(c) both DNA and RNA (d) neither DNA nor RNA

7. Viruses synthesize their protein coats:
(a) inside the host cell (b)outside the host cell
(c) both outside as well as inside the host cell (d) none of these

8. Who isolated the plant viruses first?
(a) W.M Stanley (b) E.C Stakman
(c) D.Iwanowsky (d) K.M Smith

9. Which of the following is the constituent of a bacteriophage?
(a) protein (b) DNA
(c) nucleoprotein (d) lipid and protein

10. Which of the following is the correct answer?
(a) a virion is a fully developed virus particle (b) a virion is a capsid
(c) a virion is a capsomere (d) none of the above

4.10.2 ANSWERS KEYS:

1. (a), 2.(c), 3. (b), 4. (b), 5. (b), 6. (b), 7. (a), 8.(c), 9.(c), 10.(a)

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4.12 SUGGESTED READINGS

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- Microbiology. By Joseph Pelczar, Roger Delbert Reid, Eddie Chan Sun Chan, Mc Graw Hill.
- Microbiology: An Introduction By Tortora, G.J., Funke, B.R., Case, C.L. Pearson Benjamin Cummings, U.S.A. 10th edition.

4.13 TERMINAL QUESTIONS

Q1. Give an illustrated account of the morphology and chemical structure of viruses?

Q2. Write an essay on the transmission of viruses?

Q3. What are bacteriophages?

Q4. Describe the structure of bacteriophage with the help of suitable diagrams?

Q5. Describe the different cycles seen during the replication of bacteriophages?

Q6. Write an essay on economic importance of viruses?

BLOCK-2-FUNGI AND LICHENS

UNIT-5-CHARACTERS, ECONOMIC IMPORTANCE, CLASSIFICATION AND GENERAL ACCOUNT OF MAJOR CLASSES OF FUNGI

5.1 OBJECTIVES

After reading this unit student will be able:

- To study the range of thallus structure, various types of fungal tissues and specialized vegetative and reproductive structures.
- To get familiarized with the general characters on with the classification is based.
- To study the fungi "as friends as well as foes".

5.2 INTRODUCTION

Man's interest in fungi started with the observation of the beautiful, umbrella shaped mushrooms and toad stools growing on soils forming fairy rings. The fungi (sing., fungus) are a distinct group of organisms that belong to lower plants. The name of the fungi derived from the most obvious representatives, the mushrooms (Greek- mykes, Latin- fungus). They are eukaryotes and share with plants the possession of a cell wall, liquid filled intracellular vacuoles, microscopically visible streaming of the cytoplasm and lack of motility. However, they do not contain photosynthetic pigments and are chemo-organotrophs. Most of them grow aerobically and obtain their energy by oxidation of organic substances. Compared to the plants, which are organized into stems, roots and leaves, fungi show only very limited morphological differentiation and practically no functional differentiation.

Fungi are diverse organisms. There are approximately 70,000 known species of fungi (and probably 10 to 20 times more undiscovered species). These are found in such varied environments as tropical forests, oceans and deserts. But they are primarily terrestrial heterotrophic organisms; their principle role being the decomposition of organic matter along with bacteria. Because they most often live on nonliving organic matter, hence called saprophytes (Greek: sapros-rotten, phyton- plant). The products released by the decaying action of fungi from their metabolism of organic matter are returned to the environment to be used once again by other organisms. Some fungi are parasites and often pathogens of plants and animals.

The fungi are of great economic importance on account of their both harmful as well as beneficial effects. A large number of fungi cause destructive havoc to our valuable crop and timber plants, various lines of food products. They also attack the live-stock as well as human beings. But, all of them are not harmful to the mankind, as most of the species bring about decomposition of dead bodies of plants and animals as well as of animal dung. In addition they are also useful in the production of new age medicines and other useful products.

5.3 GENERAL CHARACTERS

Thallus structure

Unicellular thallus – In some of the lower fungi such as **Chytrids** the thallus is more or less a spherical, single celled structure. At the time of reproduction it becomes a reproductive

unit. The latter produces the asexual or sexual cells. Such fungi are called **holocarpic**. In this type of fungi vegetative and reproductive phases do not occur together in the same thallus. **Plasmodiophora** has a vegetative phase consisting of a naked multinucleate, amoeboid mass of protoplasm. It is termed plasmodium. In the unicellular holocarpic forms, the mycelium is absent e.g. *Synchytrium*. Most nonmycelial fungi as *Olphidium* and Yeasts are basically unicellular with a true cell wall. In Yeast it is common to find unicellular thalli producing bud cells in succession. These bud cells may remain attached to one another in an easily dissociated chain. Such a chain of bud cells is referred to as **pseudomycelium**.

Filamentous thallus – In most true fungi, the thallus is filamentous composed of Hyphae. Loosely aggregated hyphae are collectively forms a network known as mycelium. The hyphae may be hyaline or variously coloured. Each hypha may vary in overall length and diameter, the latter ranging from 0.5 to 1mm. The apex of hyphae is a thin walled region where growth materials are added, differentiation takes place, elongation occurs in a zone behind the tip. Branching of hyphae is dichotomous when the apex of hyphae ceases elongating and forks into two equal branches. More often branching is sub apical and lateral, leaving the leading hyphal apex free to continue its growth. This type of branching may be: dichotomous, verticillate, cymose and racemose. On the basis of presence or absence of septa the hyphae of mycelical fungi are of two types (**Fig.5.1**):

(A) Nonseptate or aseptate hyphae – These are characteristic of **oomycetes** and **zygomycetes** where the mycelium contains numerous nuclei lying in a common mass of cytoplasm as in phycomycetes. Such a condition is known as **coenocytic**. There are no cross walls in the hyphae. However, septa may be laid down at the time of formation of reproductive organs to delimit them from the rest of the vegetative hyphae. **Pseudosepta** are found in **Allomyces**.

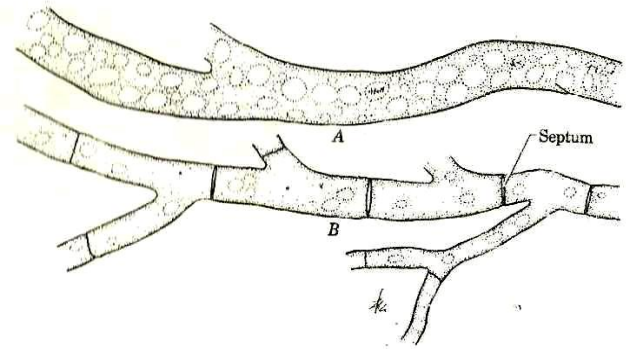


Fig.5.1: Somatic Hyphae. A. Coenocytic (nonseptate hyphae) B. Septate Hyphae

(B) Septate Hyphae – These are characteristic of Ascomycotina, Basidiomycotina, and Deuteromycotina where the hyphae are septate and hyphal segments may contain one, two or more nuclei. There are two types of septa:

a. Primary septa – Primary septa are formed in association with mitotic or meiotic nuclear division, and they separate the daughter nuclei. These types of septa are found in Ascomycotina, Basidiomycotina and their asexual states.

b. Adventitious septa – Adventitious septa are formed in the absence of mitosis or meiosis and occur especially in association with change in the local concentration of

cytoplasm. These are found in lower groups of fungi as mastigomycotina and zygomycotina.

Structure of fungal cell - With the exception of slime moulds (Myxomycotina) the fungal cell unusually consists of a strong, rigid cell wall enclosing the protoplasts.

Cell wall – The cell wall serves a number of important roles in fungi. It determines the characteristic shape of a cell. The wall acts as an interface between the protoplasts and the environment; it protects the cell from osmotic lysis and perhaps from the metabolites of other organisms: it also acts as a binding site for some enzymes. The chemical composition of the cell wall is not the same in all fungi. Cell wall composition appears to be an important criterion of fungal relationships. Table 1.1 illustrates this tendency. Chitin is characteristically present in the cell walls of most fungi. The chitin in fungal cell wall is not strictly identical with animal chitin, and the formula $(C_{22}H_{54}N_4O_{21})n$ has been suggested for the fungal chitin: It is a polymer of N-acetylglucosamine. Electron microscopic studies reveal that cellulose and chitin occur as elongated microfibrillar units.

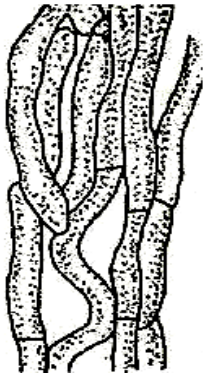
TABLE 1.1 Cell Wall Compositions in the Fungi

	Cell Wall Category	Taxonomic Group	Representative Genera
I.	Cellulose-Glycogen	Acrasiomycetes	*Polysphondylium, Dictyostelium*
II.	Cellulose-β-Glucan	Oomycetes	*Phytophthora, Pythium*
III.	Cellulose–Chitin	Hyphochytridiomycetes	*Rhizidiomyces*
IV.	Chitin–Chitosan	Zygomycets	*Mucor, Phycomyces*
V.	Chitin-β-Glucan	Chytridiomycetes	*Allomyces*
		Ascomycetes	*Neurospora*
		Deuteromycetes	*Aspergilllus*
		Basidiomycetes	*Fomes, Polyporus*
VI.	Mannan-β-Glucan	Ascomycetes	*Saccharomyces, Candida*

Fungal Tissue

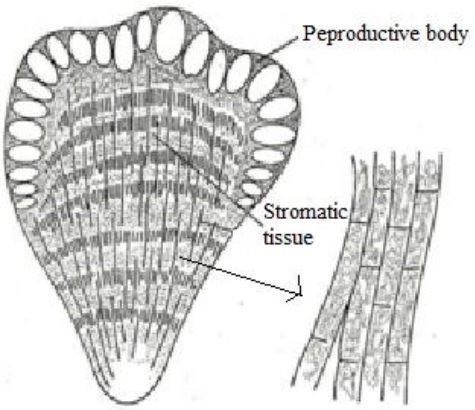
The fungal mycelium generally is a mass of hyphae interwoven loosely to form a network. During certain stages of the life history of most fungi the mycelium becomes organized into loosely or compactly woven tissues, as distinguished from the loose hyphae ordinarily composing a thallus. The term **plectenchyma** (a woven tissue) is used for all organized tissue. Two general types of plectenchyma are recognized – Prosenchyma (approaching a tissue) is a rather loosely woven tissue in which the component hyphae lie more or less parallel to one another on their typically elongated cells are easily distinguishable as such, Pseudoparenchyma (a type of plant tissue or false parenchyma) consists of closely packed, more or less isodiametric or oval cells resembling the parenchyma cells of higher plants. In this type of fungal tissue, the hyphae have lose their individuality and are not distinguishable as such. (**Fig.5.2**)

Prosenchyma and Pseudoparenchyma compose various types of somatic and reproductive structure. There are two such somatic structures the stroma (Pl. Stromata means matters) and the sclerotium (Pl. Sclerotia means hard). A stroma is a compact, somatic structure much like a mattress on which, or in which fructifications are usually formed. A sclerotium is a hard resting body resistant of unfavourable conditions; it may remain dormant for long periods of time and germinate on the return of favourable conditions.

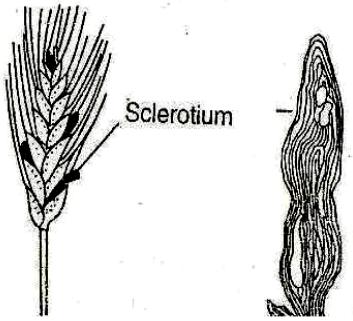


(A) Prosenchyma

(B) Pseudoparenchyma



(C) (D)



(E)

Fig.5. 2 Fungal Tissue A. Prosenchyma; B. Pseudoparenchyma; C. Stroma; D. Part of Stroma E. Sclerotia in Rye grain

Specialized somatic structures

Rhizoids – A rhizoid is a short, root like filamentous branch of the thallus, generally formed in tufts at the base of the thallus. Rhizoids may be present in both, unicellular chytrids (*Rhizophydium*) as well as mycelial (*Rhizopus*) thalli, Rhizoids function as anchoring and absorbing structures.

Rhizomorphs – There are fungi whose hyphae aggregate together, behave as an organized unit to form a root like strand in a thick, hard cortex and develop a growing tip somewhat resembling that of a root tip. Such a structure is known as rhizomorph which mainly serves the function of absorption. The rhizomorph can also survive unfavourable condition and its growth may be renewed with the return of favourable condition.

Appressoria (Sing. appressorium) – It is a terminal simple or lobed swollen mucilaginous structure of germ tubes or infecting hyphae which adheres to the surface of the host or other substratum and helps in the penetration of the infection hyphae. These are formed by some parasitic fungi such as powdery mildews and rust.

Haustoria (Sing. haustorium) – A haustorium is an organ that is developed from a hypha usually performing the function of absorption. They are characteristic of obligate parasites – Uredinales, Erysiphales, and Peronosporales. They are intercellular sac-like, filamentous or branched structure. They vary in shape and may be knob like or button shaped, elongated, finger-like or branched. They secrete some specific enzymes which hydrolyse the protein and carbohydrates of the host plant. (**Fig.5.3)**.

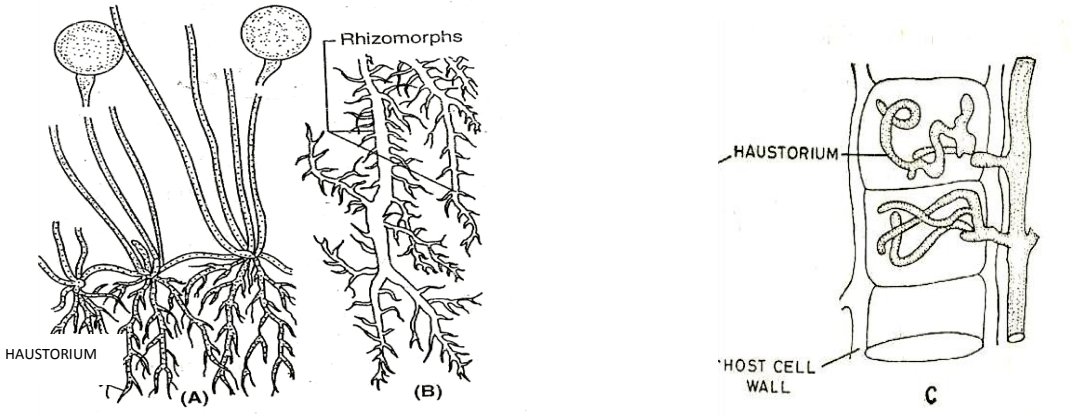


Fig.5.3: A. Rhizoids; B. Rhizomorphs; C. Haustoria

Hyphal traps (Snares) – The predacious fungi develop sticky hyphae or network of hyphal loops known as hyphal traps or Snares. They help in capturing nematodes.

Stromata – These are compact somatic structures much like mattresses. Fructifications are generally formed on or in them.

Mode of nutrition in fungi – Fungi are heterotrophic due to absence of chlorophyll. Hence, they are unable to synthesize carbohydrate food from inorganic materials and get it readymade from other sources external to themselves. They are usually grouped as saprophytes, parasites, obligate parasite, facultative parasite etc.

It is probable that, when we learn more about the physiology of the fungi, we shall be able to devise synthetic media to grow all the so-called obligate parasites. Fungi require already elaborated food in order to live because, by lacking chlorophyll; they are incapable of manufacturing their own food. But if given carbohydrates (glucose or maltose) in some form, most fungi can synthesize their own proteins by utilizing inorganic or organic sources of nitrogen and various mineral elements essential for their growth. (**Fig.5.4**)

Saprophytes – Those fungi which live on dead and decaying organic matter are known as saprophytes. They luxuriantly grow on rotten fruits and vegetables, moist leather, bread, jams, jellies, pickles, wood, dung etc. e.g., *Mucor, Rhizopus, Aspergillus, Penicillium*. Some of these are incapable of growing on living organisms and survive only as saprobes. They are known as obligate saprophytes. But there are some saprophytes which normally live as

saprophytes under favourable environmental conditions and on getting a suitable living organism can parasitize it. Such fungi are known as hemisaprophytes or facultative parasites e.g., *Pythium debaryanum*. Such fungi can easily be grown in an artificial medium.

Parasites – They obtain their food from living hosts. They are quite harmful and may cause many serious diseases in the plants e.g., rusts, smuts, mildews, blights etc. Amongst these, obligate parasites are those which can survive only in the presence of living host and they cannot be cultured on an artificial medium e.g., *Puccinia, Albugo, Peronospora*. There are some fungi which normally lead a parasitic life but under changed circumstances can live as a saprophyte. They are known as **hemiparasites** or **facultative saprophytes** (e.g. *Alternaria* spp.) and can also be grown on saprophytic substrates.

Parasitic fungi obtain their nourishment from the host in various ways. Some parasitic fungi live on the surface of the host and are known as **ectoparasites** (e.g. *Erysiphe*, *Sphaerotheca*). A large number of fungi live inside hosts and are called as **endoparasites** (e.g., *Synchytrium*). The mycelium of fungi like *Albugo* and *Pythium* may be intercellular of intracellular. In the former case the mycelium is confined to the intercellular spaces of the host cells e.g., *Albugo* spp. While in the latter it is found within the host cells e.g., *Pythium* spp. The parasitic fungi produce haustoria for the absorption of food from the host cells. The haustoria may be small, rounded, button-like as found in *Albugo* or much branched as in *Peronospora*.

A special group of parasites is that of the predacious fungi which develop mechanisms for capturing small animals such as eelworms (smaller nematodes lesser than $1/20^{th}$ of an inch), rotifers or protozoa. Predacious fungi use such animals as food. The hyphae produce a large number of small loops which form a network and become sticky due to the presence of viscid fluid. The eelworms which come in contact with such hyphae become motionless. A very fine branch develops from the hyphae and penetrates into the body of the eelworm where it swells and forms an infection bulb which gives rise to numerous branches completely filling the body of the eelworm and absorbs food e.g. *Arthrobotrys oligospora, Dactyllella cionopaga*. There are some fungi which parasitize their own kind and known as mycoparasites e.g. *Piptocephalis* spp. Parasitizing *Mucor* and other related genera in the order Mucorales.

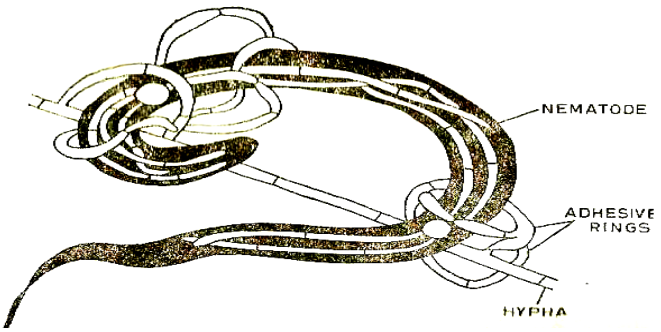


Fig.5.4: Predaceous fungus, *Arthrobotrys oligospora*

Symbionts - Fungi when live in close association with other organisms so as to be mutually beneficial to each other are known as symbionts and the phenomenon is termed as symbiosis.

Some of the common examples of fungal symbiosis are lichens and mycorrhiza. The lichens are formed by the symbiotic association of an alga and a fungus where the alga prepares food and the encamping fungus by its spongy texture retains rain water which is supplied to the alga along with mineral nutrients.

Some fungi live in close association with the roots of higher plants particularly forest trees and produce mycorrhiza which may be of two types: ectotrophic and endotrophic. In the ectotrophic mycorrhiza the mycelium covers the entire surface of the root and may sometimes penetrate it also. The mycorrhizal association increases the surface area of the root system. Some of the fungi which form the ectotrophic mycorrhiza are: *Amanita*, *Boletus*, *Tricholoma* and *Russula*. In host roots, they increase the nutrient uptake ability of the host. Such associations are commonly found within the members of the families Ericaceae and Orchidaceae.

Sometimes certain animals carry fungi (without being damaged by them) and give food and shelter to them. This condition is described as commensalism.

Saprophytic, parasitic fungi absorb their food through the hyphal cell walls, rhizoids and haustoria. The hyphal walls are permeable while the plasma membrane is semipermeable. Many fungi secrete enzymes which dissolve the cell wall and hydrolyse the compounds which become available to the fungus.

Reproduction

In fungi reproduction may take place by two methods; asexual and sexual. During both these processes spores are the essential structures (in mycology the term spore is used for any reproductive unit and is not necessarily the one after meiosis as in higher cryptogams). The spores formed after meiosis are called **meiospores** and those resulting from mitosis, **mitospores**. The ones falling under the category of meiospores are ascospores, basidiospores and sporangiospores of slime moulds and under mitospores, zoospores, aplanospores, conidia, uredospores are included under mitospores.
The diploid body produced as a result of sexual fusion is known as zygote which in lower fungi is termed as resting spore, oospore or zygospore. In higher fungi, the zygote is represented by a diploid nucleus produced in a cell (ascus or basidium). This diploid nucleus after undergoing meiosis results in the formation of haploid nuclei serving as centres for haploid sexual spores called ascospores and basidiospores.

Asexual reproduction – It takes place by following means:-

(a) Fragmentation – The hyphae break into small fragments or pieces accidently or by external force. Each piece upon getting suitable conditions, germinates to form a new mycelium.

(b) Fission – This method involves the splitting of cells into two daughter cells by the formation of a constriction followed by a cell wall formation. It is the most common method of vegetative multiplication found in bacteria and yeasts (**Fig.5.5**).

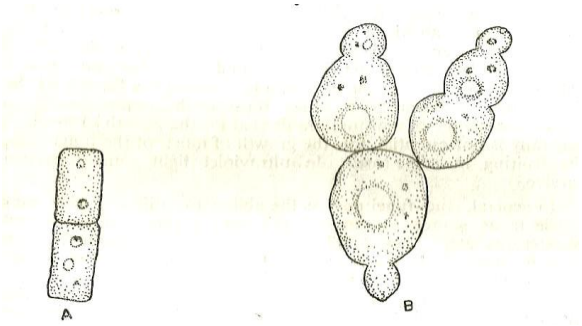


Fig.5.5: Asexual reproduction, A. Transverse cell division (fission) B. Budding

(c)Budding – In this method, a small bud is formed from the parent cell which gradually increases in size and receives a part of nucleus. A cell wall is formed which separates the daughter cell from the parent cell. Each bud after separating from the parent cell develops into a new individual. It is a common method of reproduction in yeasts(**Fig.5)**.

(d)Sclerotia – These are perennating bodies formed by the compact masses of interwoven hyphae. Sclerotia under suitable conditions germinate to form new individuals e.g. *Claviceps*, *Sclerotinia*.

(e)Rhizomorphs – These are root-like elongated mycelial strands. They remain dormant under unfavourable conditions and under favourable conditions develop into a new mycelium.

(All these above mentioned methods of reproduction are also known as methos of vegetative reproduction).

Spores – These may be grouped in two categories: Endogenous and exogenous spores.

Endogenous spores (**Fig.5.6**)**:** These are formed within a sac-like spore producing structure the sporangium which may be terminal or intercalary in position. Sporangia are produced on sporophores which are known as sporangiophores. The sporangiophores may be branched or unbranched. The entire contents or a part of the sporangium is converted into spores known as sporangiospores. The spores may be motile or nonmotile. Motile spores are called zoospores and the nonmotile aplanospores.

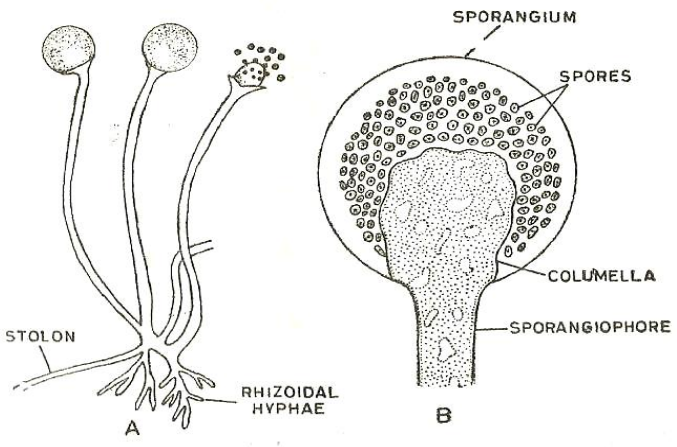


Fig.5.6 Asexual reproduction. A. Endogenous spores of *Rhizopus* B. Sporangium of *Mucor*

Zoospores (Gr. Zoon= animal + sporos =seed, spore) are commonly found in many lower fungi e.g., *Achlya*, *Saprolegnia*, *Pythium*, *Phytophthora*, and *Albugo*. They are naked spores, which after swarming, encyst, secrete a cell wall and germinate by germ tube into a thallus. They are equipped with one or two flagella (sing. Flagellum; L. Flagellum = whip). They are of two types in fungi, the whiplash and tinsel. The whiplash flagellum as the name indicates acts as a 'whip' (commonly used by a horseman) has a rigid basal portion and a short upper flexible region. The tinsel flagellum is a long hairy structure, consisting of a long rachis having hair like structures on all sides. The flagella originate from a granule like structure called **blepharoplast**, which lies deep in the **rhizoplast** (Gr. Rhiza=root+plastid). The flagellum is composed of 11 parallel fibrils, nine peripheral forming a cylinder around two central ones. Each fibril is composed of subfibrils. In a whiplash flagellum, the two central fibrils are longer than peripheral, forming the whip and the bases of the fibrils are doubled up within zoospore forming the blepharoplast. (**Fig.5.7)**

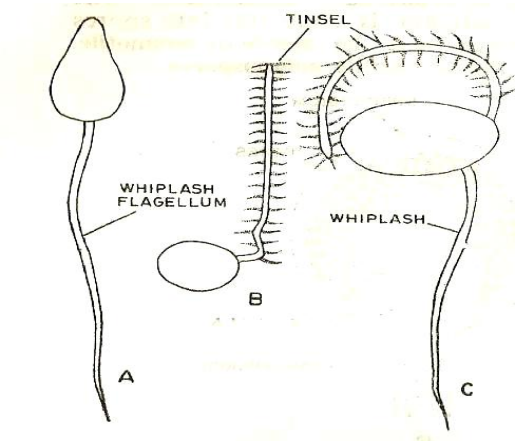


Fig.5.7: Zoospores, A. Posteriorly uniflagellate (whiplash); B. Anteriorly uniflagelate (tinsel); C. Biflagellate zoospores having both whiplash and tinsel type.

On the basis of the flagellation, 4 types of zoospores are differentiated in fungi (1) posteriorly uniflagellate zoospores (2) anteriorly uniflagellate zoospores (3) biflagellate zoospores having tinsel and whiplash attached apically or laterally (4) biflagellate zoospores with two whiplash on the anterior end.(**Fig.5.8**)

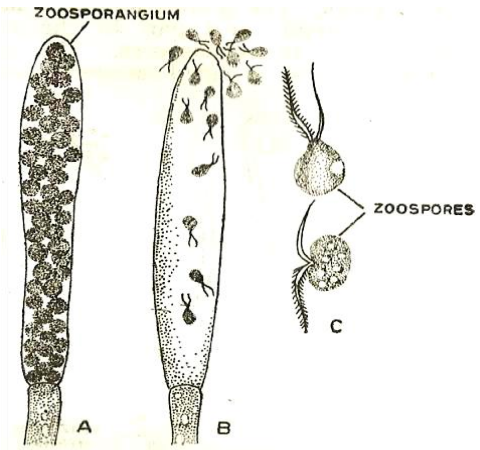


Fig.5.8: A- B. Zoosporangium containing zoospores in the process of liberation. C. Biflagellate zoospores having tinsel and whiplash attached apically or laterally

The aplanospores are nonmotile and lack flagella and are formed inside the sporangium e.g. *Mucor*, *Rhizopus*. These spores may by uninucleate or multinucleate and possess two-layered cell wall.

Exogenous spores

Conidia – All asexual spores, other than sporangiospores (zoospores and aplanospores) are called conidia. They are produced externally on branched or unbranched hyphal tips termed as conidiophores. The conidia may be formed singly or in chains. The conidial chains may be **basipetal** or **acropetal** in succession. Conidia may be uninucleate or multinucleate. The latter type is more common in the members of the form class Deuteromycetes. The shape, size and colour of the conidia vary greatly and hence have been utilised in the identification of many fungi.

There are two main types of conidia (i) thallospores and (ii) conidiospores.

Thallospores are formed by the transformation of existing cells of the thallus and are set free when the parent hyphae decay. These are of two kinds (**Fig.5.9**):

(1) **Arthrospores (oidia) –** They are produced by fragmentation of hyphae from apex to base. Each cell thus formed rounds off and separates as a spore which under favourable circumstances germinates and forms the mycelium.

(2) **Chlamydospores –** They are formed by rounding off and enlargement of terminal or intercalary cells of a hypha. These can be single or formed in chains. They do not separate from the hyphae but remain viable and germinate under favourable conditions.

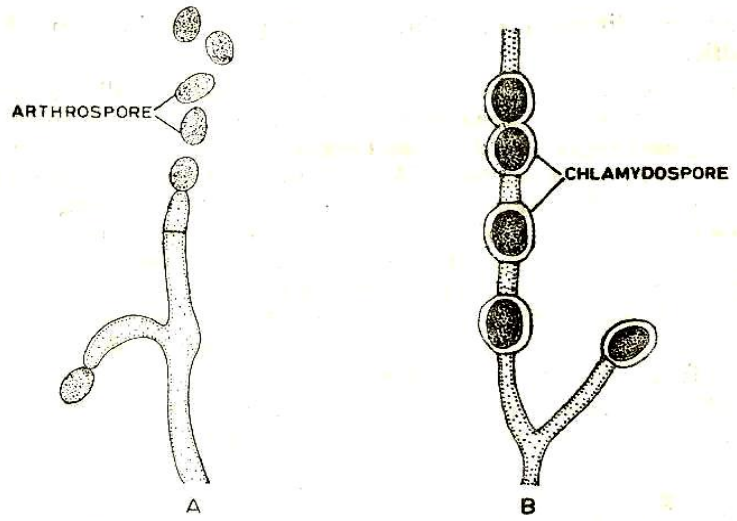


Fig.5.9: Asexual reproduction, A. Hypha fragmentation into oidia also called arthrospores; B. Chlamydospores

The conidiospores or 'true conidia' are formed as new elements from the thallus and on maturity get cut off from the conidiophores. They can be of the following three types:

(1) Blastospores – They are formed as buds from the somatic cells of a hypha or conidiophores.

(2) Aleuriospores – These conidiospores are produced by the inflation of the apex of the conidiophores and later this swollen apex gets cut off by a septum. They are generally formed sympodially (*Trichothecium*, *Arthrobotrys*).

(3) Phialospores – The phialospores are conidia which get cut off from flask-shaped, cylindrical phialides. They are generally cells of limited growth

Sexual reproduction

It involves the fusion of two compatible nuclei. Three distinct phases have been recognised on the basis of nuclear behaviour:

Phase 1. Plasmogamy – It is union of protoplasts which brings together two sexually compatible nuclei in a single cell. This process is known as plasmogamy.

Phase 2. Karyogamy – Fusion of the two compatible nuclei resulting in the formation of a diploid nucleus is known as karyogamy. In majority of the species, plasmogamy is followed by karyogamy but in higher fungi, the fusion is delayed and nuclei lie close to each other without fusion. Such a pair of nuclei is known as dikaryon and the phase termed as dikaryotic phase.

Phase 3. Meiosis – After the nuclear fusion a division takes place which reduces the chromosome number to half. The gametes taking part in the sexual fusion may be morphologically or physiologically different and they are termed as plus ($_+$) and minus (-) strains. When both male and female sex organs occur on the same mycelium, the fungus is known as **monoecious** or homothallic and when they occur on different mycelia, the fungus is said to be **dioecious** or heterothallic.

In the formation of sex organs either entire thallus (holocarpic) or only a part of thallus (eucarpic) is involved. The sex organs of fungi are called gametangia (sing. gametangium) and sex cells produced by them are known as gametes. When the gametangia and gametes are morphologically indistinguishable they are known as isogametangia and isogametes respectively. When male and female gametangia and gametes are morphologically different, they are known as heterogametangia and heterogametes respectively. In heterogametangium the male gametangium is called antheridium and the female as oogonium. Antheridium produces male gametes which are known as antherozoids or sperms and the female forms an egg or oosphere.

Plasmogamy – There are many ways by which the two compatible nuclei are brought together and any of the following methods can be involved in the process:

1. **Planogametic copulation** – In this type of copulation, two naked gametes fuse and if the gametes are motile, they are known as planogametes. In *Synchytrium* the fusing gametes are isogamous while in *Allomyces* spp. they are anisogamous. On careful biochemical and genetical analyses, it has been found that even the isogametes are physiologically and chemically different from each other. In *Monoblepharis*, however, heterogamous planogametic conjugation is found where it has been seen that the male gamete (antherozoid) is motile and the female (oosphere) non-motile and borne in a female gametangium, the oogonium.(**Fig.5.10**)

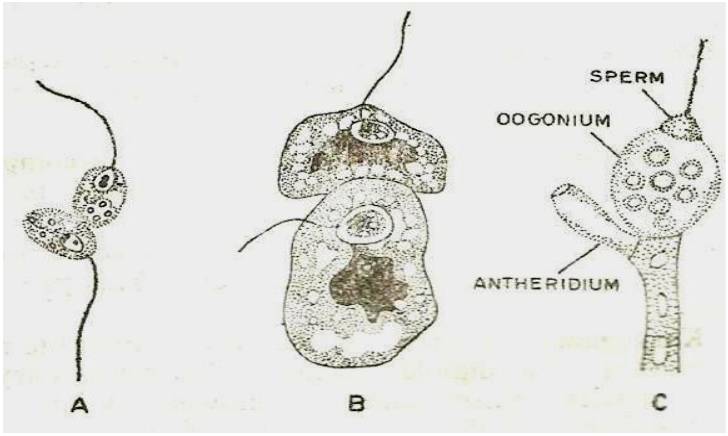


Fig.5.10 Sexual reproduction, A. Isogametes; B. Anisogametes; C. Heterogametes

2. **Gametangial contact –** Another method by which plasmogamy is brought about is the contact of male and female gametangia where both the gametes (male and female)

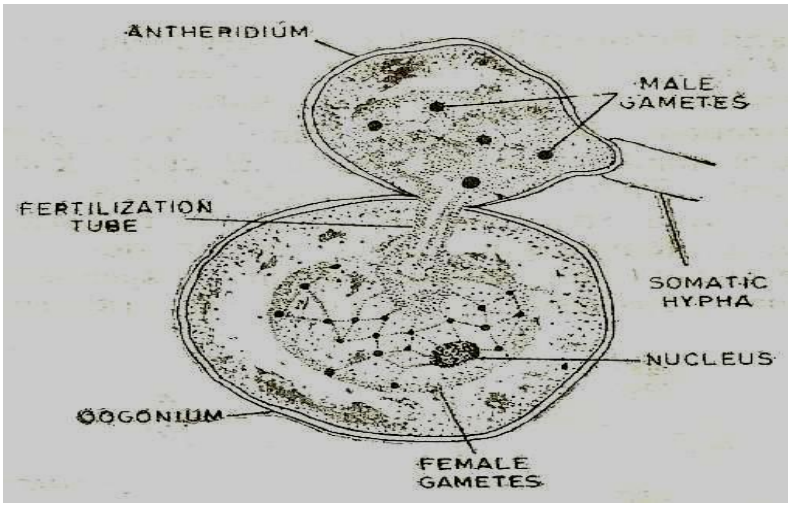


Fig.5.11 Sexual reproduction, Plasmogamy through gametangial contact in Pythium spp.

are non-motile. The nuclei of the male gametangium (the male gamete consists of nuclear material) are transferred into the female when the two gametangia of opposite sex come in close contact with each other. The nuclei from male gametangium migrate to the female one through a pore formed at the point of contact of two gametangia (*Sphaerotheca* spp.) or through a short fertilization tube formed during the process (*Phytophthora* spp.). In the latter case, after the migration of the nuclei, antheridium generally degenerates and the oogonium develops further. Sometimes as in the case of *Pyronema* sp. the contact takes place through a short or a long tubular enlargement the trichogyne formed by the female gametangium. (**Fig.5.11**)

Gametangial copulation is very common amongst lower fungi. Here the process involves the fertilization of oosphere or oospores found within the female gametangium known as oogonium. The process here is referred to as oogamy (in all Oomycetes).

3. **Gametangial copulation –** In this method, the two gametangia fuse entirely. This may take place by two ways, in the first method, the contents of the one gametangium pass into the other through a pore formed in the gametangial wall at the point of contact. In the second method, the two gametangial cells fuse by the dissolution of the wall which after fusion form a single cell as seen in Mucorales and Entomophthorales (higher members of lower fungi) while is some Chytrids (lower fungi) the contents of the smaller (male) gametangium pass into the larger (female) gametangium(Fig.5.12).

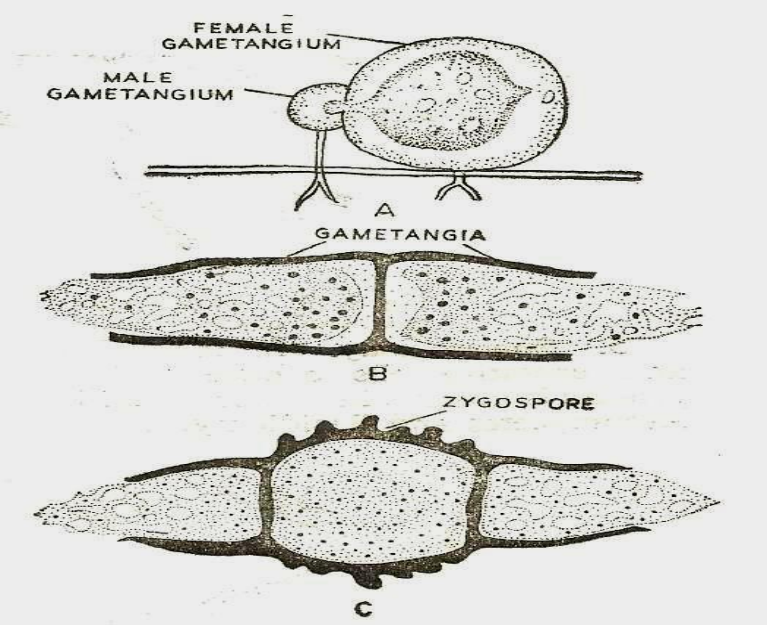


Fig.5.12 Sexual reproduction, Plasmogamy through gamentangial copulation

4. **Spermatization –** Some higher fungi (Ascomycetes adn Basidiomycetes) produce small uninucleate male structures known as spermatia (sing. spermatium). These are disseminated by insects, wind, water or by other agencies to a specialised hyphae, the receptive hyphae which behave as a reduced female gametangia. A pore develops at the point of contact and the nuclei (male) migrate from the spermatium to the receptive hyphae resulting in a binucleate (dikaryotic) cell (**Fig.5.13**).

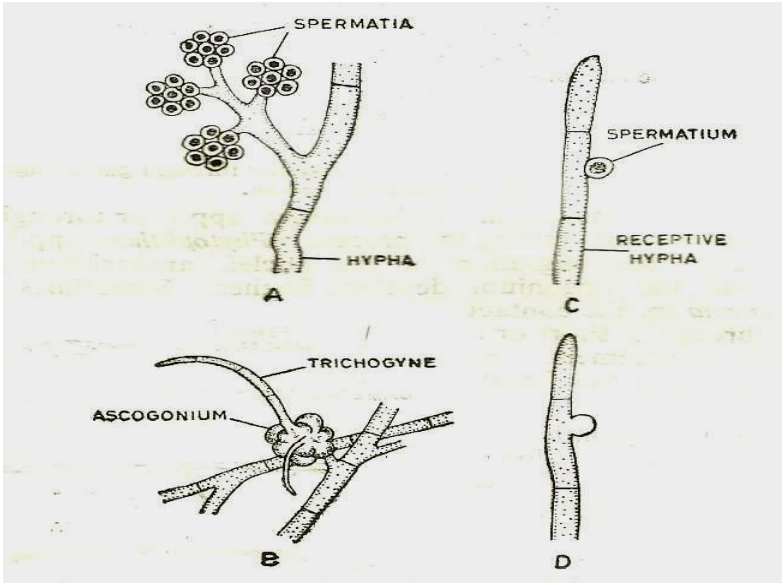


Fig.5.13 Sexual reproduction, Plasmogamy by means of spermatization

5. **Somatogamy –** In many fungi, no normal sex organs are formed and vegetative cells behave as functional gametangia. This process is absent in lower fungi but very common in the members of Ascomycetes and Basidiomycetes.

(i) Karyogamy – This generally occurs immediately after plasmogamy in lower fungi where fusion between two nuclei of opposite sex takes place. This process, however, is delayed in higher fungi where the result of plasmogamy is a dikaryotic cell (the two nuclei of opposite sex lying unfused). Sometimes the dikaryon cell may divide into more dikaryons and each time nuclei replicating the original pair. Sometimes the hyphae having dikaryotic cells form a definite tissue developing into a special layer, the hymenium. These dikaryotic cells after karyogamy develop into specialised cells **asci** in Ascomycetes and **basidia** in Basidiomycetes.

(ii) Meiosis – Karyogamy is followed by a reduction division which is the last important process in the reproduction. During meiosis of the diploid nucleus the chromosomes do not split but separate out as a whole into two complete sets. Each of the sets forms the chromosome complement of a haploid daughter nucleus. In this process some of the chromosomes in each daughter nucleus are derived from one parent and others from the other parent. Subsequently, mitotic divisions take place resulting in and increase in the number of haploid nuclei within the zygote cell. In the case of planogametes undergoing isogamy or anisogamy the resulting zygote is a resting sporangium. This resting sporangium produces zoospores which encyst individually and germinate giving rise to a germ tube. In cases where the resultant zygote is an oospore, it germinates directly giving rise to a germ tube(s) (*Phytophthora* spp.) or it indirectly results in a resting sporangium where in zoospores are produced (*Albugo* spp.). In other cases where a zygospore is formed, it germinates producing a short germ tube with an apical sporangium known as germ sporangium containing aplanospores (*Mucor* spp.).

Nuclear cycle

As in higher plants, generally there is a cycle of haploid and diploid structures, equivalent to gametophyte and sporophyte generations. However, like higher plants there may not be distinct alternation of generations. In majority of fungi, the diploid phase starts after fusion of nuclei (Karyogamy) and may occupy a very small portion of the life cycle as compared to predominantly haploid phase. They are extremely small making their study very difficult. Electron microscopy has revealed that somatic nuclei divide in a manner not directly comparable to mitosis in that no spindles or metaphase plates are formed. The meiotic divisions in fungi are typical are comparable to those of more advanced members. (**Fig.5.14**)

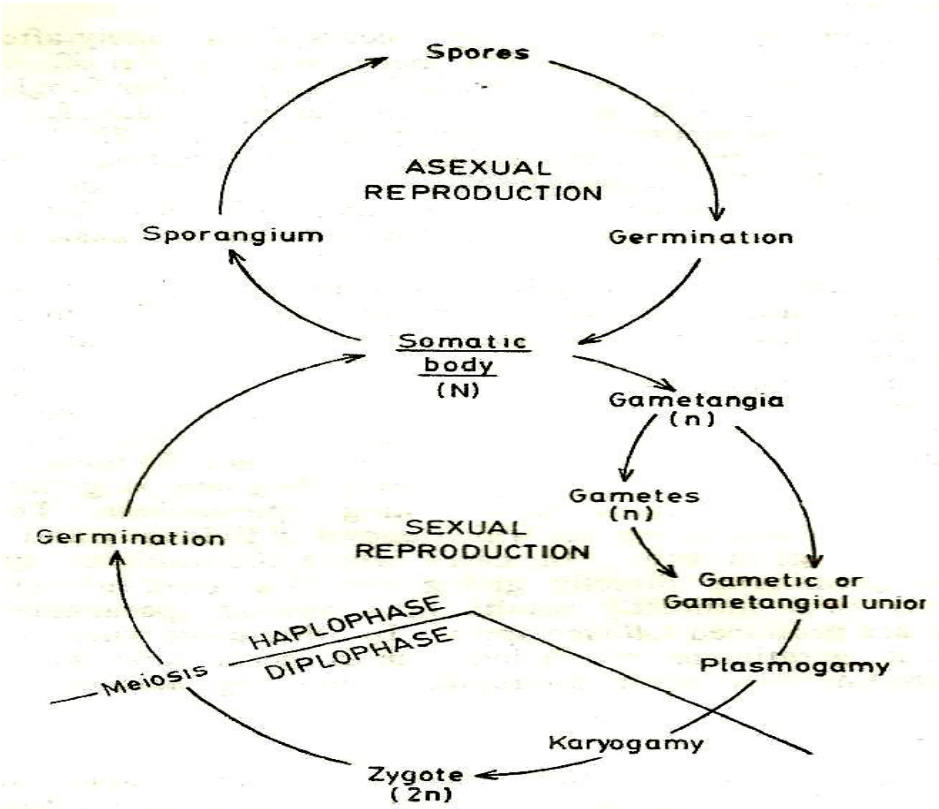


Fig.5.14 A generalised life cycle

5.4 CLASSIFICATION OF FUNGI

Although fungi are placed in the plant kingdom because of similarty in the structure of their thalli and in their reproduction by spores, mycologists now believe that most fungi have originated from some ancestral protozoan like flagellates. Ainsworth treated fungi either as a separate kingdom or as a sub-kingdom belonging to plant kingdom, with two divisions; the Myxomycota for plasmodial forms; and the Eumycota, for non-plasmodial forms which are frequently mycelial. Eumycota was further divided into five subdivisions, including the Ascomycotina (for ascomycetes) and the Basidiomycotina (for basidiomycetes). For convenience, the imperfect fungi were classified as the Deuteromycotina, although in a hierarchial classification it is incorrect to equate these fungi with the asomycetes and

basidiomycetes as they are the fungi which are still in a state of flux. Stable or ideal system is yet to be proposed although several systems of classificcation have been proposed from time to timy by taxonomists. In the present section for the classification of fungi the scheme proposed by Ainsworth (1971), and adopted in The Fungi volume IVA and IVB (Ainsworth, Sparrow and Sussman, 1973) has been given. Following is an outline of scheme of classification of fungi (Ainsworth, 1973).

Kingdom fungi

Key to divisions of fungi

Plasmodium or pseudoplasmodium present.....Myxomycota

Plasmodium or pseudoplasmodium absent,

assimilative phase typically filamentous.....Eumycota

I. Myxomycota

Key to classes of Myxomycota

1. Assimilative phase free-living amoebae
   which unite as a pseudoplasmodium before
   reproduction.....Acrasiomycetes
   Assimilative phase a plasmodium.....
2. Plasmodium forming a network
   (net plasmodium).....Hydromyxomycetes
   Plasmodium not forming a network.....
3. Plasmodium saprobic, free-living.....Myxomycetes
   Plasmodium parasitic within cells of the
   host plant.....Plasmodiophoromycetes

II. Eumycota

Key to subdivisions of Eumycota

1. Motile cells (zoospores) present, perfect
   state spores typically oospores.....Mastigomycotina
   Motile cells absent.....
2. Perfect state absent.....Deuteromycotina
   Perfect state present.....
3. Perfect state spores zygospores.....Zygomycotina
   Zygospores absent.....
4. Perfect state spores ascospores.....ascomycotina

Perfect state spores basidiospores.....Basidiomycotina

III. Mastigomycotina

Key to classes of Mastigomycotina

1. Zoospores posteriorly uniflagellate

   (flagella whiplash type).....chytridiomycetes

   Zoospores not posteriorly uniflagellate.....

2. Zoospores anteriorly uniflagellate
   (flagella tinsel type).....Hyphochytridiomycetes
3. Zoospores biflagellate (posterior flagellum)
   whiplash-type; anterior tinsel type); cell-
   wall cellulosic.....Oomycetes

IV. Zygomycotina

Key to classes of Zygomycotina

1. Saprobic or, if parasiticc or predacious, having
   mycelium immersed in host tissue.....Zygomycetes
2. Associated with arthropods and attached to the
   cuticle or digestive tract by a holdfast and not
   immersed in the host tissue.....Trichomycetes

V. Ascomycotina

Key to classes of Ascomycotina

1. Ascocarp and ascogenous hyphae absent;
   thallus mycelial or yeastlike.....Hemiascomycetes

   Ascocarps and ascogenous hyphae preset;
   thallus mycelial.....
2. Asci bitunicate; ascocarp an ascostroma.....Loculoascoycetes
   Asci typically unituniccate; if bitunicate,
   ascocarp an apothecium.....
3. Asci evanescent, scattered within teh
   astomous ascocarp which is typically
   a cleistothecium, ascospores aseptate.....Plectomycetes
   Asci regularly arranged within teh ascocarps
   as a basal or eripheral layer.....
4. Exoparasites of arthropods, thallus reduced;
   ascocarp a perithecium, asci inoperculate.....Laboulbeniomycetes
   Not exoparasites of arthropods.....

5. Ascocarp typically a perithecium which is usually ostiolate (if astomous, asci not evanescent); asci inoperculate with an apical pore or slit.....Pyrenomycetes
6. Ascocarp an apothecium or a modified apothecium, frequently macrocarpic, epigean or hypogean; asci inoperculate or operculate.....Discomycetes

VI. Basidiomycotina

Key to classes of Basidiomycotina

1. Basidiocarp lacking and replaced by teliospores or chlamydospores (encysted probasidia) grouped in sori or scattered within the host tissue; parasitic on vascular plants.....Teliomycetes
   Basidiocarp usually well- developed; basidia typically organized as a hymenium; saprobic or rarely parasitic.....
2. Basidiocarp typiccally gymnocarpous or semiangiocarpous; basidia phragmobasidia (Phragmobasidiomycetidae) or holobasidia (Holobasidiomycetidae); basidiospores ballistospores.....Hymenomycetes
3. Basidiocarp typically angiocarpous;
4. basidia holobasidia; basidiospores not ballistospores.....Gasteromycetes

VII. Deuteromycotina

Key to classes of Deuteromycotina

1. Budding cells with or without pseudomycelium characteristic; true mycelium lacking or not well developed.....Blastomycetes
   Mycelium well developed, assimilateve budding cells absent.....
2. Mycelium sterile or bearing spores directlly or on special branches (sporophores) not iin pycnidia or acervuli.....Hyphomycetes
3. Spores in pycnidia or acervuli.....Coelomycetes

5.5 GENERAL ACCOUNT OF MAJOR CLASSES OF FUNGI

I. Division – Myxomycota

i. Class – Myxomycetes

These are commonly called the true or plasmodial slime molds and are sometimes also reffered to as acellular slime molds. The trophic phase in these fungi is a free-living, mobile, acellular (coenocytic) multinucleate, saprobic plasmodium. The spores develop in masses with a persistent peridium inside a fructification. On germination, each spores liberates one to four flagellated swarm cells. Asexual reproduction occurs by binary fission. Sexual reproduction takes place by fusion forming zygote that develop into plasmodia.

ii. Class – Plasmodiophoromycetes

These are commonly known as the endoparasitic slime molds, consists of a naked holocarpic plasmodial thallus with plasmodial movement and feeding. Members are obligate parasites of algae, aquatic fungi and higher plants. The somatic phase is plasmodium which develops inside the host cells. The zoospores are bilflagellate with two anteriorly inserted whiplash flagella of unequal size. These zoospores possibly fuse (but remain binucleate) before infecting the host, to form a cystogenous plasmodium, whicch gives reise to thick walled cysts (resing spores). Cyst formation is probably preceded by karyogamy and followed by meiosis.

II. Division – Eumycota

a. Subdivision – Mastigomycotina

i. Class - Chytridiomycetes

The motile cells of these fungi have a single posterior whiplash flagellum. The flagellum is attached to a structure termed blepharoplast within the cell. Sexual reproduction takes place usually by the fusion of motile isogamous gametes or anisogametes; or by the fusion of a nonmotile femate gamete with a otile male gamete; or by the fusion of rhizomycelia ; or by the conjugation of a small and a large thalli , resulting in the formation of a thick walled resting body.

ii. Class – Oomycetes

These are typically aquatic, either free-living or parasitic on algae, water molds, small animals and other forms of aquatic life, although some are terrestrial. The Oomycetes are characterized by:

1. Production of biflagellate zoospores with two types of flagella; the shorter flagellum of the tinsel type directed forward; and the whiplash type directed backwards. The flagella may be anteriorly or laterally inserted on the zoospores; two types of zoospores formed in some species; one pear-shaped and the other reniform;
2. An advanced type of oogamous reproduction takes place ;
3. Meiosis is gamentangial rather than zygotic, and the vegetative thallus is diploid;
4. The life cycle is of the haplobiontic type.

b. Subdivision – Zygomycotina

i. Class – Zygomycetes

This class is represented by the presence of coenocytic hyphae, absence of motile cells and sexual reproduction that occurs by gametangial copulation and results in the formation of a zygospores. Asexual reproduction occurs typically by non-motile sporangiospores.

c. Subdivision – Ascomycotina

i. Class – Hemiascomycetes

(hemi = half + part) The class is characterized by the absence of an ascocarp so that the asci are naked and lack sterile cells. The wall of the asci are generally thin and release the ascospores by bursting. Cells may often remain attached to each other for varying periods of time. These are most primitive among Ascomycotina.

ii. Class – Plectomycetes

These are characterized by the globose evanescent asci arising at different levels from the ascogenous hyphae in a closed fruiting body, the cleistothecium. The asci are typically 8-spored. The wall of the cleistothecium varies from loosely woven to distinct peridium that may be one- to several layers thick. The majority of members are saprobic. However, a few are parasitic on plants, and few on animals, including man. The fungi beloging to this class are of great economic importance.

iii. Class – Pyrenomycetes

These are defined as ascomycetes with ascocarps entirely surrounded by a peridial wall and containing unitunicate asci which are primarily arranged in a hymenial layer. The ascocarps are provided with an opening, the ostiole. The members grow on wide range of habitats. Some are important plant pathogens, while others are fungal symbionts of lichens.

iv. Class – Discomycetes

These ascomycetous fungi produce fructifications with an exposed hymenium, called apothecia (sing. apothecium; Gr. apotheke = store house). Most of these are cup-shaped or even mushroom – shaped. All the Discomycetes eject their spores forcibly except the Tuberales.

d. Subdivision – Basidiomycotina

i. Class – Teliomycetes

This class includes two important gorups of plant pathogens, the rusts and the smuts. The characteristic feature is the production of a thick-walled resting spore, the teliospore in which karyogamy takes place. In teliomycetes a simple pore similar to those of the Ascomycotina and the characteristic septum found in other Basidiomycetes, the dolipore septum, is absent.

ii. Class – Hymenomycetes

This is the largest class of the subdivision Basidiomycotina. It includes the fungi popularly called toadstools, bracket fungi, polypores, mushrooms, fairy clubs, jelly fungi, coral fungi. The characteristic features are the formation of basidia in a hymenium,

gymnocarpous or semiangiocarpous nature of the basidiocarp and the explosive discharge of the basidiospores.

e. Subdivision – Deuteromycotina

i. Class – Hyphomycetes

In these imperfect fungi conidia are formed from aggregated or separated modified hyphae borne on the exterior face of substrates and enclosed by additional fungal or host tissue.

ii. Class – Coelomycetes

These are imperfect fungi in which conidia ae formed within a cavity lined by either fungal tissue, host tissue, or a combination of both. These are mainly the asexual states of Ascomycotina, however, the possibility that some may yet be correlated with the Basidiomycotina cannot be excluded.

5.6 ECONOMIC IMPORTANCE OF FUNGI

The fungi are of great economic importance on account of their both harmful as well as beneficial effects. A large number of fungi cause destructive havoc to our valuable crop and timber plants, various types of food products. They also attack the live-stock as well as human beings. But, all of them are not harmful to the mankind, as most of the species bring about decomposition of dead bodies of plants and animals as well as of animal dung. In addition they are also useful in the production of new age medicines and other useful products.

There are several species of fungi which are of tremendous economic importance. They are beneficial as well as harmful to man.

(a) Beneficial activities of fungi

(i) Edible Fungi – Fungi provide us food that is rich in proteins. Dried yeasts contain about 50 per cent protein. Besides, they are rich in vitamin and B-complex. Mushrooms are generally members of Basidiomycetes. Fruiting bodies of about 105 saprophytic mushrooms are edible; they are preferred for both their taste and food value. Most of the edible fungi are the members of Basidioycetes and Ascomycetes, for example:

Edible fungi of Ascomycetes-

- Saddle fungi – *Helvella* and *Gyromitra*
- Morels – *Morchella* and *Verpa*
- Truffles – Species of *Tuber* and *Cyttaria*

Edible fungi of Basidiomycetes -

- Jew's ear fungi – *Hirneola auriculajudae* adn *Hirneola polytricha*
- Mushrooms – Species of *Agaricus*

- Pore fungi – *Boletus, Strobilomyces* and *Fistulina*
- Teeth fungi – Species of *Hydnum*
- Giant puffball – *Clavatia mexima* and *Lycoperdon* species

(ii) Role of Fungi in Agriculture:

Fungi and nitrogen fixation – Some soil fungi are beneficial to agriculture because, a small amount of atmosphere nitrogen is also fixed by non-symbiotic fungi such as *Rhodotorula* and *Saccharomyces*.

Soil fertility – Some soil fungi maintain the fertility of soil. The saprophytic fungi particularly in acid soils where bacterial activity is at its minimum cause decay and decomposition of dead bodies of plants and their wastes taking up the complex organic compounds (cellulose and lignin) by secreting enzymes. The enzymes convert the fatty, carbohydrate and nitrogenous constituents into simpler compounds such as carbon dioxide, water, ammonia, hydrogen sulphide etc. Some of these return to the soil to form humus and rest to the air from where they can again be used as raw material for food synthesis. Some fungi like *Aspergillus, Cladosporium, Rhizopus, Penicillium*, etc. have soil binding property. This is achieved by the secretion of mucilaginous substances. Some common fungal inhabitants of the soil help to combat diseases caused by soil borne fungi. *Trichoderma lignorum* and *Gliocladium fimbriatum* are found in damp soils. They have an inhibitory effect on the growth of the mycelium of *Pythium*. They serve to suppress fungi causing the damping off disease of seedlings and thereby influence favourable the growth of crops. There are some predacious fungi in the soil. They trap and destroy the nematodes.

(iii) Role of Fungi in Industry:

Baking industry – *Saccharomyces cerevisiae* (yeast) popularly known as baker's yeast is widely used in baking industry. Alcoholic fermentation is the basis of baking industry, because the fermentation of sugar solutions by yeasts produces ethyl alcohol and carbon dioxide. Carbon dioxide is collected, solidified and sold as dry ice. In the baking industry CO2 is the useful product. It serves two purposes: (i) causes the dough to rise, (ii) makes the bread light.

Production of alcoholic beverages – The other by product of fermentation of sugar or malt solution is alcohol. The enzyme zymase present in yeast cells convert hexose sugars into alcohol.

Acid production – Several fungi are helpful in the commercial production of many organic acids, for example, *Aspergillus niger* in citric and oxalic acid, *A. Gallomyces* in gallic acid, *Penicillium purpurogenum* in gluconic acid, Mucor in fumeric acid, *Rhizopus oryzae* in lactic acid.

Enzyme production – Many fungi produce enzymes which have industrial uses, for example, amylase from *Aspergillus*, invertase from *Alternaria* and *Saccharomyces*, and zymase from *Saccharomyces*.

Cheese making – *Penicillium camemberti* adn *P. Roquefortie* are used in cheese making. These moulds add a special flavour to the cheese.

Vitamin extraction – the yeasts are the best sources of vitamin B complex, Vit. B12 is extracted from *Eremothecium ashbyji* and Vit. A from *Rhodotorula gracilis.*

Source of hormones – Gibberellins are plant hormones produced by the fungus *Gibberella fujikoroi* which causes a disease of rice accompanied by abnormal elongation. Gibberellin is used to accelerate growth of several horticultural crops.

(iv) Role of fungi in medicine – At present there are more than 700 fungal species which secrete antifungal and antibacterial substances. These substances are called antibiotics. The first antibiotic penicillin was extracted from *Penicilliium notatum* by Sir Alexander Flemming, for which he was awarded Nobel Prize in 1945. Some important antibiotics and their sources are as follows:

	Antibiotics	Fungal source
I	Streptomycin	*Streptomyces griseus*
II	Penicillin	*Penicillium notatum and P. Chrysogenum*
III	Ramycin	*Mucor rammannianus*
IV	Brefelidin	*Penicillium brefedianum*
V	Fumigallin	*Aspergillus fumigates*
VI	Clavacin	*Calvaria*
VI	Griseofulvin	*Penicillium griseofulvum*
VII	Ergotin	*Claviceps purpurea*

Some of the cholesterol and blood pressure lowering drugs are also obtained from certain fungi, e.g. mevastatin from *penicillium citrinum* and lovastatin from *monnascus ruber*.

(v) Fungi in Biological Research – Use of microorganisms in determining the potency of drugs, detection and estimation of various chemicals in given samples is known as the biological assay. Amongst fungi, *Aspergillus niger* is used to detect very minute quantities of Zn, Ca, Pb, Mn, Cu, etc. in given samples. *Neurospora* is an ideal material for genetic and biochemical studies. It is popularly known as 'Drosophila of Plant Kingdom', because of its suitability in the studies of biological sciences.

(b) Harmful activities of fungi

(i) Plant diseases – Fungi have a negative value because they are the causative agents of different diseases of our crop, fruit and other economic plants. These fungal diseases take a heavy toll and cause tremendous economic losses. Some important plant diseases and their causative agents are given in table.

Table:1- Some Important Plant Diseases

	Name of disease	Fungus
1.	Early blight of potato	*Alternaria solani*
2.	Late blight of potato	*Phytophthora infestans*
3.	Loose smut of oat	*Ustilago avenae*
4.	Brown leaf spot of rice	*Helminthosporium oryzae*
5.	Black or stem rust of wheat	*Puccinia graminis tritici*
6.	Loose smut of wheat	*Ustilago nuda tritici*
7.	Powdery mildew of wheat	*Erysiphe graminicola*
8.	Ergot of bajara	*Claviceps microcephala*
9.	Loose smut of barley	*Ustilago nuda hordei*
10.	White rust of crucifers	*Albugo candida*
11.	Green ear disease of bajara	*Sclerospora graminicola*
12.	Tikka disease of groundnut	*Cercospora arachidicola*

(ii) Deterioration of food and other articles – Saprophytic fungi for example *Rhizopus*, *Mucor*, *Aspergillus* grow on food articles such as bread, jam, pickles, and make them inedible. They also destroy leather articles. Tubber, wool and painted surfaces are also get damaged by species of *Aspergillus*, *Penicillium*, *Alternaria* and *Rhizopus*.

(iii) Decay of wood – In India the commercial timber yielding plants such as sal, teak, sisam are destroyed by *Polyporus*, *Ganoderma* etc. These fungi secrete cellulose and lignin decomposing enzymes and cause 'heart rot'.

(iv)Fungal toxins – Mushrooms like *Amanita phalloides*, *A. Virosa* are poisonous. Poisoning of these mushrooms causes abdominal pains with vomiting, sweats, diarrhoea etc. *Claviceps purpurea*, a parasitic fungus contains a powerful poison and causes gangrenes. LSD (Lysergic acid diethylamide), a hallucinogenic and hypnotic compound, is also obtained from *Claviceps*. Besides this, some fungi secrete a group of toxic/carcinogenic compound called aflatoxins.

(v) Human and animal diseases – Some species of *Aspergillus* such as *A. fumigates*, *A. flavus* and *A. niger* are human pathogens. They cause disease collectively known as aspergilloses. The symptoms of this disease are similar to tuberculosis. Many parasitic fungus Imperfecti live in the mucous membranes of throat, bronchi and lungs and cause infection of mouth and lungs. A few fungi cause serious diseases of domestic animals. Some fungal disease of humans are given in table.

Table :2- Some Fungal Diseases of Humans

	Disease	Pathogen
1.	Ringworm	*Microsporon ianosum*
2.	Dobhi-itch	*Epidermophyton floceosum*
3.	Candidiasis	*Candida albicans*
4.	Athelete foot	*Trichophyton interdigitate*
5.	Blastomycosis	*Blastomyces dertimidis*
6.	Aspergilliosis	*Aspergillus flavus, A. Fumigates, A. Niger*
7.	Penicillosis	*Penicillium* sp.

5.7 SUMMARY

This chapter has highlighted the physiological biodiversity of fungi in terms of morphology, growth, metabolism and cell reproduction. Understanding the diverse vegetative as well as reproductive structure produced by different fungi and the ways in which fungi interact with their growth environment is crucial for the identification and their management for human welfare.

While classification is very important for systematic study of any organism, there are different views/criteria used by different scientists in classifying fungi. This leads to many useful ways of grouping them, with the groups created by one definition of similarity different to those created by another. Stable or ideal system for fungal classification is yet to be proposed although several systems of classificcation have been proposed from time to timy by taxonomists. In this unit, general key characteristics at class level is given to look at some aspects of fungal classification and identification so as to get an idea of how mycologists have gone about studying fungi over the past two century.

Fungi have been utilized for thousands of years for the production of various foods and beverages. While these applications are still important, fungi are now being used in novel ways for the production of antibiotics and enzymes. It is very obvious that fungi are economically very important and continued use of fungi on large scale by humans is guaranteed.

5.8 GLOSSARY

Acervulus (pl. Acervuli) - a mat of hyphae giving rise to short conidiophores closely packed together forming a bed-like mass.

Acropetal - refers to spore formation where the most recently formed spore is at the tip of a chain of spores. Usually seen as being smaller than the immediate neighbour. Note that a continuous cytoplasmic link exists from base to tip.

Acervulus (pl. Acervuli) - a mat of hyphae giving rise to short conidiophores closely packed together forming a bed-like mass.

Apical - at the apex or end.
Apothecium - cup shaped ascocarp. Hymenium is exposed at maturity.
Appressorium - a swelling on a germ tube or hyphae, often terminal, which may attach to the surface, especially in development of colonisation of a cell.
Ascocarp - a fruiting body containing one or more asci.
Ascomycota - one of the four divisions within the Fungal Kingdom. Contains three classes, Laboulbeniomycetes, Protoascomycetes, Euascomycetes. Characterised by the formation of ascospores.
Ascospore - a sexual spore formed following meiosis in an ascus.
Ascus (asci) - a sac-like cell generally containing a definite number of ascospores, typically 8, formed after karyogamy and meiosis.
Asexual - mitotic reproduction, not involving fusion of nuclei and meiosis, offspring the same as the single parent.
Basidiocarp - a fruiting body that bears basidia.
Basidiospore - a sexual spore formed following meiosis, borne on a basidium.
Basidium (basidia) - a structure bearing on its surface a definite number of basidiospores (usually four) that are formed following karyogamy and meiosis.
Chitin - principle microfibrillar component of cell walls of fungi, composed of (1 - 4) linked polymer of N-acetyl-glucosamine.
Clamp connection - a bridge-like hyphal connection between two adjacent cells, found only in some Basidiomycota.
Class - fungal divisions are divided into classes. Classes are divided into orders.
Columella (pl. columellae) - a sterile extension of the stalk into the sporangium of some zygomycetes.
Conidiophore - a simple or branched hyphae arising from somatic hyphae which bears at its tip or sides, cells which form or become conidia.
Conidium (pl. conidia or conidiospore) - a nonmotile asexual spore formed on a conidiophore, formed from or as an extension of the hyphal walls. May be single or multicelled, simple or complex, round, elongated or spiral in shape. Found only in the Ascomycota or Basidiomycota.
Dikaryon - a hypha or portion of hyphae which contains two haploid nuclei in each cell.
Dolipore - a central pore in a septum surrounded by a barrel shaped swelling of the septal wall. Common in the Basidiomycetes.
Ectomycorrhiza - type of mycorrhiza in which fungal hyphae grow around the root and between cells of the epidermis.
Endophyte - general term to describe a fungus which lives within healthy plant tissue. May specifically refer to Balansioid fungi colonising grass leaves.
Flagellum (pl. flagella) - fine long thread projecting from a cell having a lashing or undulating motion which enables the cell to move when in water. Two types discussed in the fungi, true fungi may have a whiplash flagellum and organisms now placed in the Chromista may have whiplash or tinsel flagella.
Haustorium - a specialised hyphal invagination of plant cells.
Heterokaryotic - a mycelium which contains genetically different mating types.

Heterothallic - a fungus which requires two different mating types to form sexual fruiting bodies.
Holdfast - projection from the thallus which attaches the thallus to a surface, may be called an appressorium in the higher fungi.
Holocarpic - all the thallus is used for the fruit body.
Hyaline – colourless.
Imperfect stage - the asexual stage of a life cycle.
Karyogamy - fusion of two nuclei.
Mycelium - mass of hyphae constituting the body of the thallus or fungus.
Mycorrhiza - mutually beneficial association between plant root and fungus.
Perithecium - a closed ascocarp with a true wall and an ostiolate opening.
Pycnidium - an asexual fruiting body that is hollow, and partially lined inside with conidiophores.
Rhizomorph - a thick strand of organised hyphae resembling a fine root.
Saprophyte - organism which obtains its organic nutrients in solution from dead or dying tissues of any other organism.
Sclerotium (sclerotia) - a hard, aggregation of hyphae which functions as a resting body. In extreme cases, the body is surrounded by melanised hyphae forming a skin, in other cases, the body tends to be diffuse.
Sporangiolum (Sporangiola) - small sporangium containing a few sporangiospores.
Sterigma - pointed projection on the outer surface of basidia from which basidiospores emerge and are dispersed.
Stroma (pl. stromata) - a compact, matress like somatic structure in or on which reproductive structures form.
Zoospore - motile naked spore formed within a sporangium.
Zygospore - sexual spore resulting from the conjugation of gametes, found within a zygosporangium, contains a diploid nucleus.
Zygote - cell in which two nuclei of opposite mating type have fused.

5.9 SELF ASSESSMENT QUESTIONS

5.9.1 Multiple choice Questions:

1. Columella is a specialized structure found in the sporangium of:

(i) Ulothrix (ii) Spirogyra
(iii) Rhizopus (iv) none of these.

2. A lichen can be described as a mutualistic relationship between an ascomycete and a(n):

(i) angiosperm root (ii) chytrid
(iii) archeabacterium (iv) green alga

3. Mycorrhiza exhibits the phenomenon of:

(i) symbiosis (ii) Parasitism
(iii) antagonism (iv) endemism

4. The black rust of wheat is a fungal disease caused by:
(i) Albugo Candida (ii) Puccinia graminis tritici
(iii) Melampsora lini (iv) Claviceps purpurea

5. A mycelium is:
(i) a mutualistic relationship between a fungus and a plant.
(ii) a mass of connected fungal hyphae.
(iii) a partition between the cells of fungal hyphae.
(iv) a specialized reproductive structure of a fungus

6. Which of the following is not a characteristic of the fungi?
(i) Mitosis takes place within the nuclear membrane
(ii) They have cell walls made of chitin.
(iii) They are all absorptive heterotrophs.
(iv) They are all motile

7. Absorptive heterotrophic nutrition is exhibited by:
(i) bryophytes (ii) fungi
(iii) pteridophytes (iv) Algae

8. Mycorrhiza is correctly described as:
(i) symbiosis of algae and fungi
(ii) symbiotic relationship between fungi and roots of some higher plants
(iii) parasitic association between roots and some fungi
(iv) relation of ants with the stem of some trees.

9. Puccinia forms uredia and:
(i) telia on wheat leaves (ii) pycnia on barberry leaves
(iii) aecia on barberry leaves (iv) aecia on wheat leaves.

10. An ascomycete can be distinguished from other fungi:
(i) by the presence of gills on the mycelium.
(ii) by the presence of eight sexual spores in an ascus.
(iii) because ascomycetes lack a dikaryotic phase.
(iv) because ascomycetes are mainly diploid.

11. A basidium is typically observed in the common:
(i) bread mold (ii) gilled mushroom.
(iii) lichen (iv) chytrid

12. A wildlife pathologist is examining some skin tissue from a dead frog. She observes the presence of a fungus. She cultures some of the fungal cells and notices that some of the cells are flagellated. She concludes that the frog has a fungal disease caused by:

(i) a zygomycete (ii) a basdiomycete
(iii) an ascomycete (iv) a chytrid

13. How many species of fungi are thought to exist?

(i) 1.5 billion (ii) 150,000
(iii) 1500 (iv) 1.5 million

14. Organisms which are indicator of SO2 pollution of air:

(i) mosses (ii) mushrooms
(iii) lichens (iv) puffballs

15. Most of the lichens consist of:

(i) brown algae and higher plant (ii) blue green algae and basidiomycetes
(iii) green algae and ascomycetes (iv) red algae and ascomycetes

16. Lichens indicate SO2 pollution because they:

(i) grow faster than others
(ii) are sensitive to SO2
(iii) show association between algae and fungi
(iv) flourish in SO2 rich environment

17. Black rust of wheat is caused by:

(i) Ustilago (ii) Puccinia
(iii) Albugo (iv) Phytophthora

18. You are walking in the woods and see a fungus that is unfamiliar to you. You remove a reproductive structure, and take it home to examine further. When you look at it under the microscope, you find a zygosporangium. Based on this information alone, this fungus is an:

(i) zygomycete (ii) chytrid
(iii) ascomycete (iv) basidiomycete

19. Which one of the following statement about lichens is wrong?

(i) these grow very rapidly (2 cm per day)
(ii) some of its species are eaten by reindeers
(iii) they show fungal and algal symbiotic relationships
(iv) these are pollution indicators

20. Adhesive pad of fungi penetrate the host with the help of:

(i) mechanical pressure and enzymes (ii) hooks and suckers
(iii) only by mechanical pressure (iv) softening by enzymes

21. Which pair of the following belongs to basidiomycetes?

(i) Peziza and stink horns (ii) Puffballs and Claviceps
(iii) Morchella and mushrooms (iv) Birds nest fungi and puffballs

22. In Fungi food materials are digested:

(i) within food vacuole (ii) Outside the body
(iii) By Lysosomes (iv) By Mitochondria

23. Fungi penetrate their host by:

(i) Mechanical means (ii) Enzymatic action
(iii) Mechanical and enzymatic action (iv) By hook and suckers

24. In Penicillium, the hyphae are:

(i) Septate and coenocytic (ii) Septate and uninucleate
(iii) Septate and multiinucleate (iv) None of these

25. The walls of fungi are composed of:

(i) Starch (ii) Cuttin
(iii) Cellulose (iv) Chitin

26. Fungi that use in baking industry respire?

(i) Aerobically (ii) Anaerobically
(iii) Both (i) and (ii) (iv) None of these

27. Which fungal disease spreads by seed and flowers?

(i) soft rot of potato (ii) covered smut of barley
(iii) loose smut of wheat (iv) corn smut

28. Coenocytic hyphae are:

(i) Septate with heavy cytoplasm
(ii) Uninucleated with common cytoplasm
(iii) Multinucleated with separated cytoplasm
(iv) Aseptate, multinucleate with common cytoplasm

29. Which of the following secretes toxins during storage conditions of crop plants?

(i) Colletotrichum (ii) Aspergillus
(iii) Albugo (iv) All of these

30. A loosly woven fungal tissue is called:

(i) Prosenchyma (ii) Pseudoparenchyma
(iii) Stroma (iv) Plectenchyma

5.9.1 Answers key:

01. (iii)	**07. (ii)**	**13. (iv)**	**19. (i)**	**25. (iv)**
02. (iv)	**08. (ii)**	**14. (iii)**	**20. (i)**	**26. (ii)**
03. (i)	**09. (i)**	**15. (iii)**	**21. (iv)**	**27. (iii)**
04. (ii)	**10. (ii)**	**16. (ii)**	**22. (ii)**	**28. (iv)**
05. (ii)	**11. (ii)**	**17. (ii)**	**23. (iii)**	**29. (ii)**
06. (iv)	**12. (iv)**	**18. (i)**	**24. (ii)**	**30. (i)**

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5.11 SUGGESTED READING

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5.12 TERMINAL QUESTIONS

5.12.1: Long answer type questions:

Q1 Describe range of thallus structure and nutrition in fungi.

Q2 Describe distribution and mode of nutrition in fungi as a whole.

Q3 Describe various methods of asexual reproduction in fungi.

Q4 What is mycelium? Give an account of different types of mycelia met within the fungi.

Q5 Give a concise account of sexual reproduction in the fungi.

Q6 Describe distinguishing features of each class of fungi.

Q7 Give general characters and classification of fungi.

Q8 Write an essay on economic importance of fungi.

Q 9 Explain the beneficial and harmful roles of fungi.

5.12.2: Short answer type questions:

Q 1 Write mode of nutrition in fungi.

Q2 White notes on-

(i) Gametangial contact
(ii) Spermatization
(iii)Gametangial Coupulation
(iv) Any five fungal plant disease and their symptoms
(v) Edible fungi
(vi) Role of fungi in industry
(vii)Cell wall and structure of flagella in fungi

UNIT- 6- GENERAL ACCOUNT, HABIT, STRUCTURE AND METHODS OF REPRODUCTION IN MASTIGOMYCOTINA, ZYGOMYCOTINA, ASCOMYCOTINA

6.1 OBJECTIVES

After reading this unit student will be able:

- To know the distinguishing features, general account of Mastigomycotina, Zygomycotina and Ascomycotina
- To study habit, structure , methods of reproduction and life cycle in the representative genera of Mastigomycotina, Zygomycotina and Ascomycotina
- To study the symptoms of disease caused by species of *Albugo, Mucor* and *Erysiphe* and their economic importance.

6.2 INTRODUCTION

In Whittakar's five kingdom system of classification (1969), fungi have been placed under advanced kingdom (among the five kingdom two are advanced and three are primitive kingdoms). Earlier in 1937 Gwune- Vaughan and Barnes kept all the three (Mastigomycotina, Zygomycotina and Ascomycotina) as a class in sub- division named Fungi. This is because at that time fungi did not get the status of a kingdom. In 1962, Alexopoulos kept all these three i.e. Mastigomycotina, Zygomycotina and Ascomycotina under subdivision Eumycotina. At that time, Mastigomycotina was not known because fungi of this sub-division were studied in separate classes like Chytridiomycetes, Hyphochytridiomycetes, Oomycetes and Plasmodiophoromycetes of Eucomycotina. However Zygomycotina and Ascomycotina were known as a classes (zygomycetes and Ascomycetes).

Another system of classification which came after Whittaker's five kingdom system of classification of 1969 is that of Ainsworth, Sparrow and Susman (1973). In his system of classification Mastigomycotina, Zygomycotina and Ascomycotina got the status of separate sub-divisions.

His classification was based on the presence/absence of motile cells in the life cycle and also methods of plasmogamy and formation of fertilized diploid structure.

Summary of status of Mastigomycotina, Zygomycotina and Ascomycotina

Gwyne- Vaughan and Barnes (1937)	Alexopoulos(1962)	Ainsworth, Sparrow and Susman(1973)
Kingdom : Plantae	Kingdom : Plantae	Kingdom: Fungi
Division: Thallophyta		Division : Myxomycota
Sub-division : Fungi	Division: Mycota	Division ; Eumycota*

Mastigomycotina, Zygomycotina and Ascomycotina as sub-divisions.

6.3 MASTIGOMYCOTINA- *Albugo*

6.3.1 – General account

The sub-division Mastigomycotina includes fungi which have been classified in one of the division (i.e. Eumycota) of kingdom fungi. The main features of these fungi are:

- Presence of definite cell wall in their somatic structures (a few exceptions)
- Absorptive mode of nutrition.
- This sub-division has been divided into classes like Chytridiomycetes, Hyphochytridiomycetes, Oomycetes etc. on the basis of type of flagella, their number and place of attachment in the motile cells.

Now, the class Oomycetes to which *Albugo* belongs has been divided into four orders i.e. Saprolegniales, Leptomytales, Lagenidiales and Peronosporales. So the systematic position of *Albugo* is:

Fungi
Eumycota
Mastigomycotina
Oomycetes
Peronosporales
Albuginaceae
Albugo

- This is the only genus of the family and its species cause a disease called White Rust Disease, in plants.
- The genus earlier named as *Cystopus.*

6.3.2 – Habit and Habitat

The species of this genus are all obligate parasites on vascular plants e.g.

Albugo candida on plant of Crusiferae
A. bliti on plants of Amranthaceae
A. evolvulii on Evolvulus
A. ipomoeae-panduranae on sweet potato
A. portulacae on Portulaca

You may recognize the genus *Albugo* in nature by observing the symptoms on host plants. The symptoms are:

a) White / creamy small, shining, irregular, raised **pustules/ blisters** on leaves **/ stems of host plants (Fig.6.1).**

Fig.6.1 Infected leaf

b) Deformed inflorescence of host plants i.e. hypertrophied inflorescences. This is because of increase in the size of cells of host plants due to infection (**Fig.6.2**).

Fig.6.2 Hypertrophied inflorescence

6.3.3 – Structure

- The thallus of *Albugo* consists of mycelium which is made up of well developed, branched, intercellular and **coenocytic** hyphae. Nuclei are diploid.
- Hyphal cell walls are made up of cellulose, multinucleate and filled with vacuolated granular protoplasm. Because the mycelium is intercellular it takes nutrition from host cells after penetrating their cell walls through minute perforations, and expanding on the inside of the cells by globose or knob like structure, the **haustorium**.

6.3.4 – Reproduction

Albugo reproduces by asexual and sexual methods of reproduction.

Asexual reproduction in *Albugo* takes place by conidia/sporangia.

- **Asexual reproduction** starts when the hyphae have attained certain stage of maturity. At this time, the hyphae aggregate or collect at certain places just below the epidermis of host and produce-
  - Short
  - Thick walled, thin at the tips
  - Club shaped
  - At right angles to the epidermis of host
  - Closely packed vertical structures called **conidiophores / sporangiophores**. These are in a solid layers / beds.
  - When sporangiophores / conidiophores reach at the certain stage of maturity they begin to cut-off conidia / sporangia at their tips in the basipetal succession on below the other i.e. in chain. There is a gelatinous disc (disjunctor cell) between two conidia / sporangia.

- As conidia / sporangia mature they become detached and are free in the space between the conidiophores / sporangiophores below the epidermis of host.
- After the formation of sporangiophores / conidiophores and conidia in large numbers below the epidermis of host, a pressure is created towards the epidermis. This results in the bulging and ultimately bursting of the epidermis over the growing sorus. Upon bursting of the epidermis conidia / sporangia are released and form a white crust over the surface of host. Conidia / sporangia when produced are globose in shape. Later on, these become cuboid or polyhedral due to pressure during their formation. These are thin walled and multinucleate and are disseminated by wind, water and any other agency. A large number of conidia / sporangia die out.

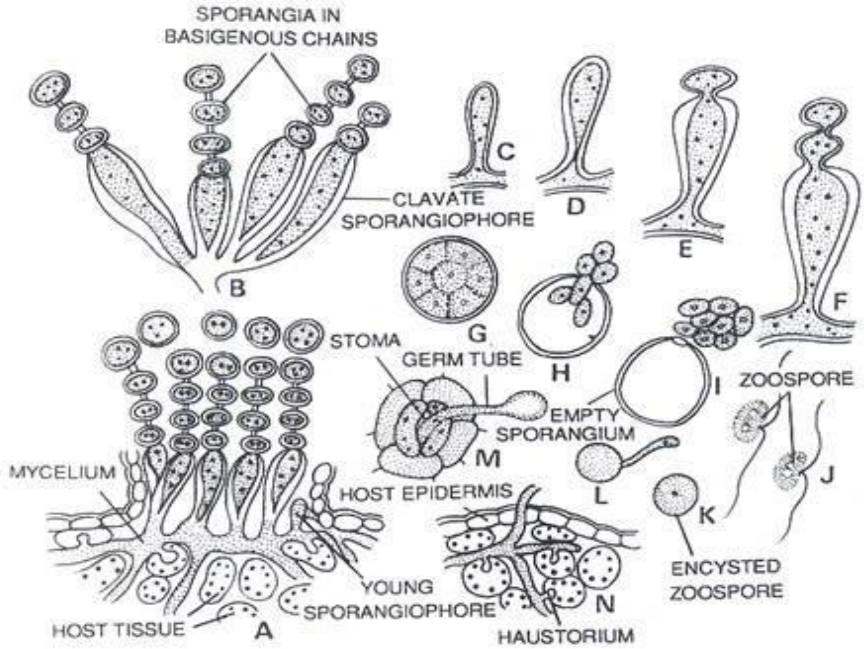

Fig.6.3 Asexual Reproduction in *Albugo candida*

There are the two methods of germination of conidia/sporangia:

1. Direct- when temperature is high
2. Indirect- when temperature is low.
    1. **Direct germination** is by the germ tube, the asexual spores are called conidia.
    2. **Indirect germination** Takes place by zoospores (showing in **Fig.6.3 G-N**). Here the content of asexual spores which are sporangia divides into uninucleate parts. Each such part gets transformed into uninucleate, biflagellate kidney shaped zoospores. A papilla develops at the end of the zoosporangium. This papilla gets transformed into sessile vesicle**.**

       Zoospores escape / come out into this vesicle from the zoosporangium. They swim/move in this vesicle for a short period then lose their flagella, encyst (change their shape) and germinate by germ tube.
3. **Sexual method** of reproduction is oogamous which after plasmogamy, karyogamy and meiosis results in the formation of a resting spore called oospore. Meiosis takes

place at the time of development of antheridia and oogonia. In all species sexual reproduction is similar but the cytological details of fertilization probably fall into three patterns which are as follows:

- e.g. *A. bliti, A. protulacae* etc there are many functional nuclei, a large receptive papilla and a small coenocentrum.
- e.g. *A trapogonosis* all nuclei except a single male and a single female nucleus degenerate after migration into periplasm. There is a small papilla and a large coenocentrum.
- e.g. *A. candida* a pair of functional nuclei fuses after disintegration of all other nuclei. The size of papilla and coenocentrum is intermediate between those of the other two types.

Following is the method of sexual reproduction based on A. *candida:*

Gametangia (reproductive structures) are formed when the host is matured or at the end of the growing season of the host. This is manifested by hypertrophied or deformed inflorescence of host. Gametangia are antheridia and oogonia, the male and female respectively. Both develop close to each other and are multinucleate, one celled structures.

Anthredium is club shaped and situated to the side of oogonia i.e. in paragynous condition and is cut off from the rest of hyphae by a septum.

Oogonium terminal in position (rarely intercalary), and globose in shape. It is also cut off from the rest of the hyphae by a septum. The nuclei divide by mitotic division. When mature, the oogonium contains a periplasm and a ooplasm. All the nuclei, except one which is the only functional nucleus migrate into periplasm and gradually disintegrate. The one functional nucleus in the ooplasm is called egg or oosphere. Fertilization is paragynous with the help of a specialized structure called fertilization tube. The fertilization tube grows from the anthredium, which gets laterally attached to the wall of oogonium and at the point of attachment a swelling , called receptive papilla , develops over the surface of oogonium . By piercing the wall of the receptive papilla the fertilization tube enters into the oogonium and releases only one anthredial nucleus/male nucleus. Now the male nucleus fuses with the egg or oosphere making it diploid oospore (2n). Soon after the fertilization the coenocentrum is lost.(**Fig. 6.4 A, B, C**)

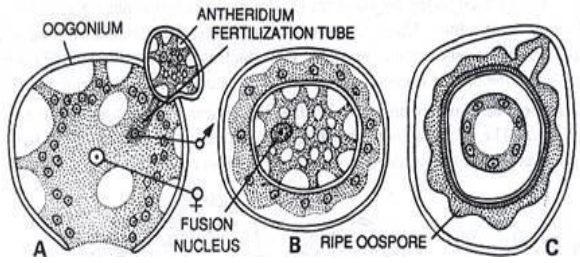

Fig.6. 4 ***Albugo candida*****. A, oogonium and anthredium with fertilization tube; B, the oospore wall has begun to form, the fusion of nucleus; C, the mature oospore**

Structure of oospore is globose in shape. It develops thick a wall which is ornamented and is called exospore. Inner wall is thin and is called endospore. Oospores are released after the disintegration of host tissue. The oospore enters a resulting stage. After resting period, and under favourable conditions, the nuclei resume mitotic activity , and the protoplast of the oospore divides into a large number of uninucleate sections, each of which develops into a bifllagelate, reniform zoospore. Zoospores are liberated, after cracking of outer thick exospores, in a thin vesicle which is the expansion of thin endospore. The vesicle after some time ruptures and then the zoospores swim for some time, then they discard their flagella, change their shape and encyst and germinate by forming a germ tube (**Fig. 6.5**). Thus a diploid mycelium is developed.

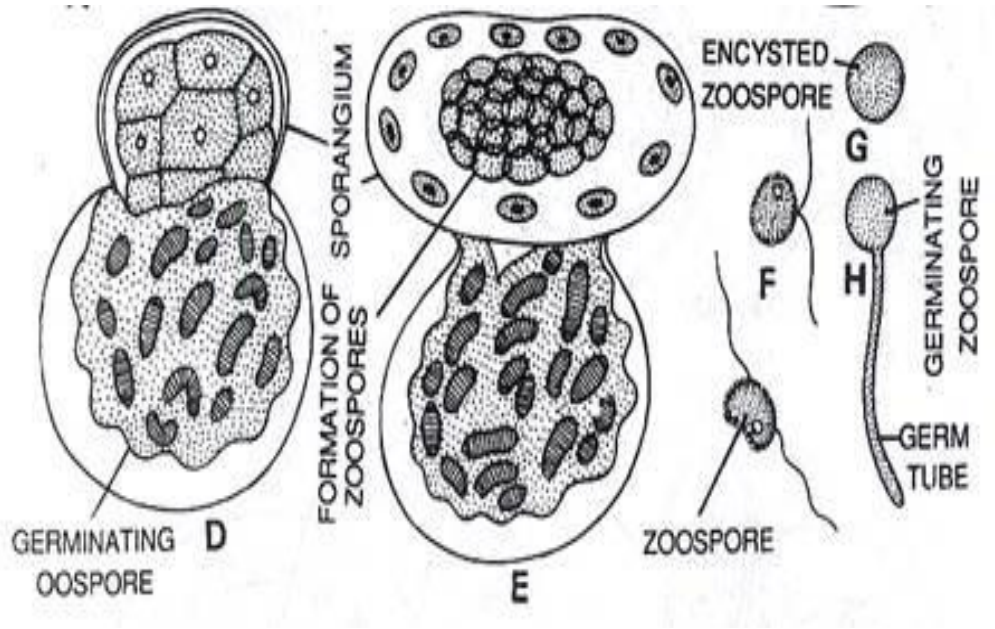

Fig. 6.5 *Albugo candida* D&E, showing germinating oospore and formation of zoospores; F, Biflagillate kidney shaped zoospore; G, Encysted zoospore; H, Germinating zoospore.

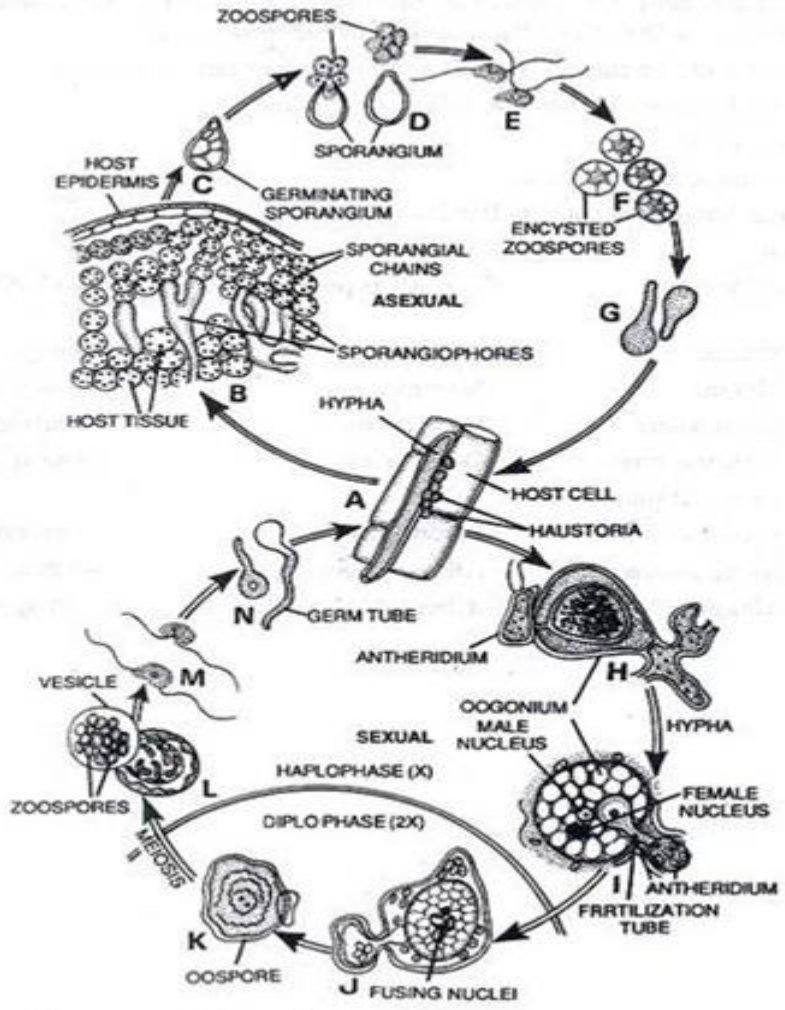

Fig.6.6. Albugo *candida* Diagrammatic Life Cycle. A, Hypha within host cell showing globular haustoria; B, Infected leaf in vertical section showing sporangiophores and sporangial chains; C, Germinating sporangium; D, sporangia releasing zoospores; E, zoospores; F, encysted zoospores; G, Germination of encysted zoospores; H, antheridium and oogonium; I, Plasmogamy; J, Karyogamy; K, Oospore; L, Germination of oospore producing zoospores within vesicle; M, Zoospores; N, Germination of encysted zoospore.

6.4 ZYGOMYCOTINA- MUCOR

6.4. 1 - General account

This section deals with the chief characteristics of sub-division zygomycotina with reference to the life cycle of *Mucor.*

- Zygomycotina includes fungi, which produce thick walled, resting spores called zygospore that develop within zygosporangium formed as a result of complete fusion of two gametangia.
- Flagellated cells are absent. The sub-division has been divided into two classes i.e. Zygomycetes and Trichomycetes. *Mucor* belongs to zygomycetes so the systematic position of *Mucor* is :

- Fungi
- Eumycota
- Zygomycotina
- Zygomycetes
- Mucorales
- Mucoraceae
- *Mucor*

6.4. 2 - Habit and Habitat

Mucor is commonly known as black mould. Most of the species are saprobic occurring on product containing simple carbohydrate e.g. breads, jams, jellies and other food stuffs. Species are also coprophillous i.e. growing on dung. Some species can causes break down organic matter in soil. Some are human pathogens causing zygomycosis e.g. *Mucor pusillus*. *M. javamicus* is used in the production of alcohol. Some common species are:

- *Mucor hiemalis*
- *Mucor indica*
- *Mucor mucedo*
- *Mucor javanicus*
- *Mucor silvasticus*

6.4. 3- Structure

Thallus is made up of mycelium which consist of coenocytic (aseptate) branched hyphae. Hyphae grow over the substratum and send five branches within the substratum for the absorption of nutrients. These are absorptive hyphae. These hypae also bear reproductive structures. Stolons are not found in *Mucor* (**Fig.6.7, 6.8, 6.9**).

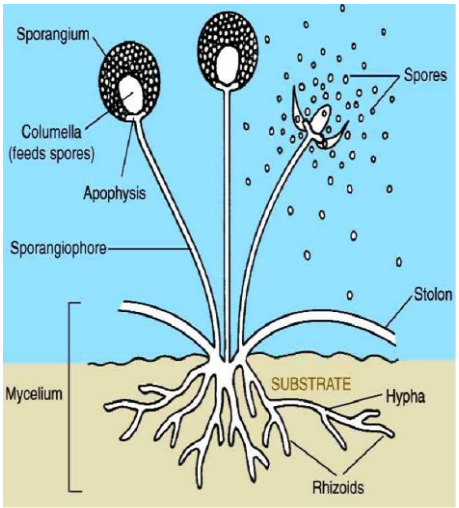

Fig. 6.7 Thallus Of *Mucor*

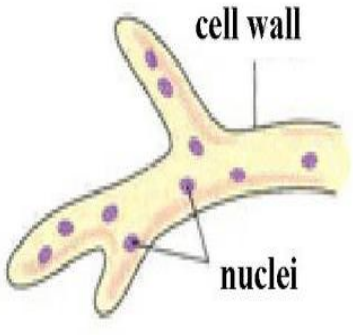

Fig.6.8 Showing coenocytic (aseptate) branched hyphae

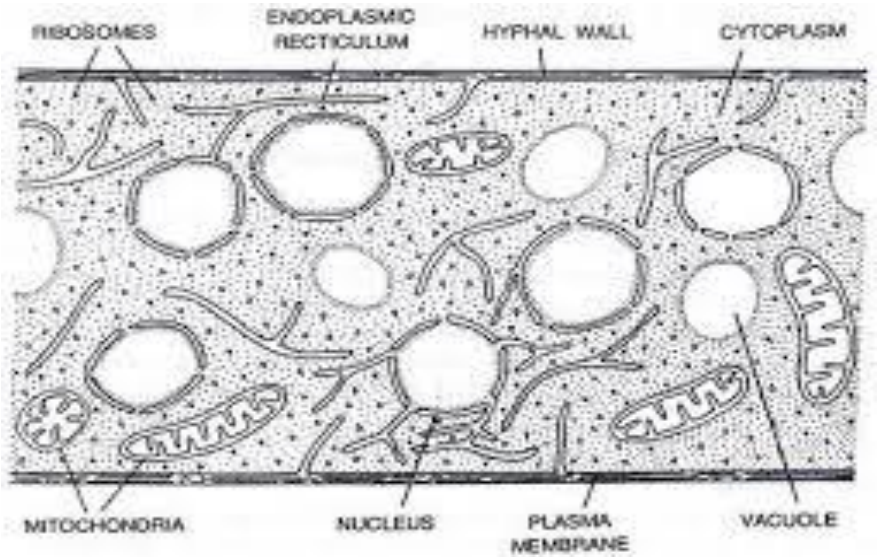

Fig.6.9. Showing part of hypha of *Mucor* sp. (As seen under electron microscope)

6.4. 4- Reproduction

Mucor reproduces by asexual and sexual methods

Asexual reproduction in *Mucor*:

It takes place by sporangiospores, chlamydospores and oidia.

By Sporangiospores – These are endogenously formed non motile asexual spores. These develop inside a sac like structure called sporangium. Sporangia borne at the tip of erect unbranched sporangiophores. The sporangiophores of *Mucor mucedo* are branched.

From the hyphae a large number of nuclei with cytoplasm and food material migrate into the tip. Now gradually such tips enlarge in size and the nuclei divide repeatedly by mitotic division. Now most of the protoplasmic contents remain in the periphery of the growing sporangium.

So in the centre, there are few nuclei and much less protoplasm. This is followed by appearance of vacuoles between central and peripheral portions. These vacuoles unite with one another resulting in the formation of a dome shaped septum separating the peripheral fertile and central sterile portion. This dome shaped sterile structure is called as **columella**. After the formation of columella cleavage takes place in the peripheral protoplasm resulting into numerous small spores with several nuclei. These are s**porangiospores**.

When sporangium is fully matured its wall becomes fragile and disintegrated by disturbance of air current. Now it ruptures and spores are scattered by air currents.

Spores are small, dark colored either ellipsoidal or globose in shape and dry. Each spore germinate by germ tube when they reach a substratum or host. Germ tube gives rise to a new mycelium (**Fig.6.10**). The collumella is persistant structure. It persists even after the bursting of the sporangium.

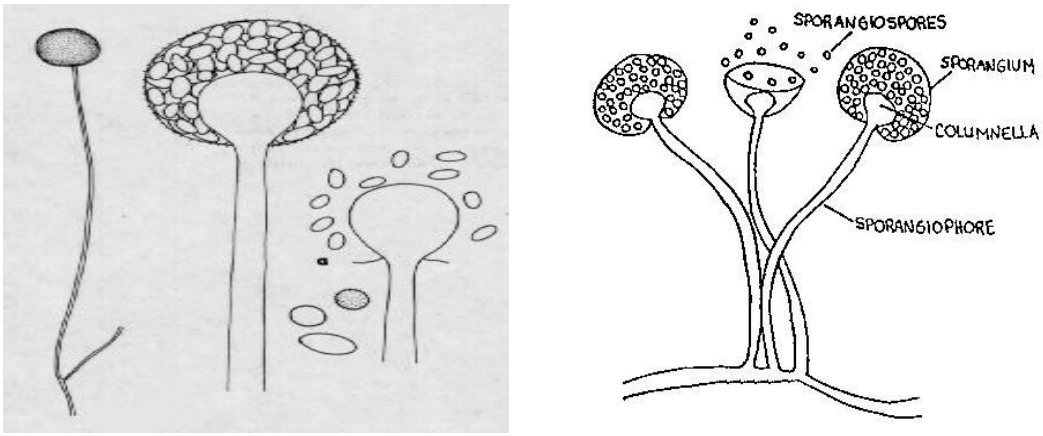

Fig.6.10. Showing development of sporangiophore and formation of sporangiospores and collumella within sporangium

By Chlamydospores : When the mycelium of *Mucor* is grown in a nutritive liquid medium, some of the hyphae break up by transverse walls into chains of cells. These cells round off and develop thick walls around themselves. These are **chlamydospores**. Chlamydospores are resting spores. Each such spore under favourable condition gives rise to new mycelium (**Fig. 6.11**).

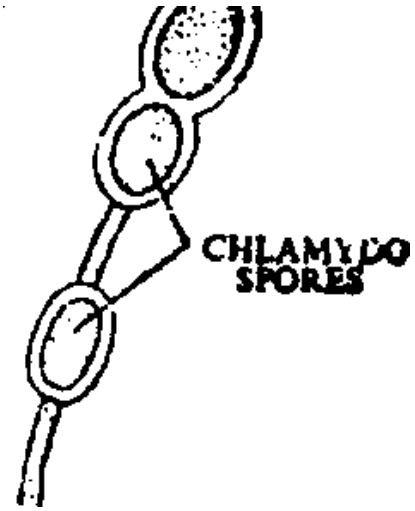

Fig.6.11. Showing Chlamydospores

By Oidia: species like *Mucor racemosus* if cultured in high sugar concentration, their hyphae breakup into short segments called oidia. These oidia may bud like yeast and called **torula condition** (**Fig. 6.12**).

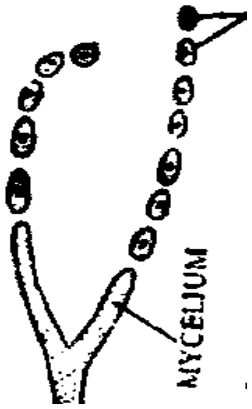

Fig. 6.12 Showing Oidia

Sexual Reproduction: Sexual reproduction requires the presence of two physiologically different and compatable mycelia designated by + and – signs. These physiologically different hyphae develop after the germination of physiologically different sporangiospores.

When such two compatable hyphae (+ and -) come in contact with each other, copulating branches (progametangia) are formed. This is followed by the flow of cytoplasm and many nuclei into the contacting tips of these organs. The progametangia are separated by the rest of hyphae due to the formation of septa near the tip. Now there are two cells i.e. a terminal cell called gametangia and the other one is the suspensor cell (**Fig. 6.13 & 6.14**).

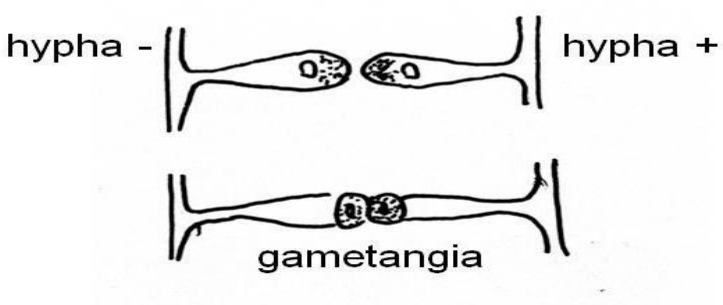

Fig. 6.13 Formation of progametangia

At the point of the contact, the walls dissolve and mixing of the two protoplasts takes place, which is followed by pairing of compatible nuclei (+and -). The two nuclei fuse and form diploid nuclei. Remaining nuclei probably disintegrate (Cutter, 1942b). The new cell which is formed by the two copulating gametangia enlarges in size; its wall thickens with rough warty surface, and a zygospore is formed.

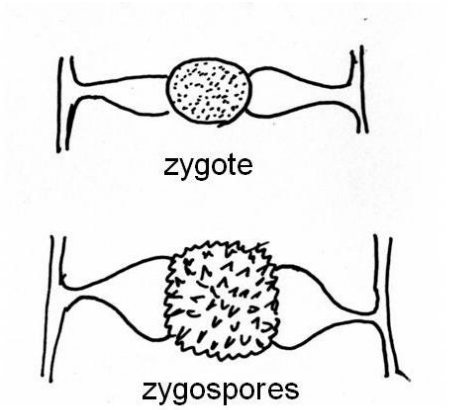

Fig. 6.14 Showing formation of Zygospore

Zygospores undergoes for a resting period. Now meiosis takes place when conditions are favourable. The outer wall splits and inner one grows out to form a hypha like germ tube or pro mycelium. It grows erect to form sporangiophore bearing a sporangium. This type of sporangium is also called **germ sporangium** or **zygosporangium**. These structure produce spores after meiosis. Thus the spores are haploid. These spores after reaching a suitable substratum or host, form new haploid mycelia.

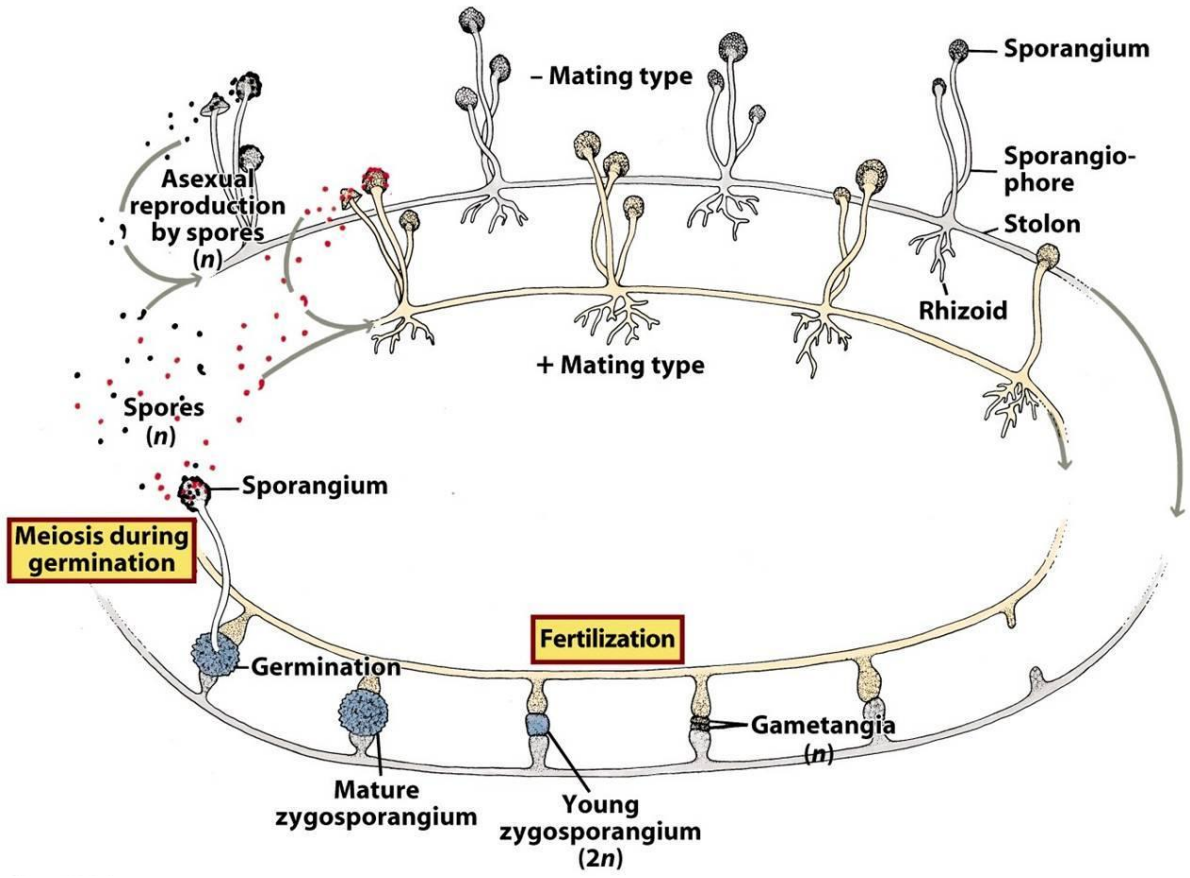

Fig. 6.15 Life cycle of *Mucor*

6.5 ASCOMYCOTINA- *Sacchromyces* and *Erysiphe*

6.5.1 General account

In this section, characteristics of Ascomycotina in the reference of *Saccharomyces* and *Erysiphe* will be discussed.

- Ascomycetes are the fungi, in which spore resulting from karyogamy and meiosis are enclosed in a sac like cell called ascus. The spores are ascospores which develope by free cell formation method.
- These fungi do not have motile cells.
- Chitin is the chief composition of the cell wall. There is no cellulose.
- There is short dikaryotic stage interspread between plasmogamy, karyogamy and meiosis.

Saccharomyces and *Erysiphe* are two fungi of this class which will be discussed in detail:

6.5.1.1-*Saccharomyces*-

- Mycota
- Eumycotina
- Ascomycetes
- Hemiascomycetedae
- Endomycetales
- Saccharomycetaceae
- *Sacchromyces*

6.5.1.2- Habit and Habitat

It is commonly known as Yeast. These fungi are widely distributed over the surface of earth , particularly over such substratum which are rich in sugar e.g. nectar of flowers, surface of fruits. That is why the name *Saccharomyces* (Saccharum =sugar + myketes = fungi) is given. Species also grow in soil, animal excreta and milk etc. *Saccharomyces* has useful as well as harmful importance. The useful property is, their role in fermentation. Therefore these fungi are economically important in bakery and brewery. This useful property of *Saccharomyces* if not handled properly, causes loss to the industrialists. They also destroy cheese, causing bad flavor. Common species of Yeasts are:

- *Saccharomyces cerivisiae*
- *Saccharomyces apiculatus*
- *Saccharomyces frnctuum*

6.5.1.3 Structure

In case of *Sacchromyces* the vegetative body is unicellular not made up of hyphae. Sometimes, many cells of Yeast unite in chain and form a pseudomycelium. Thus, this genus is polymorphic. This nature depends on the type of medium in which the fungus is growing.

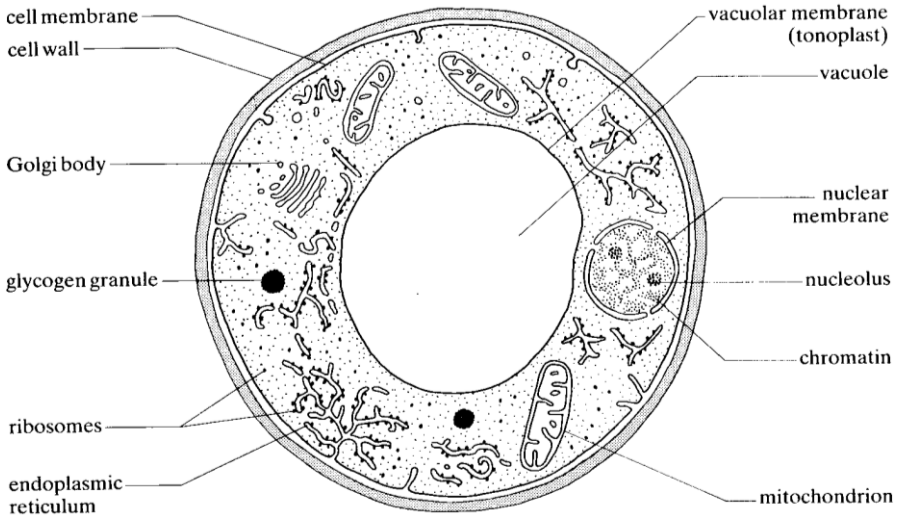

Fig. 6.16 Structure of Yeast cell

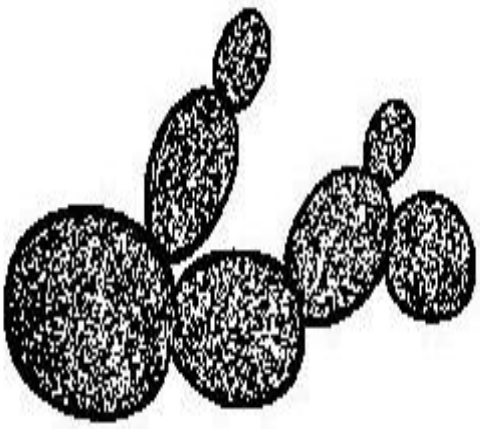

Fig. 6.17 Pseudomycelium

The Yeast cell is colourless in nature but when it is grown in the medium in laboratory, the cells may be pink, white or cream colored. Normally shape of cell is spherical, oval or cylindrical. There is a definite thin cell wall composed mainly of chitin along with carbohydrates, mannan and glucan. There are two layers in a cell wall. The outer one is dense and approximately 0.05 micron thick, and an inner layer which is less dense and nearly 0.2 micron thick, containing microfibrils. Inside the cell wall, there is cytoplasm which is granular. In mature or old cells the cytoplasm is differentiated into an outer ectoplasm and an inner endoplasm. Ribosomes, mitochondria and endoplasmic reticulum are also found in the cell. Glycogen, oil globules and protein particles are the reserve food materials. There are

different views about the nature of vacuole, nucleus and nucleolus (**Fig 6.16**). The views are as follows:

Ist view – The centrally placed hyaline area is nucleus and there is a deeply stained body to one side of it is the nucleolus. The central area is not a vacuole. There is a centromere on one side of nucleus.

IInd view– Central hyaline area is a vacuole and not a nucleus. Nucleus is present opposite to it which was identified as nucleolus by the supporters of Ist view.

IIIrd view– Nucleus is surrounded by a definite membrane and is distinct from vacuole.

6.5.1.4 Reproduction

Saccharomyces reproduces by two methods, i.e. Asexual and Sexual.

Asexual reproduction

1) **Budding:** This method is adopted when there is abundant food / nutrients. During budding, nucleus of mother cell divides mitotically. This is followed by appearance of small growth over the surface of the vegetative cell called bud. One of the nuclei passes into this bud, and the bud is separated from parent cell by the formation of a wall between the two cells. Thus there is formation of new individual. This process continues and at times the bud may also start forming new buds. This results formation of a pseudomycelium (**Fig 6.17, 6.18**).

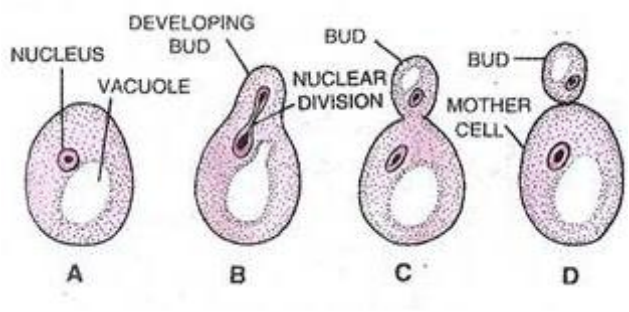

Fig. 6.18. Budding in Yeast

2) **Endospore formation**- This process is adopted when there is scarcity of food or there is chance of desiccation. The protoplasm of vegetative cell divides usually into four portions. Later on, each portion gets surrounded by a thick wall. These are endospores. When conditions are favorable for growth, they germinate and form a chain of cells.

Sexual reproduction – The process takes place by conjugation between two haploid cells. Cells may be similar or dissimilar and are called gametangia. So, there are no specialized sex organs. This process is termed as hologamy. The diploid zygote itself function as ascus and produces ascospores after meiosis. Ascospores are generally globose or ovoid and differ in number. Ascospores upon liberation multiply by budding to produce haploid cells. Zygote

may also bud forming large number of diploid cells. Each diploid cell behaves as ascus (**Fig 6.19**).

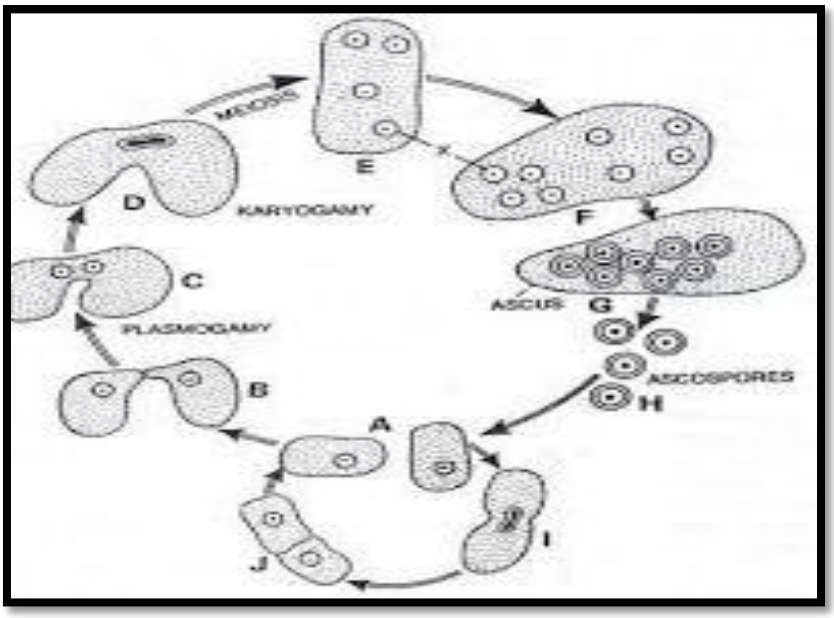

Fig.6.19. Sexual reproduction in Yeast

Life cycle of *S. cerevisae* is haplodiplobiontic type. The haploid and diploid phases are equally well represented. Students therefore there is clear alternation of generations in this species of Yeast (**Fig.6.20**).

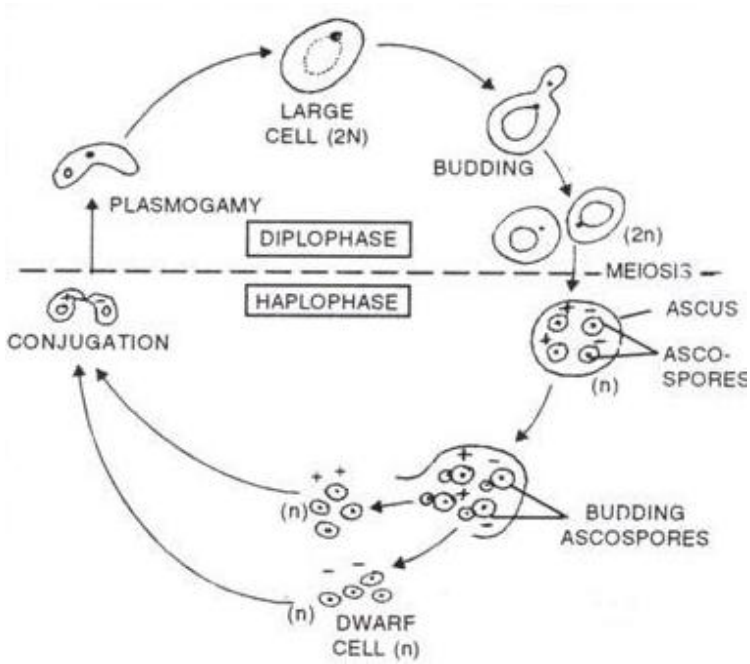

Fig. 6.20. Haplodiplobiontic life cycle of *Sacchromyces cervisae*

6.5.2.1 *Erysiphe* - HABIT AND HABITAT

The systematic position of *Erysiphe* is as follows:

- Mycota
- Eumycotina
- Ascomycetes
- Hamenoascomycetidae
- Pyrenomycetes
- *Erysiphe*

Erysiphe is an important fungus and causes powdery mildew disease. Some mycologists classify it under Plectomycetes because of completely closed nature of ascocarp (fruiting body),while others give more emphasis to the position of asci inside the ascocarp and place it under Pyrenomycetes. Thus the difference in classifying this fungus is just because of the different criteria taken by the mycologists.

Many species of this genus attack the host and develop white powdery coating over the surface of host, hence the name powdery mildew. Following are some of the species of this genus:

- *Erysiphe polygonii*
- *Erysiphe graminis*
- *Erysiphe cichoracearum*

6.5.2.3 STRUCTURE

Thallus of *Erysiphe* is made up of hyphae and which are hyaline abundantly present over the surface of the epidermis of host. The hyphae send haustoria into the epidermis of host for absorbing nutrients. Haustoria arise from the lobed swellings of the hyphae adjacent to leaf surface and are called appresoria. In *Erysiphe polygoni* haustoria are almost knob shaped and in *E. graminis* these are finger like or branched. But in any case haustoria are restricted only in the epidermal cells of host **(Fig.6.21**).

6.5.2.4 REPRODUCTION

Erysiphe reproduces by asexual and sexual methods. Asexual method of reproduction is commom by conidia. It starts after few days of infection when the hyphae produce erect, long, septate conidiophores. The upper most cell is called generative cell. This cell produces conidia. The conidia in some species are abstricted before the maturation of next one, while in other species conidia are not abstricted and remain attached to each other and thus form chain of conidia. These are hyaline, one celled and may vary in shape. Mostly the shape is oval or cylindrical with rounded edges. They are disseminated by wind, water, insects or other agencies.

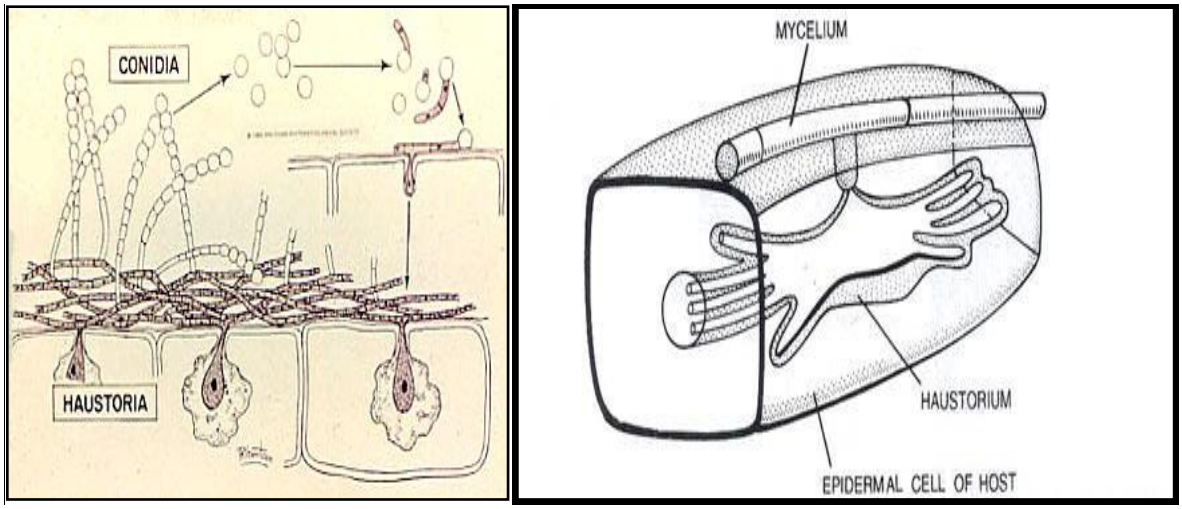

Fig.6.21 Showing mycelium, conidia and haustoria found in genus *Erysiphe*

The conidia germinate and do not require free water for germination. The distal end of each conidium develops a flat appressorium over the surface of the host. A peg-like structure develops from appressorium and penetrates the epidermis of the host. The tip of the peg like projection swells resulting in the formation of a vesicle which gives rise the finger like haustorium. Asexual reproduction takes place during growing season.

Sexual Reproduction

At the end of growing season of host, black colored round structures are seen on the white powdery surface of the host. These are the symptoms of the onset of sexual reproduction. The black structures are cleistothecia. Newly formed cleistothecia are first white, then turn orange, reddish brown and finally black when mature.

Most of the species are homothallic except few i.e. *E. graminis* which is heterothallic. The uninucleate gametangia develop from the hyphae which are closely present. Male gametangium is more slender than the female gametangium ie. the ascogonium. These are so close to each other that they press each other. Mycologist disagree as to the cytological details which follow. But probably the further course of development follows the usual pattern. The antheredium nucleus passes into the ascogonium through a pore. Process of karyogamy is postponed until the ascus mother cell is formed. Many sterile hyphae develop from the base of ascogonium and surround the sex organs completely. The surrounding sheath is thick and multilayered.

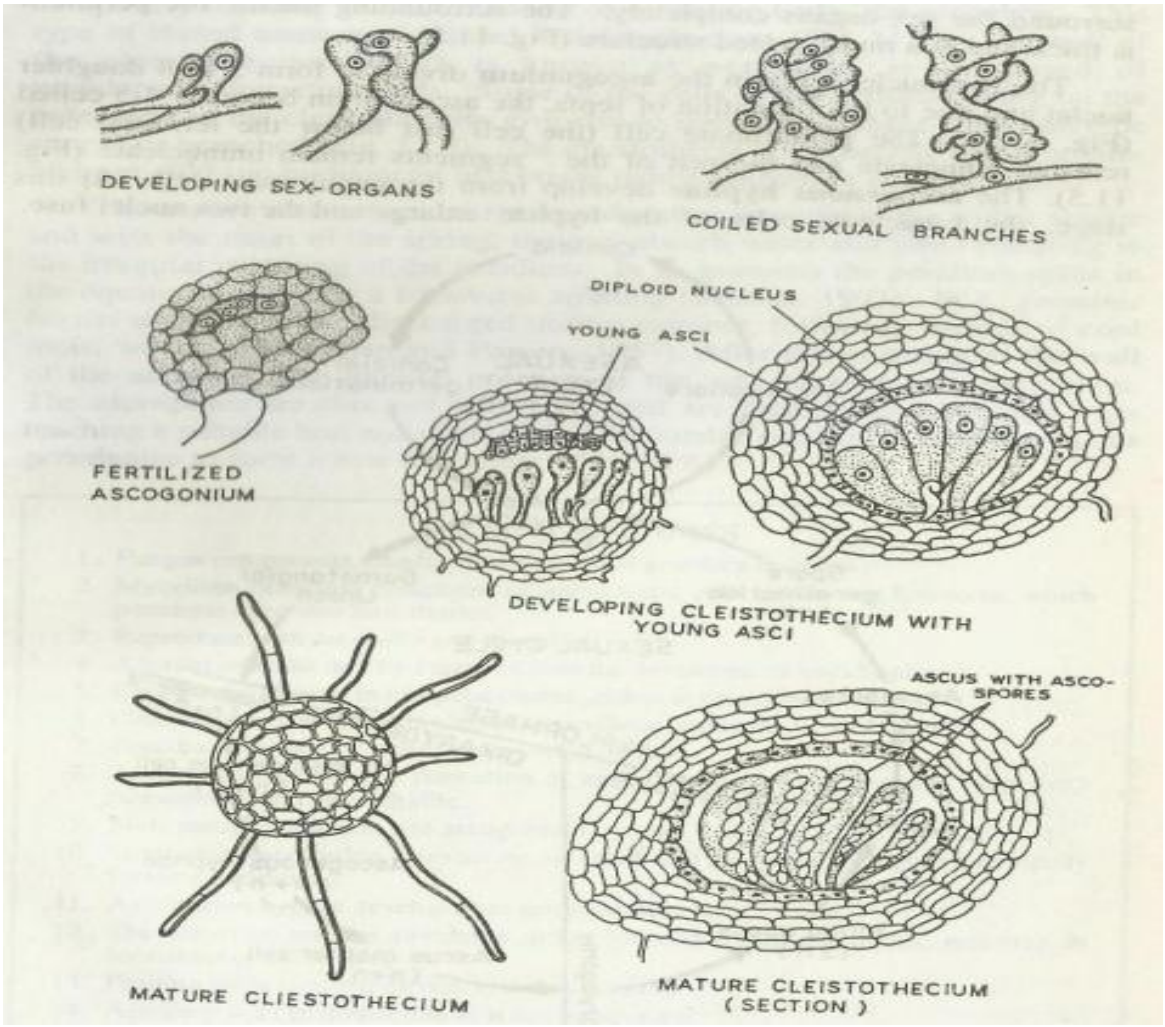

Fig.6.22 Showing gametangial contact and formation of ascus

The two nuclei lying in the ascogonium divide to form 5 to 8 daughter nuclei and due to formation of septa, the ascogonium becomes 4-5 celled. The cell just below terminal cell i.e. penultimate cell remains binucleate and rest of the segments remain uninucleate. The penultimate cell forms hyphae called ascogenous hyphae. Now the binucleate cells of the ascogenous hyphae enlarge and the fusion of nuclei takes place. The diploid nucleus divides by meiosis. This is followed by mitotic division, which results in the formation of eight haploid nuclei. Each nucleus gets surrounded by a small amount of cytoplasm and a wall. There are eight haploid ascospores. In *E. cichoracearum*, each ascus contains only 2-3 ascospores. Ascospores are hyaline, one- celled, uninucleate and ellipsoidal in shape. Ascospores remain inside ascocarp within asci. The ascocarp is called cleistothecium (**Fig.6.22**).

The cleistothecium is a sub-globular or round, completely closed structure. Large number of hyphae surrounds the surface of cleistothecium. These hyphae are called appendages, which are either simple or branched (**Fig.6.23**).

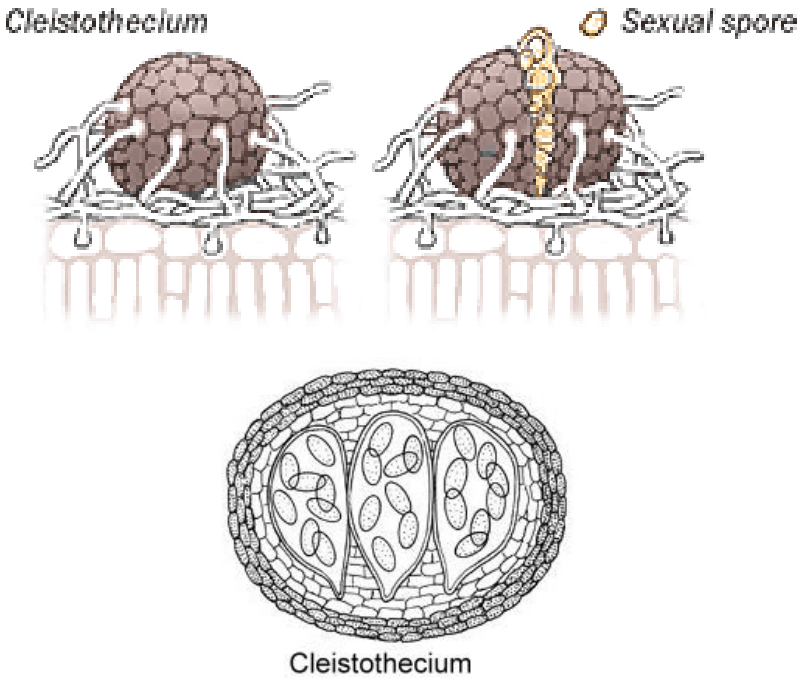

Fig.6.23 Showing Cleistothecium

The mature cleistothecium remains dormant throughout the winter season and with the onset of the spring, the asci absorb water and swell resulting in the irregular rupturing of the peridium. In *E. graminis* the peridium splits in the equatorial plane by transverse splitting. Asci absorb moisture, swell and then burst releasing the ascospores. These are disseminated by wind. On reaching a suitable host germinate and produce haploid mycelium.

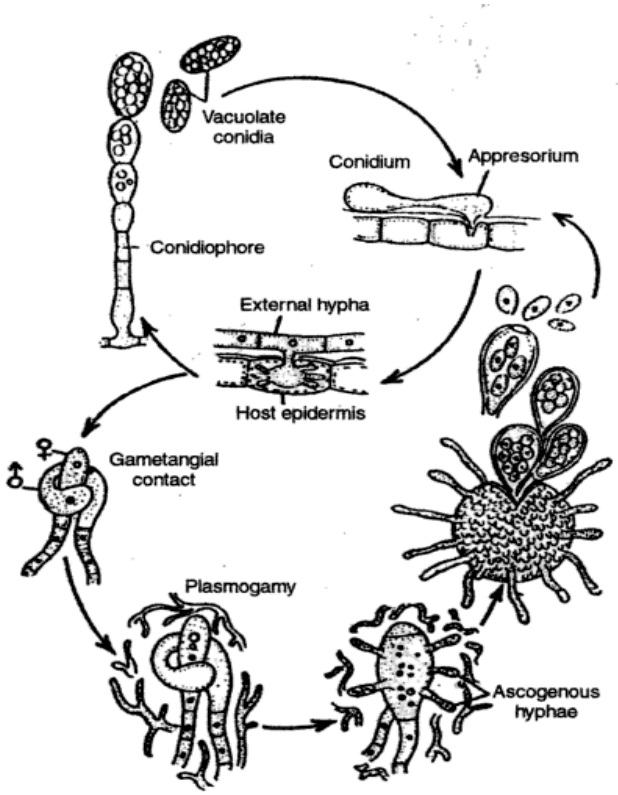

Fig.6.24 Showing Life cycle of *Erysiphe*

6.6 SUMMARY

Status of the Mastigomycotina, Zygomycotina and Ascomycotina in fungal world.

- Mastigomycotina, Zygomycotina and Ascomycotina have got the status of sub-division after the five kingdom classification of Whitaakar(1969). All the three groups show a definite cell wall.
- Mastigomycotina have flagellated fungi.
- Zygomycotina and Ascomycotina are non- flagellated fungi.
- Mastigomycotina after plasmogamy, karyogamy and meiosis produce oospores formed from fertilized egg.
- Zygomycotina produce zygospores after plasmogamy, karyogamy and meiosis. The fusing gametangia may be equal or unequal.
- Ascomycotina produce ascospores after plasmogamy, karyogamy and meiosis inside a sac like structure called ascus.
- *Albugo* is an obligate genus causes a disease called white rust of crucifers. It produces symptom of disease on host which differ during asexual and sexual stages. Symptoms of asexual stage are recognized by white pustules on leaf of host, while symptoms of sexual stage are recognized by hypertrophied inflorescence of host. Mycelium of *Albugo* is diploid and is made up of hyphae which are coenocytic , intercellular and produce knob shaped haustoria for absorption of food from host. It reproduces both by asexual and sexual methods. Asexual reproduction takes place by conidia/sporangia borne on conidiophores. Asexual spores germinate by two methods. Sexual reproduction is oogamous resulting into a diploid thick walled oospore. Meiosis takes place at the time of formation of gametangia.
- *Mucor* has species both parasitic and saprobic . *Mucor* is commonly known as black mold. Species spoil food, particularly rich in carbohydrates. Some species are pathogenic. *Mucor* has mycelium made up of branched haploid hyphae. Its reproduces by asexual and sexual methods. Asexual reproduction is by sporangiospores, chlamydospores and oidia. Sexual process results in formation of diploid thick walled spore called zygospores. Due to fusion of two gametangia phenomenon of heterothallism has been reported in the species of this genus.
- *Saccharomyces* commonly called Yeast, is of great industrial importance. Species are used in bakery and brewing. It is one celled, colourless in nature. There is controversy about the nature of vacuole present in the cell. It reproduces by asexual and sexual methods. Asexual reproduction takes place by budding and endospore. Sexual reproduction by conjugation in between two haploid cells i.e. hologamy. Sexual reproduction results in the formatation of ascospores inside the ascus.
- *Erysiphe* is also parasitic genus. It causes a disease known as Powdery Mildew on economically important crops. It consist of superficial mycelium which grows on the surface of leaf of host and only haustorium enters into the epidermis of host for the absorption of food.

Asexual reproduction is by conidia on short conidiophores. Sexual reproduction results in the formation of ascospores inside asci. These are borne inside the cleistothecium. These fruiting bodies have a number of appendages on its surface.

6.7 GLOSSARY

Achlorophyllous: lacking chlorophyll.

Aeciospores : a binucleate spore produced in an aecium.

Aecium : a structure consisting of binucleate hyphal cells with or without a peridium that produces spore chain consisting of aeciospores alternating with disjuncture cells, following the successive conjugate division of the nuclei.

Antheridium : a male gametangium.

Appresorium : a flattened, hyphal, organ from which a minute infection peg usually grows.

and enters the epidermal cell of the host.

Ascocarp : a fruit body containing asci.

Ascospores : a meiospore borne in an ascus.

Ascostroma : a stromatic ascocarp bearing asci directly in locules within the stroma.

Ascus : a sac like cell generally containing a definite number of ascospores (typically eight) formed by free cell formation usually after karyogamy and meiosis, characteristic of class Ascomycetes.

Aseptate : lacking cross walls.

Asexual : reproduction not involving karyogamy and meiosis.

Budding : the production of a small outgrowth (bud) from a parent cell; a method of

asexual reproduction.

Chlamydospores : a thick walled thallic conidium that generally functions as a resting spore.

Coenocytic : a non – septate referring to the fact that nuclei are embedded in the cytoplasm without being separated by cross-walls.

Collumella : a sterile structure within a sporangium or other fructication, often an extension of the stalk.

Conidiophore : a simple or branched hypha arising from a somatic hypha and bearing at it

stip or side one or more conidiogenous cells.

Conidium : a non-motile asexual spores usually formed at the tip or side of a sporogenous

cell; in some instance a pre – existing hyphal cell may be converted to a conidium.

6.8 SELF ASSESSMENT QUESTIONS

A. Tick the right answer:

1. White rust in crucifers is caused by:
(i) *Albugo campestris* (ii) *A. bliti*
(iii) *A. candida* (iv) *A. rodmani*

2. Hypertrophied inflorescence of mustard represents on set of which stage of *Albugo candida*:
(i) Asexual (ii) Sexual
(iii) Vegetative (iv) Either vegetative or sexual stage

3. Process of Plasmogamy in *Albugo* takes place by:
(i) Gametangial contact (ii) Gametangial copulation
(iii)Spermatization (iv) Somatogamy
4. Species of *Mucor* do not have:
(i) Stolen (ii) Columella
(iii) Sporangiophore (iv) Sporangia

5. Plasmogamy in *Mucor* is by:
(i) Somatogamy (ii) Gametangial contact
(iii) Gametangial copulation (iv) Plasmogametangial copulation

6. *Saccharomyces* belongs to the class:
(i) Ascomycetes (ii) Zygomycetes
(iii) Basidiomycetes (iv) Oomycetes

7. *Erysiphe* forms haustoria in:
(i) Cortex of host (ii) Epidermal cells of host
(iii) Vascular bundle of host (iv) Spongy Parenchyma of host

B. Fill up the blanks:

1. Succession of conidia on conidiophores in *Albugo* is ----------
2. Mycelium of *Mucor* has…….. hypha.
3. Thallus of *Mucor* is…….
4. Columell is……..after dehiscence of sporangia in Mucor.
5. Diploid spore of *Mucor* is called…….
6. *Saccharomyces* is…… fungus.
7. Ascocarp of *Erysiphe* is called……
8. *Mucor* is commonly known as …… and *saccharomuces* is called…….

Answers:

A. 1. (i), 2. (ii), 3. (i), 4. (i), 5 (iii), 6. (i), 7. (ii)

B. 1. Basipetal succession 2. Coenocytic 3. Haploid 4. Exposed 5. Zygospore 6. Unicellular 7. Cleistothecium 8. Black mold, *Yeast*

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6.11 TERMINAL QUESTIONS

1. Mastigomycotina, Zygomycotina and Ascomycotina have been classified under which system of classification? Give a detailed account.
2. What are chief characteristin of Ascomycotina and Zygomycotina?
3. How *Albugo* reproduces by asexual and sexual methods of reproduction?
4. What are symptoms of infection caused by *Albugo*?
5. What is torula conditions of *Mucor*?
6. How does zygospore formation takes place in Mucor?
7. Give an illustrtraited account of sexual reproduction in *Mucor* with special reference to heterothallism.
8. Name the disease caused by the species of *Erysiphe*. Discuss its sexual stages of reproduction.
9. Write characteristic features of *Saccharomyces*.
10. Discuss method of reproduction in *Saccharomyces*.

UNIT-7 GENERAL ACCOUNT, HABIT, STRUCTURE AND METHODS OF REPRODUCTION IN BASIDIOMYCOTINA, DEUTEROMYCOTINA AND MYCOPLASMA

7.1- OBJECTIVES

The objectives of this unit are to get you familiar about the differences between Basidiomycotina, Deuteromycotina and Mycoplasma. These are as follows:

- Basidiomycotinaincludes fungi which reproduce both by sexual and asexual methods of reproduction, while Deuteromycotina group includes imperfect fungi which reproduce only by asexual methods.
- Mycoplasma are prokaryotic organisums and do not resemble fungi belonging to Basidiomycotina or Deuteromycotina.
- After reading this unit you will know about the living place of members of these groups, the variations in their vegetative / somatic structures as well as the methods of reproduction.
- You will know the disease caused by *Puccinia, Agaricus* and *Alternaria.*

7.2 INTRODUCTION

In **unit 6.2**The status of Mastigomycotina, Zygomycotina and Ascomycotinawas described in few important systems of classification of fungi . In this unit, the status of Basidiomycotina and Deuteromycotina will be discussed.

These two groups were also treated as a class of sub-division fungi by Gwune-Vaughan and Barnes 1937. Then according to Alexopoulus system of classification (1962), these were also treated as class, but of sub-division named Eumycotina. In 1969, Basidiomycotina and Deuteromycotina also got the status of a separate sub-division when Whittakar system of five kingdom classification was accepted.

7.3 BASIDIOMYCOTINA

(A) *Puccinia*

7.3.1 General account

In this section you will learn about the classification of Basidiomycotina with particular reference to *Puccinia* and *Agaricus.*

- Basidiomycotina are those fungi in which spores resulting from plasmogamy, karyogamy and meiosis are borne on club shaped structure called basidium. Motile cells are absent in the members of this group.
- Dolipore septa occur in most Basidiomycetes.
- There is long dikaryotic stage interspread between a Plasmogamy, karyogamy and is represented by extensive dikaryotic mycelium which produces sporophores (Basidiocarps).
- *Puccinia* and *Agaricus*are two important fungi belonging to basidiomycotina. The systematic position of *Puccnia*is as follows:

- Mycota
- Eumycotina
- Basidiomycetes
- Hetrobasidiomycetidae
- Uredinales
- Puciniaceae
- *Puccinia*

7.3.2 Habit and habit

Puccinia is very important and widely distributed genus of this group. It includes about 700 species from India Most of the species are obligate parasites. The fungus is characterized by its spores which are orange/ black in colour and this is the reason that the disease caused by various species is called **Rust disease**. The fungus attacks important crop plants e.g. oat, rye, barley and wheat etc. Some important species are as follows:

Puccinia graminis	- Black or stem rust
P. recondita	- Brown rust
P. striiformis	- Stripe rust or yellow rust

The most important species of the genus is *Pucciniagraministritici*and is being taken as an example to explain the structure and reproduction of the genus. It is obligate parasite an the fungus is heterocious, polymorphic and macrocyclic. The meanings of all above adjectives used for this species are as follows:

Obligate: Life cycle is completed only on hosts.

Parasite: Life cycle is completed on host.

Heteroecious: Life cycle is completed on two entirely different hosts i.e.

- Barberry (*Barberis vulgaris*) which is a secondary alternate host.
- Wheat (*Triticumvulgare*) which is a primary host.

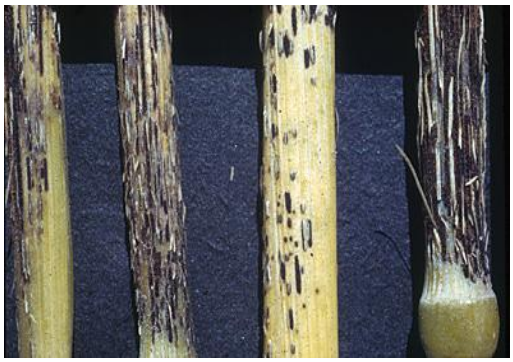
Polymorphic: Life cycle shows five distinct stages at regular intervals and are represented by five types of spores. These are as follows:

Stage I- 0-stage – represented by Spermogonia/ Pycnia/ pycnidia containing:
spermatia/ pycnidiospores = Male reproductive structure
receptive hyphae/ flexnous hyphae = Female reproductive structure

Stage II-	**1-stage-**	Aecia/ Aecidia	Aeciospores/ Aecidiospores
Stage III-	**2-stage**	Uredia/ Uredosori	Uredospores/ Uredospores
Stage IV-	**3-stage**	Telia/ Teleutosori	Teleutospores/ Teliospores

Stage V-	**4-stage**	Basidia/ Pro-mycelium	Basidiospores/ sporidia

The Stage I, II and V occur on *Barberry* host (which is an alternate host of the pathogen), while, the stage III and IV occur on the primary host i.e. wheat plant (**Fig. 7.1**).



(i)

(ii)

(iii)

Fig.7.1-Showing Symptom of Rust on Wheat stem (i & ii) and Berberry leaf (iii)

7.3.3 Structure

Mycelium well developed and consist of hyphae which are septate, branched moderately. There are two types of hyphae. Primary hyphae are those which form after the germination of basidiospores. These primary hyphae are unicellular and multinucleate in the beginning but due to formation of septa they become multicellular and uninucleate. These are haploid and are of two strains. One is represented by + ve sign and other by – ve sign. These hyphae form monokaryotic mycelium. Secondary hyphae are multicellular and binucleate (n+n),form dikaryotic mycelium.

The monokaryotic mycelium grows on Barberry (secondary host) and secondary mycelium on wheat (primary host).

Both types of mycelia are not very deep seated but they grow close to epidermis of host.

7.3.4 Reproduction

Reproduction and life cycle of *Puccinia graminis* is represented by following stages:

0-Stage- I Represented by spermogonia containing male (spermatia) and female (receptive hyphae) structures.

Reproductive organs of *P. graminis* are spermatia (Pycniospores/ Pycnidiospores) and receptive hyphae/ flexous hyphae, the male and female structure respectively. These are borne in spermogonia / pycnia/ pycnidia. These are haploid. Spermogonia are produced on upper surface of Barberry leaf by primary monokaryotic mycelium.The primary monokaryotic mycelium which develops after the germination of haploid basidiospore(+ or – strains).Thespermogonia are of two types. One containing all the structures of one strain or factor (+) and the other having all the structures of opposite strains or factor (-). In nature spermagonia of opposite strains or factor are produced close together.

The basidiospores germinate on the surface of leaf of barberry in the presence of moisture. The spores produce germtube which penetrate directly into the epidermis of leaf. After penetration of germ tube, it freely branches and forms haploid monokaryotic mycelium. The mycelium carries either (+) or (-) factor / strains which depends upon the strain of basidiospores. The mycelium now vigorously grows producing dense mats of uninucleate hyphae. These are primordial of the spermogonia (pycnia / pycnidia). The formation of spermogonia may be observed by small yellow or red patches on the upper surface of the infected leaves.

The mycelium which is not involved in the formation of spermogonia penetrates deep towards the lower epidermis of leaf of *Barberry*. A mature spermogonia is small, oval to flask shaped structureThe wall of spermogonium is called **peridium** and it is made up of pseudoparenchymatousplectenchyma. There is an opening called **ostiole** which opens at the leaf surface. From the base of spermogonium arise a large number of uninucleate, unicellular hyphae called **spermatiaphores**. These have rounded base and blunt tips (**Fig.7.2**).

The spermatiophores cut off from their tips a large number of male cells called **spermatia or pycnia orpycnidia**. Spermatia are unicellular, globose and uninucleate and are formed in chain. From the neck of spermogonia arise two types of hyphae, one is called **flexous hyphae or receptive hyphae**. These are female reproductive structures. Receptive hyphae are also uninucleate one celled with flat base and pointed tips. The other type of hyphae are**sterile hyphae called paraphysis**. Receptive hyphae and paraphysis grow out of the epidermis.

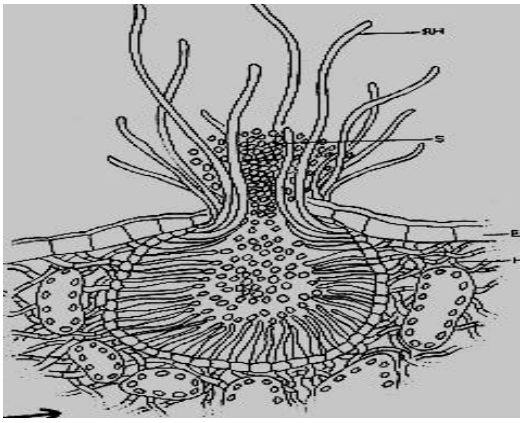


Fig.7.2: Showing Spermagonium of wheat, receptive hyphae (RH), spermatium (S), and wheat epidermis (E)

Plasmogamy- In *Pucciniagraminis* process of plasmogamy is with the help of flies. The flies or insects get attracted towards the sweet nectar of leaf and when they suck the nectar some spermatia stick to their legs and proboscis.When such insects / flies visit another spermogonia on the leaf, the spermatia are taken away by the receptive hyphae of these spermogonia. When the spermatia contact the receptive hyphae or of spermogonia of opposite strain, the dikaryotization takes place resulting in dikaryotic receptive hyphae. The spermatia of one strain enter into the receptive hypha of opposite strains through a pore which develops at the point of contact between them (**Fig.7.3**).

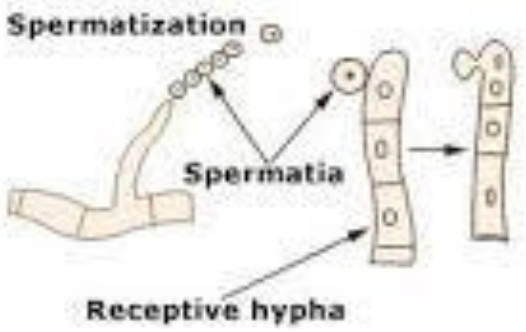


Fig.7.3: Showing process of Plasmogamy

1- Stage -II Represented by Aecia and aeciospores (Aecial stage) on barberry leaf.

At the time of spermatization which results in dikaryotization, the primary monokaryotic mycelium penetrates entire leaf of berberry and hyphae near the lower epidermis form a number of aecial primordial. Spermatial nuclei which passes from spermatia into receptive hyphae pass through the septal perforation of mycelium and reach the cells of aecial primordial, rendering them binucleate. It has been demonstrated that the aecialprimordia fail to grow further unless and until spermatization takes place.

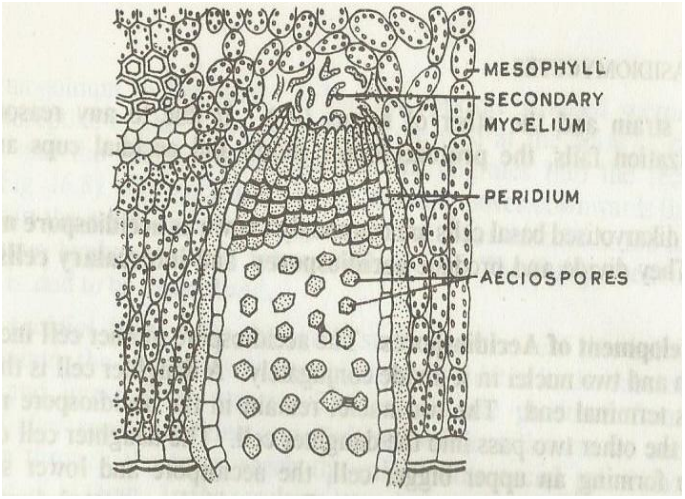


Fig.7.4:Showing Aecia & aceiospores

Aecia are globose and completely closed structure. The wall or aeciumis called peridium and made of pseudo parenchymatous fungal tissue. The cells present at the base of aecia are called aeciospores mother cells. These are the first binucleate cell in the life cycle. Aeciospore mother cells divide and form aeciospores at the terminal position alternating with sterile cells called disjunctor cells. Aeciospores are polyhedral, unicellular and binucleate. They are formed in chains. Peridium covers the entire aecial cup but as the cup grows and aeciospores are formed the pressure is created resulting into breaking up off the peridium. Then the aeciospores are released. The broken part of the peridium lies over the surface of lower epidermis and called lip of the peridium. Aeciospores are disseminated by wind and germinate under favourable conditions on a susceptible primary host i.e. wheat plant and produce binucleatemycelium. But the aeciospores fail to grow further if they do not reach a grass host. So, a binucleate aeciospores produced on barberry leaf can grow further only on wheat host (**Fig.7.4**).

2- Stage –III Represented by uredia forming uredospores on wheat plant.

In the month of March / April (spring season) reddish orange coloured spots / pustules appear over the leaf of wheat. These are uredia or uredosori. Uredia are made up of secondary dikaryotic mycelium which develops after the germination of aeciospores on wheat leaf. The secondary dikaryotic mycelium grows, ramifies just below the epidermis of wheat leaf. From the base of uredosori arise a large number of sporophores which bear at their apices oval thick walled, brown coloured, binucleate, one celled, stalked urediospore Each urediosporesposses four germ pores which are equatorially arranged. Outer wall of urediospore is thick and ornamented/rough while inner wall is smooth. Uredospores remain compactly arranged with in the uredosori. As the spores mature, pressure is exerted towards the growing epidermis of host which ultimately breaks away releasing the spores above the surface of epidermis. The germ tube of uredospore does not enter directly into the host cells. The tip of the germ tube swells up form an elongated **appresorium.** The appresorium is cut off from the spore and sends fine hyphae into the stomata. These hyphae grow and ramify within the intercellular space of host tissues.

As soon as the spores mature (3-4days) they germinate by forming germ tube and the mycelium again infect the wheat host on which they were produced. Thus the spores spread the disease from plant to plant and from field to field. That is why the uredospores are also called **repeating spores (Fig.7.5)**.

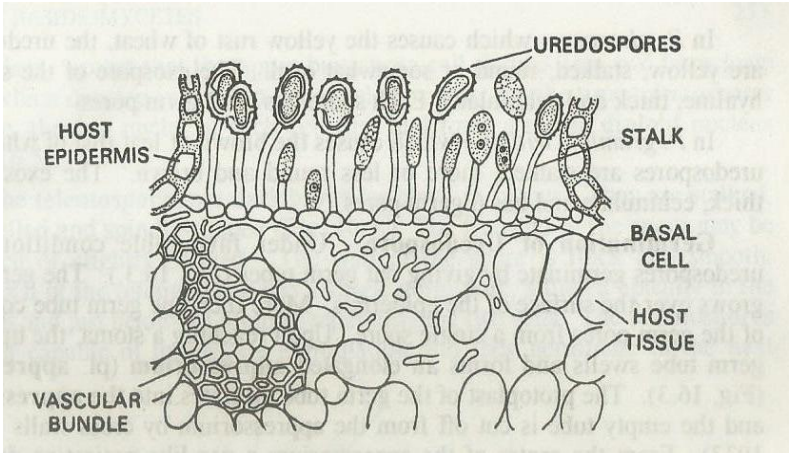


Fig.7.5: Showing Uredosorus in various stage of development

3-Stage –IV Telia / teleutosori forming teleutospores

About the time wheat grain is maturing instead of brown/ orange spots or pustules black coloured streaks develop on the leaf of wheat host. These are telia / teleutosori.

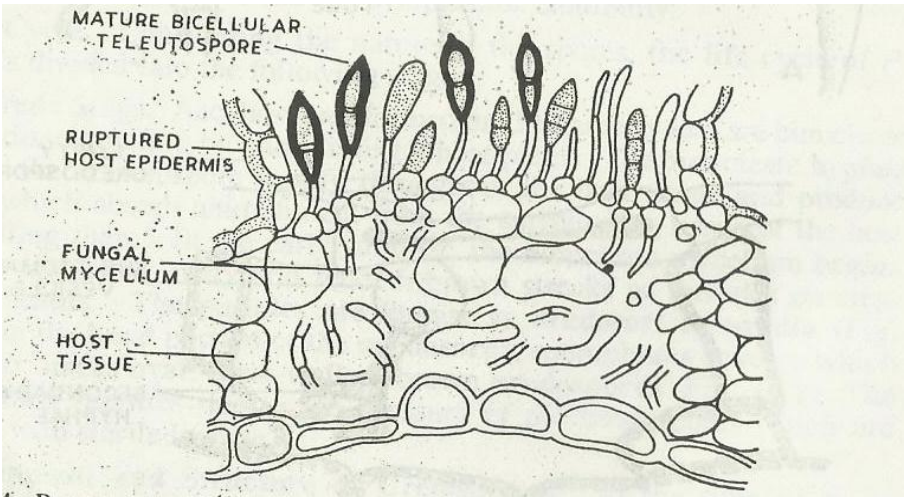


Fig.7.6. Showing bicelled Teleutosorus in various stage of development

The dikaryotic mycelium collects below the surface of epidermis of wheat stem and forms teleutosori in the same manner as it formed uredosori on leaf of wheat. Sporophores produce at their tips bicelled, thick walled, dikaryotic, stalked teleutospores. Each cell contains a pair of nuclei of opposite strains (+,-). Tip of teleutospore is either rounded or pointed. There are two germpores present in spore, one at the tip of upper cell and one at the junction of two cells. The two nuclei (+,-) of each cell fuse together making the spores diploid. The Teleutosporedo not germinate immediately. They survive during winter and germinate in next spring. Thus over wintering takes place in diploid teleutospores (**Fig.7.6**).

4-Stage –V Represented by promycelium forming basidium and basidiospores

Early in the spring each cell of the teleutosporegerminates and produce pro mycelium into which passes the diploid nuclei where it undergoes for meiosis and for four haploid nuclei (n).

Nowthe pro mycelium becomes dividedinto four uninucleate cells. From the side of which a single small tube the sterigmata on which basidiospore is fomed. The nucleus and cytoplasm moves into uredospore through sterigmata. *P.graminis* is hetrothallic and out of the four basidiospores thus formed two one of (+) strain and other two are of (-) strain. The basidiospores are one celled, uninucleate, stalked, oval in shape and brown yellow in colour. Soon after their formation the basidiospores are forcibly ejected by water droplet method ane are carried away by the wind. If they fall on a barberry plant (host) then only they germinate and produce mono karyotic primary mycelium of either + or – strains depending upon the nature of basidiospore from which its germinates (**Fig.7.7**).

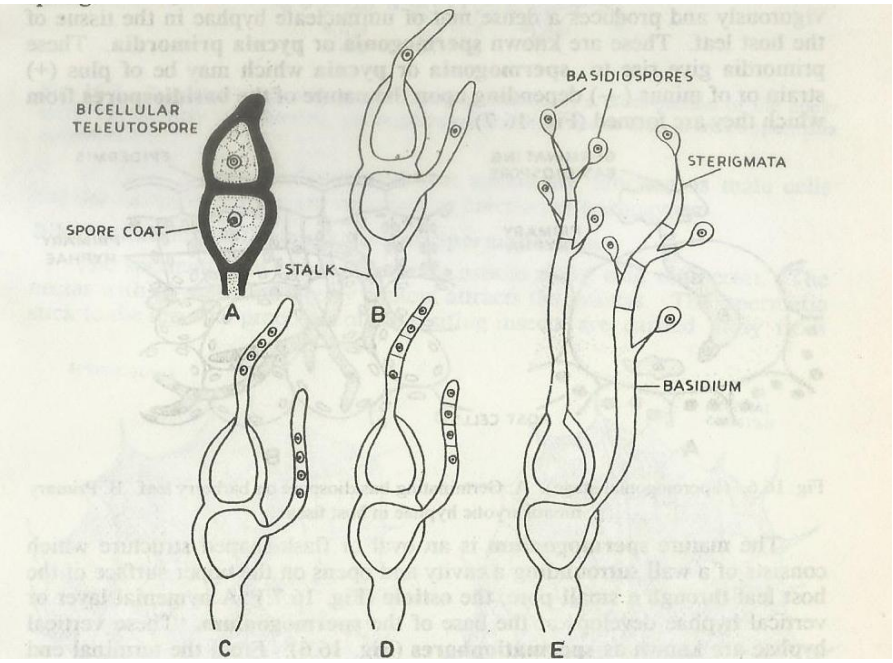
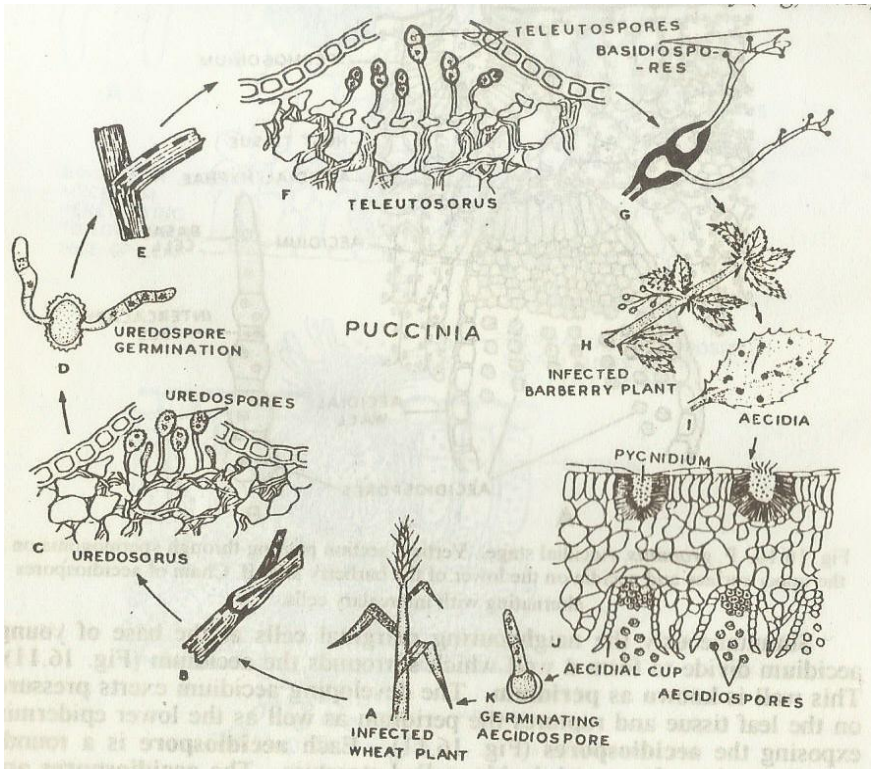
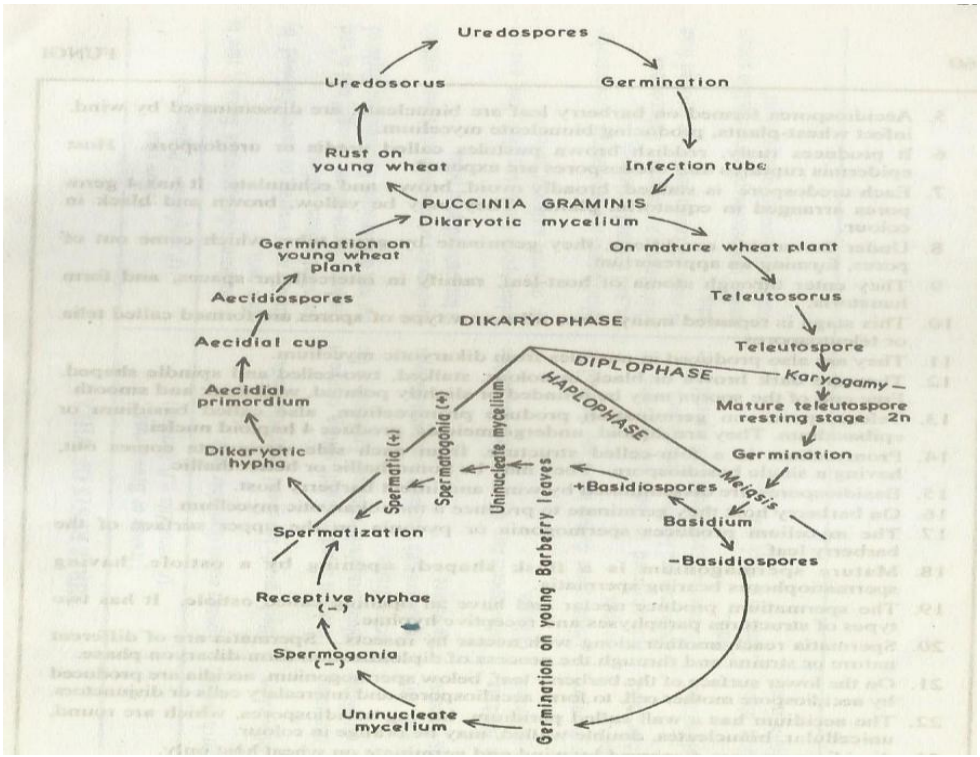


Fig.7.7: Showing bicellular teleutospore (A), Stages in the germination of teleutospore (B,C,D) and Germinated teleutospore (E)



8(A)



8(B)

Fig.7.8: (A) & (B) Showing Life Cycle of *Puccinia graminis triticii*

(B) General account- *Agaricus*

*Agaricus*is a saprorphytic fungus and commonly called mushroom. It forms fairy rings. *Agaricus spp*. are both poisonous and edible, some common species are as follows:

- *Agaricusbisporus* edible mushroom
- *A. campestris* field mushroom
- *A. sylvastris*
- *A. rodmani* lawn mushroom

Genus in the field is recgonised by its fruiting body called basidiocarp. These are umbrella like stalked structure having stalk (stipe) and a cap (pielus). Species are rich in some of vitamins and minerals.

Fig.7.9. Showing Fairy ring of *Agaricus*

A.bisporous

A. campestris

A.***silvastris***

A. rodmani

Fig.7.10. Showing structure of *Agaricusbisporous, A. campestris, A. sylvastris and A. rodmani*

7.4.2 Habit and habitat

Species of *Agaricus* grows on damp, moist places, rotten wood, dead and decaying organic matters, on manure piles and on well manured grasses i.e. Lawns.

7.4.3 Structure

Vegetative bodies are made up of mycelium whose hyphae pass through two phases.

Phase I – Is represented by monokaryotic mycelium. This is primary mycelium whose hyphae are short lived much branched septate. These hyphae are loosely tangled and grow below substratum. Cells contain highly vacuolated granular protoplasm. These are uninucleate and have oil globules. This mycelium is produced by the germination of basidiospores. Primary mycelium contains two types of strains represented by + ve and – ve signs.

Phase II – is represented by the mycelium whose cells are binucleate. This develops after the dikaryotization of cells of hyphae of primary mycelium.

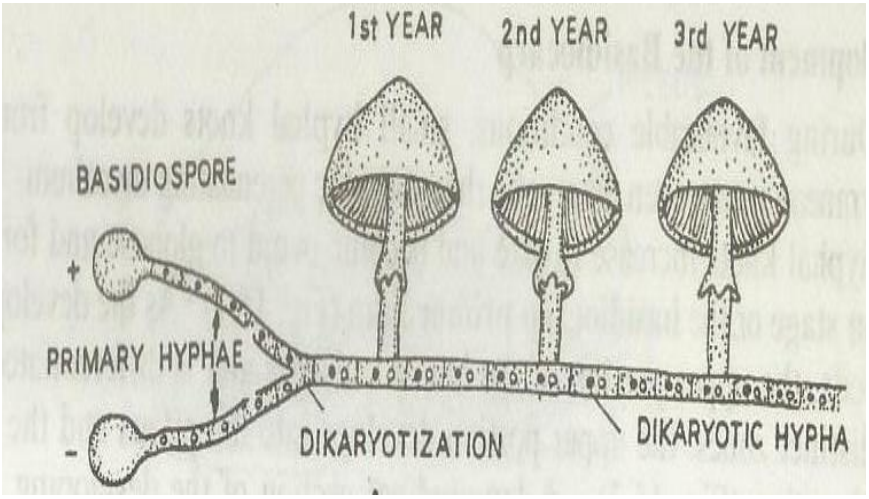


Fig.7.11. Showing hypha bearing fruiting bodies of 1st, 2nd and 3rd year

These are persistant and survive on the substratum for very long duration. It has a tendency to grow in all direction. It consist of septate, profusely branched hyphae. Hyphae have dolipore septum through which connectivity of cells is maintained. It forms rhizomorphs and fruting body i.e. basidiocarps (**Fig.7.9, 7.10, 7.11**).

7.4.4 Reproduction

Agaricus reproduce by asexual method of reproduction by forming chlamydospores. Sexual reproduction result in the formation of basidiospores after plasmogamy, karyogamy and meiosis.

Plasmogamy by somatogamy between the cells of compatible hyphae. Most of the species are heterothallic. Heterothallic species may be both bipolar or tetrapolar. *A. compestris* is bisporous and homothallic.

Some times dikaryotization may also occur due to oidization i.e. fusion of protoplast of cells of hyphae and an oidium of opposite strains (+ve and –ve). The new dikaryotic cell grows, ramifies and forms dikaryotic mycelium. The mycelium spreads below the substratum and at certain places forms small knot like structures called hyphal knots.

When the surface of substratum is moist the hyphal knots come above the substratum as small globose or ovoid structures constituting the 'button stage' of the life cycle.

Button grows and its upper end enlarges resulting into the distinct regions. The upper and low parts. The upper part developing into pileus or cap and lower into stipe or stalk. At the junction of upper and lower parts to cavities appear consisting of downwardly growing hyphae.

These cavities are called pre lamellar cavities. From the roof of these cavities the downwardly growing hyphae push themselves into the gill cavities to form a series of radiating plates called gills. The margin of the button is connected with the stalk by a membrane called veil or velum.

Due to the growth of gills and stalk the velum bursts and the two cavities colasces with each other and a ring like remnant called annulus attached to the upper part of the stipe.

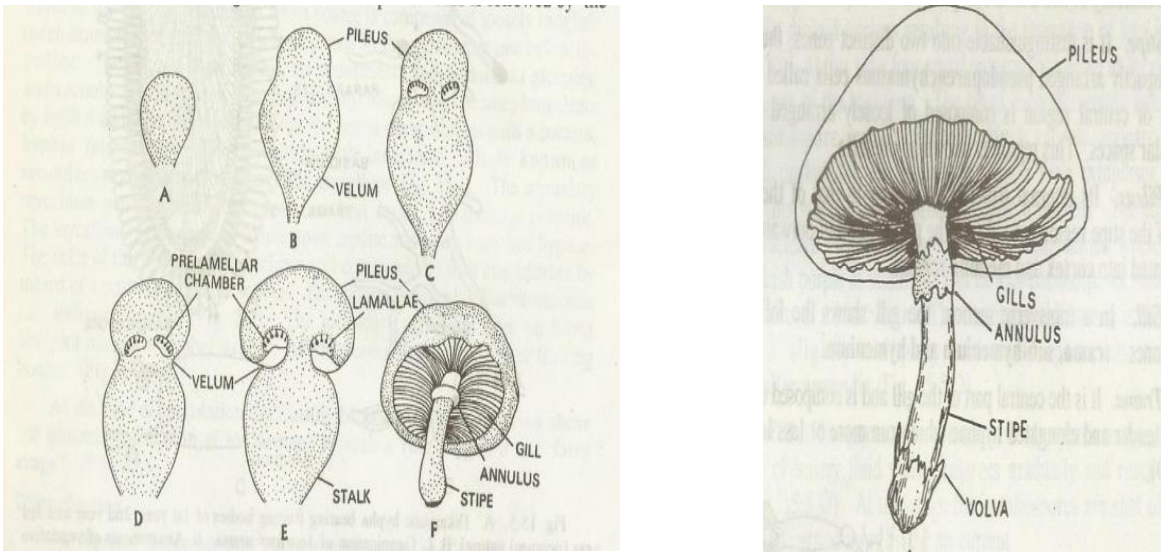


Fig.7.12. ShowingDevelopment of basidiocarp. A,B,C,D,E. Development stages of sporophore. F. Mature basidiocarp

A mature basidiocarp consists of a fleshy cylindrical pinkish while coloured stipe holding the umbrella like pileus. At the way of stipe an annulus a ring or collar like structure (remains of velum) is found. Pileus has a diameter of 2-4 inches. Its upper surface is convex light brown colored. Lower surface is concave having a large number of vertical plate like structure called gills or lamellae. Lamellae radiate from the stipe towards the edge of pileus. Pink in colour when young but as they mature they become dark brown in colour. Each gill is covered on both sides by a spore bearing layer, the hymenium (**Fig. 7.12**).

V.L.S. of gills shows following structure (**Fig.7.13**)

- Trama
- Sub – hymenial layer
- Hymenial layer

Trama: It is the innermost or middle part of the gill consist of interwoven mass of slender and elongated hyphae. Hyphae run parallel to each other. Hyphae of trama after attaining certain growth bend outwards and terminates in a layer of small rounded cells forming sub – hymenial layer.

Sub-hymenial layer on both sides have smaller pseudoparenchymatousplectenchymatous cells.

Hymenial layer: The cells are elongated club shaped dikaryotic cells. These are of two types. One called basidia and other is cystidia. Karyogamy takes place in mature basidium making it diploid. The diploid nucleus undergoes meiosis resulting ito four haploid nuclei. At this stage four small outgrowth appears over the surface of the basidium called sterigmata. The tip of each sterigmata swells. The four nuclei are pushed along with a little of cytoplasm into these swelling due to the formation of a large vacuole in the basidium. Now the swelling on sterigmata are called basidiospores. Basidiospores are rounded , haploid, unicellular at first pink colour later on purple brown in colour. Thus, from each basidium, four basidiospores are produced, the two belongs to (+) strain and the other two to (-) strains (**Fig.7.14**).

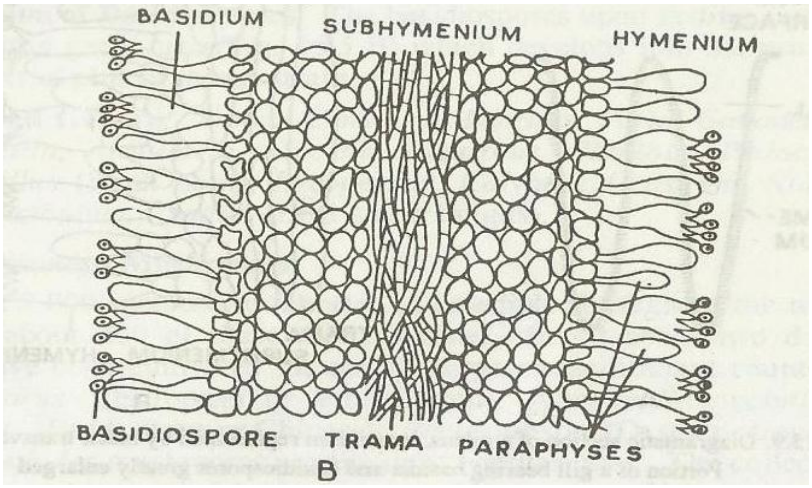


Fig.7.13. Showing Section of hymenium

Discharge of spores takes place by water droplet method. When the basidiospores are matured small projection called hilum appear at the junction of body of basidiospores and strigma. A small water drop about one ourth of the size of basidiospores grows and then the basidiospore is suddenly shot away from the sterigmacarriying the drop with it.

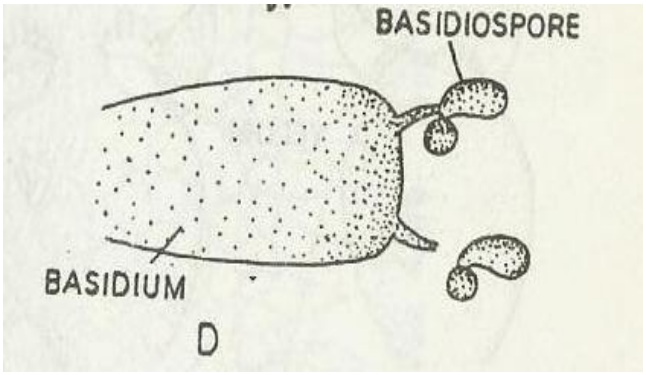


Fig.7.14. Showing Basidium with a pair of sterigmata, one bearing a basidiospore from hilum of which a drop pf fluid has been exuded just before spore discharge. From the second sterigma, spore has been shot off

When the basidiospores fall on suitable substratum, germinate to form the primary monokaryotic mycelium either or (+) strain or of (-) strains **(Fig.7.15)**.

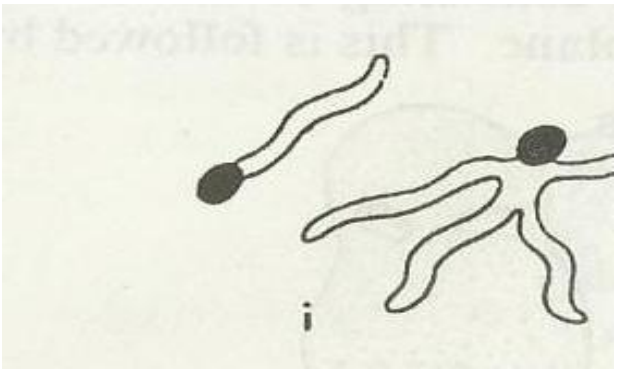
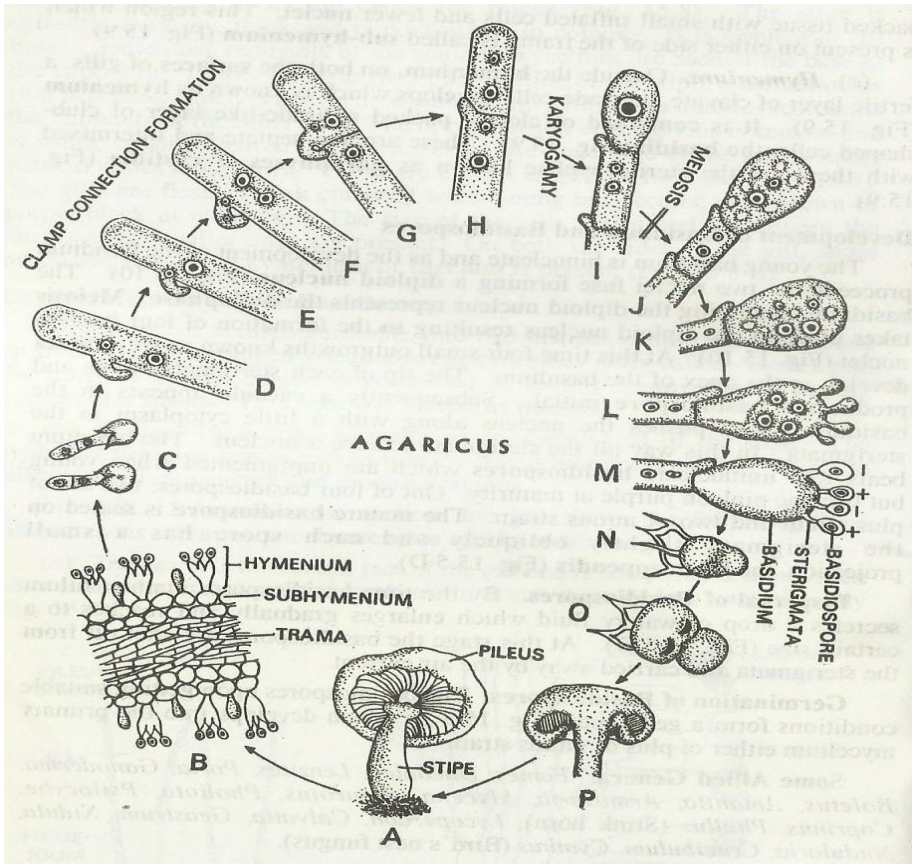
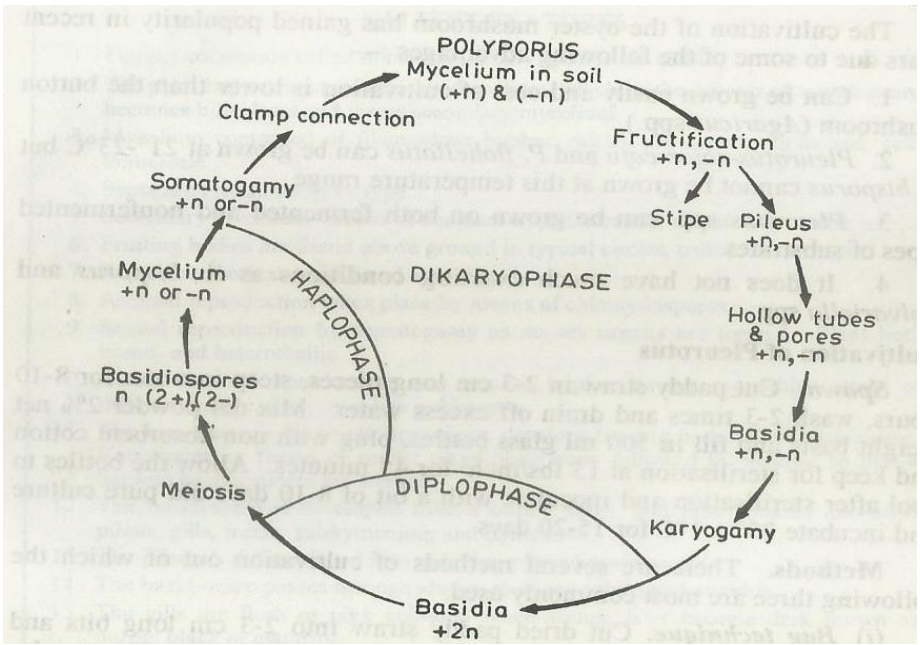


Fig.7.15: Showing germination of Basidiospores



(i)



(ii)

Fig.7.16. Showing Life cycle of *Agaricuscampestris*

7.5 DEUTROMYCOTINA

Alternaria

7.5.1 General account

In this, student will learn about the characteristic of Deuteromycotina with particular reference to *Alternaria.*

Deuteromycotina includes those fungi which reproduce only by asexual methods producing asexual spores called conidia. That is why Deuteromycotina is called **fungi imperfectii**. Their conidia are singly or in groups and are formed on four different types of structures. These are called **Synnema, Sporodochium, Acervulus** and **Pycnidium**.

Systematic position of *Alternaria* is as follows:

- Mycota
- Eumycotina
- Deuteromycotina
- Moniliales
- Dematiaceae
- *Alternaria*

7.5.2 Habit and habitat

It is a cosmopolitan strongsaprobic and weak parasitic form genus. Some common species are as follows:

A. solani causing Early blight of potato.

A. tenuis causing black spot disease on wheat

A. Brassicae & *A. brassicola* both cause **leaf spot of crucifers** i.e. cabbage, mustard, cauliflower etc.

Some of the parasitic species cause Hay-fever and few saprobic species are common contaminants, also occur on culture media and dead plant parts.

A.solani on potato leaflets produce spots which give a blighted appearance. Spots are small isolated scattered pale brown to dark coloured and are angular or oval in shape. There is narrow chlorotic zone which gradually invade the normal green area of the leaflets (**Fig.7.17**).

Fig.7.17: Showing early blight symptom on potato leaf and potato skin

7.5.3 Structure

Mycelium consists of hyphae which are light brown coloured at early stages but latter on they become dark brown, slender, septate, profusely branched. At early stages of growth the hyphae are intercellular latter on become intracellular within the host cells.

7.5.4 Reproduction

The genus*Alternaria* also reproduce only by asexual spores called the conidia. These are produced on conidiophores. The conidiophores are straight or flexuous, short, dark coloured. They come out of the host through stomata or through dead, damaged parts of the host surface. There are multi septate conidia and are borne on the conidiophores not by construction and subsequent enlargement of a terminal cell but from a bud which is formed on that cell.

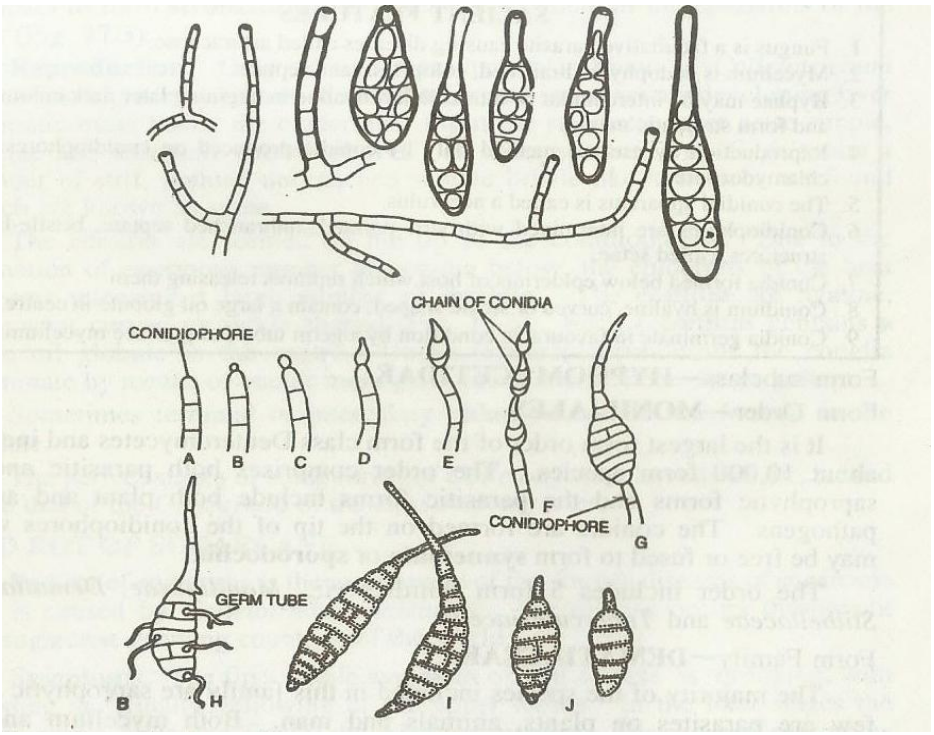


Fig.7.18: Showing stages in the development of mycelium, conidiophores and conidia of *Alternaria alternate, A. solani*(G-H), *A. brassicae*(I)and *A. brassicola*(J)

Conidia are large, dark coloured, obclavate(rounded base and pointed tip) terminating into a beak like structure. Conidia are divided by septa. Septa are transverse as well as longitudinal. Matured conidia get detached from conidiophores and are disseminated by wind. In the presence of moisture they germinate quickly by sending 5-10 germ tubes (**Fig.7.18, 7.19**).

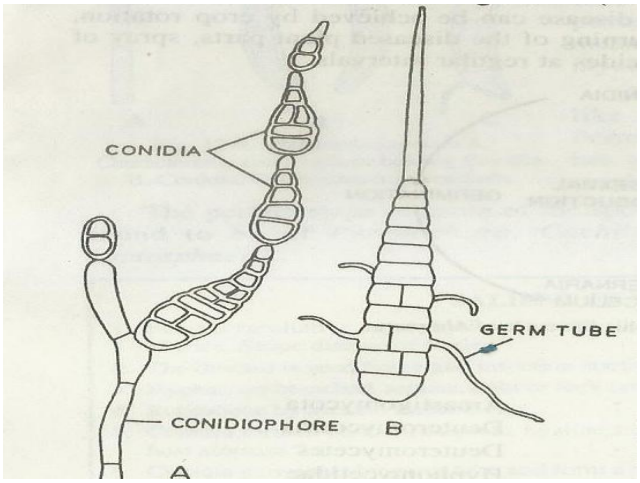


Fig.7.19: Showing conidiophores and conidia of *Alternaria*

7.6 MYCOPLASMA- GENERAL ACCOUNT

Systematic position (Frenndt, 1955)

- Kingdom: Bacteria
- Phylum: Tenericutes
- Class: Mollicutes
- Order: Mycoplasmatales
- Family: Mycoplasmataceae
- Genus: *Mycoplasma*

Nocard and Raux French scientists in 1898 observed association of Mycoplasma with a disease called bovine pneumonia. This is highly contagious disease. It was only in 1956 the International Committee of Nomenclature of Bacteria placed them in a seprate class Mollicutes on the basis of absence of a rigid cell wall. So these are bacteria like organisms lacking cell wall and posses a three layered cellular membrane. Mycoplasma is made up of two words:

Greek Mykes + Plasma

Fungus + Forms (Albert Bernhard Frank, 1889)

Mycoplasma (Tulian Nowak)

These are also called Pleuropneumonia like organisms (PPLO) because these organisms resembled with the causal organism of Pleuropneumonia in cattles.

Importance

Species are saprophytic or parasitic. Parasitic spp. have been reported to cause a large number of diseases in humans and animals. Some are as follows:

- *M. genitalium* – Pelvic inflammatory infection.
- *M. pneumonia* – primary atypical pneumonia in man.
- Bovine pleuro pneumonia in animals
- Alfa yellow disease

- Papaya bunchy top disease

Morphology and Structure:

Because they lack rigid cell wall, they do not have a definite shape. It changes from rounded to oblong, and thus, they are called pleomorphic. These organisms are unicellular, prokaryotic, normally non motile and have fried egg shaped colonies.

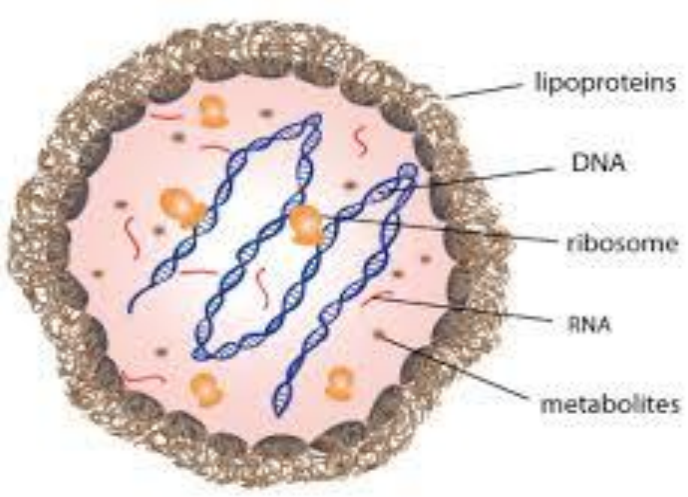


Fig.7.20: Showing structure of Mycoplasma

They can pass through bacterial filters. Cells are delimited by a lipoproteinaceous unit membrane which is triple layered i.e. Plasma membrane. Both DNA (base) compounds from 23 to 36 mole percent GC (cf. L-forms).

RNA and ribosomes are present. Cells are resistant to antibiotic like penicillin. Cells are inhibited by tetracyclines because they react on metabolic pathways. Size varies (300nm to more) and diameter about 0.2µm. Internal structures is those of prokaryons and generally resembles with true bacteria. Growth requirements are eg. Carbohydrates and Arginine. Metabolism substrate is fermentation. Motility is gliding or non- motile. Although they resembles with prokaryotes but they have one feature common with Eukaryotes i.e. have sterols lipid, cholesterol and cholesterol esters that are characteristic of animal cells and found in mycoplasmas. These are absent in the cells of Bacteria (**Fig.7.20**).

Reproduction:

Reproduction takes place by binary fission, budding or forming small spherical elementary units within the cells. These minute bodies may form chains or filaments of minute spheres,which are small in size. After liberation these minute bodies enlarge in size. Inside these new elementary bodies are formed. These bodies liberate after the rupture of membrane of large body and become free.

7.7 SUMMARY

1. *Puccinia* (Rust) is an obligate parasitic, heteroceious polymorphic and macrocyclic fungus.
2. *Puccinia* has mycelium consist of primary monokaryotic hyphae and secondary dikaryotic hyphae.
3. Reproductive structure borne in sporangium (receptive hyphae and spermatia).
4. Plasmogamy is by spermatization. Aecia are first binnucleate cells of the life cycle. Uredospores are one celled and teleutospores are bicelled. Karyogamy takes place in teleutospores and meiosis in promycelium/probasidium. Basidiospores are haploid one celled. Out of four basidiospores two are of one strain(-) and two are of (+) strain.
5. *Agaricus* (Mushroom) is saprobic. Species growing in well mannured lawns, on wood and in field. *****Some species are edible. *Agaricus*consists of mycelium which is primary, secondary and tertiary. Primary mycelium is monokaryotic. Tertiary mycelium forms basidiocarps. Basidiocarps is umbrella liked structure. Sexual reproduction results in the formation of haploid basidiospores.
6. *Alternaria*is saprobic and pathogenic fungus. It is a common contaminants of laboratories.
7. Mycoplasma are prokaryotic organism resembles bacteria in many characters except few. The most striking difference is absence of cell wall.
8. They resemble eukaryotes in having sterols in cytoplasmic membranes, which stabiles the membrane and protect the cells against the osmotic lysis. Cholesterol and cholesterol esters characteristic of animal cell are found in mycoplasma.
9. Absence of cell wall provides resistance against enzyme and penicillin. One species *M.gallisepticum* is different from other mycoplasma in many properties eg. in having membrane bound vesicle (bleb).
10. Reprduction is by binary fission, budding or formation of special elementary units within the cells.

7.8 GLOSSARY

Agaric: common name for any member of the order Agaricales(Basidiomycota); a mushroom.

Alternate host: used in reference to one of the hosts of a heteroecious rust fungus; the host upon which stages 0 and 1 are produced.

Annulus: the ring found on the stalk of certain species of mushrooms; a remnant of the inner veil.

Antibiotic: a substance produced by living organism which injuries or kills another living organism.

Basidiocarp: a fruit body that contains basidia.

Basidiospores: a spore borne on the outside of a basidium, following karyogamy and meiosis.

Basidium: a structure bearing on its surface a definite number of basidiospores (typically four) that are usually formed following karyogamy and meiosis.

Basipetal: a chain of spores with the youngest at base or proximal end of the chain(cf. acropetal)

Bipolar heterothallism: used to describe a type of sexual compatibility that is controlled by one pair of genes.

Clamp connection: a bridge like hyphal connection characteristic of the secondary mycelium of many basidiomycota; involved in maintaining the dikaryotic condition.

Coenocytic:nonseptate; referring to the fact that nuclei are present in the cytoplasm without being separated by cross-walls, that is, the nuclei lie in a common matrix.

Colony: a group of individual of same speices living in close association; in fungi, the term usuallyrefers to many hyphae growing out of a single pointand forming a round or globosethallus or a groupof cells that presumably are derived from a single cell.

Compound oosphere : an oosphere with many functional gamete nuclei in Oomycota.

Conidiogenous cell: a hyphal compartment or cell from which a conidium is formed.

Conidiophore: a simple or branched hypha arising from a somatic hypha and bearing at its tip or side one or more conidiogenous cells; previously used interchangeably with conidiogenous cell.

Conidium: a nonmotile asexual spore usually formed at the tip or side of a sporogenous cell; in some instances a preexisting hyphal cell may be converted to a conidium.

Deuteromycetes: Imperfectfungi that only reproduce asexually.

Dictyospore: a pair of closely associated, sexually compatible nuclei; each may or may not be derived from a different parent hypha or cell.

Dioecious: refers to species in which the sexes are segregated in different individuals; the use of this term is often restricted to plants.

Epigean: above the ground (cf. hypogean).

Fairy ring: a ring of mushrooms produced at the limits of the underground mycelium.

Frucification: any complex fungal structure that contains or bears spores; a sporocarp.

Gametangial contact: a method of sexual reproduction in which two gametangia come in contact but do not fuse; the male nucleus migrates through a pore or fertilization tube into the female gametangium.

Gametangial copulation: a method of sexual reproduction in which two gametangia or their protoplasts fuse and give rise to a zygote that develops into a resting spore.

Germ tube : the hyphal structure that first emerges from a germinating spore in most fungi; germ tubes either develop into hyphae or, in the case of some pathogenic species, give rise to specialized infection structures.

Haustorium :an absorbing organ originating on a hypha of a parasite that penetrates the host cell wall and invaginates the host cell plasma membrane; most often formed by obligate parasites.

Heterothallic:self-sterile (self-incompatible) individuals requiring the union of two compatible thalli (of different mating types) for sexual reproduction; obligate outcrossing (cf. homothallic).

Heterothallism: the condition exemplified by heterothallic species.

Host: a living organism harboring a symbiont (usually in a parasite connotation).

Hyaline: transparent or translucent.
Hymenomycetes: general term used to refer to Basidiomycotatht produce their basidia in adefinite layer or hymenium.
Hyperplasia: excessive multiplication of cells, abnormal rate of cell division.
Hypertrophy: excessive enlargement of cells.
Hypha:the unit of structure of most fungi; a tubular filament.
Hypogean: growing below ground(cf. epigean).
Imperfect fungi: see deuteromycetes.
Imperfect state: the asexual (usually conidial) state of afungus; also known as anamorph in a life cycle with a sexual state (telelomorph) (cf. perfect state).
Indirect germination: in Oomycota, germination of sporangia by zoospores; in Ascomycota and Basidiomycota, germination of spore to form a secondary spore without germ tube formation.
Karyogamy: the fusion of two nuclei (cf. plasmogamy).
Macrocyclic: a rust fungus that typically exhibits all five stages of the rust life cycle.
Macrocyclicconidiation: formation of conidia following the development of a mycelium from a germinated spore.
Medium: substratum of a balanced chemical composition employed in the laboratory for growing microorganisms; media may be used in the liquid state or solidified with agar, gelatin, or other solidifying agents.
Monokaryotic: condition of having a single nucleus in each hyphal compartment (cf. homokaryotic; dikaryotic).
Muriform: term used to describe a spore with both transverse and longitudinal septa, as in a dictyospore.
Mushroom: a fleshy, sometimes tough, umbrella like basidiocarp of certain Basidiomycota.
Mycelium: mass of hyphae constituting the body(thallus) of a fungus.
Mycology: the study of fungi.
Oidium: a thin walled, free, hyphal dead cellderived from the fragmentation of somatic hypha into its component cells or from an oidiophore; it behaves as a spore or as a spermatium; normally used in reference to such cells produced by certain Basidiomycota.
Oidization: the union of an oidium with a somatic hypha, resulting in the dikaryotization of the latter.
Oogamous: refers to a type of fertilization in which two heterogametangia come in contact, and the contents of one flow into the other through pore or tube.
Oogonium: a female gametangium containing one or more eggs.
Ooplast: membrane bound cellular incision in the oospore of some of Oomycota.
Oosphere: a large naked, nonmotile, female gamete.
Oospore: a thick walled spore that develops from an oosphere through either fertilization or parthenogenesis.
Parasite: an organism that lives at the expense of another, usually invading it and causing disease.
Perithecium: a closed ascocarp with a pore at the top, a true ostiole, and a wall of its own.
Plasmogamy: the fusion of two protoplasts (cf. karyogamy).
Primary appendage: an outgrowth that develops from the ascospore cell in Laboulbeniales.

Primary septum: a septum formed in association with a nuclear division; laid down between daughter nuclei.

Promycelium: a germ tube issuing from the teliospore in which meiosis takes place an that bears basidiospores; technically, the metabasidium.

Pycnidiospore : a conidium borne in a pycnidium.

Pycnidium: a hollow conidioma lined with conidiophores.

Pycniospores: another designation for the spermatium of the rust fungi.

Pycnium : another designation for the spermogonium of the rust fungi.

Receptive hypha: the structure with which a rust spermatium fuses.

Saprobe: an organism that utilizes dead organic matter for food.

Secondary heterothallism: apparent homothallism that actually involves the presence of two compatable mating types in a single spore, also known as amphithalism.

Secondary zoospore: kidney-shaped zoospore produced in Oomycota; the flagella are inserted laterally on the spore (cf. primary zoospore).

Self-compatible: self-fertile; refers to a thallus that reproduces sexually without outcrossing.

Self-incompatible:self sterile; refers to a thallus that cannot reproduce sexually without outcrossing.

Septal pore cap: see dolipore septum.

Septate : with more or less regularly occurring cross-walls.

Septum: a cross-wall in a hypha that develops centripetally.

Somatic: refers to the assimilative phase in fungi; a structure or function as distinguished from the reproductive; also referred to as vegetative.

Somatogamy: fusion of somatic cells during plasmogamy.

Species: a taxonomic rank; a group of closely related individuals resembling one another in certain inherited characteristics;it is designated by a binomial consisting of the generic name and the specific epithet.

Spermatiopores: a specialized hypha that produces spermatia.

Spermatium : a nonmotile, uninucleate, sporelike male structure that empties its contents into a receptive female structure during plasmogamy; spermatia are regarded variously as gametes or gametangia.

Spermogonium : a structure resembling a pycnidium and containing minute, rod shaped, or oval spore like bodies that in some cases have proved to be functional spermatia.

Sporangiophore: a specialized hypha that bears a sporangium.

Sporangiospore : a spore borne wihin a sporangium.

Sporangium: a saclike structure, the entire protoplasmic contents of which become converted into an indefinite number of spores.

Spore:a minute propagative unit functioning as a seed, but differing from it in that a spore does not contain a preformed embryo.

Stipe : the stalk of a stipitatebasidiocarp or ascocarp.

Substrate: any substance or material from which a fungus can obtain nutrients.

Trama: the fungal tissue composing the pileus or bearing the hymenium of some basidiomycota.

Urediniospore:a binucleate, repeating spore of Uredinals or rust fungi.

Uredinium: a group of binucleate cells that give rise to urediniospores; produced on tissues of a host plant.
Vegetative hyphae: hyphae present in some basidiocarps; they lack septa and are calssified as either skeletal or binding hyphae(cf. generative hyphae).
Yeast: Economically important single-celled fungus that reproduces by budding or fission. Saccharomycetales, often designated as true yeasts, do not produce an ascocarp; the term yeastlike often is used also to refer to other groups with budding cells.
Zoosporangium: a sporangium that contains zoospores.
Zoospore: a motile, asexually produced spore.
Zygophore : a special hypha capable of developing into a progametangium in Zygomycota.
Zygosporangium: a sporangium containing a zygospore; develops following the fusion of two gametangia.
Zygospore: a resting spore that results from thefusion of two gametangia in Zygomycota and apparently some chytriomycota.
Zygot: a diploid cell resulting from union of two haploid cells.

7.9 SELF ASSESSMENT QUESTION

7.9.1:Tick the right answer:

1. *Puccinia graminis* is:

(i) Autoecious (ii) Heteroecious
(iii) Both autoecious and Heteroecious (iv) none of the above.

2. How many stages are there in the life cycle of *Pucciniagraminis*?
(i) one (ii) two
(iii) three (iv) five

3. Alternaria belongs to class:
(i) Ascomycetes (ii) Basidiomycetes
(iii) Zygomycetes (iv) Deuteromycetes

4. Fairy rings are found in:
(i*) Pezziza* (ii) *Agaricus*
(iii) *Aspergillus* (iv) *Mucor*

5. Sterile cell between the basidia in *Agaricus* are called:
(i) Disjunctor cell (ii) Cystidia
(iii) Paraphysis (iv) Albuminous cell

6. Apical cell of conidium of *Alternaria*:
(i) Branched (ii) Unbranched
(iii) coenocytic (iv) Septate

7. Basidiocarp of *Agaricus* is like:
(i) Fan (ii) Umbrella
(iii) Stick (iv) Ball

8. Which layer in a V.L.S. of gill of *Agaricus*is fertile:
(i) Hymenial (ii) Sub-hymenial
(iii) Excip… (iv) None of the above

9. Out of five types of spores of *P. graminis* which is dikaryotic and bicelled:
(i) Uredospore (ii) Teleutospore
(iii) Aeciospore (iv) Basidiospore

7.9.2: Fill in the blanks:

1……..disease caused by different species of *Puccinia.*
2. At the ……..stage of life cycle of *P. graminis* in which karyogamy take place.
3. *Agaricus* is also called……
4. Conidia of *Alternaria* are ……
5. PPLO stand for……
6. Mycoplasma differs from prokaryotes in……
7. List some disease caused by Mycoplasma……

Answers Keys: 7.9.1:

1. (ii), 2. (iv), 3. (iv), 4. (ii), 5. (ii), 6. (iii), 7. (ii), 8. (i), 9. (ii)

Answers Keys: 7.9.2:

1. Black rust, yellow rust, stripe rust 2. Telial, 3.Mushroom, 4.Multicellular, 5.Pleuropneumonia like organisms, 6. Usually requires sterol for growth 7. Alfaalfa, Witches broom, Papaya bunchy top, Clover Phyllody, Grassy Shoot of Rice etc.

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7.12 TERMINAL QUESTIONS

1. How many stages are there in the life cycle of *Pucciniagraminis*. Give their names and host on which these stage are found.

2. Describe the life cycle of *Pucciniagraminis* with the help of figures only.

3. Differentiate between uredospores and teleutospores.

4. Draw well labeled diagrams of section passing through uredia, telia, aecia and pycnidia.

5. What are fairy rings?

6. Draw morphology of basidiocarp of *Agaricus*.

7. Draw V.L.S. of gill of *Agaricus*.

8. What is button stage in the life cycle of *Agaricus*.

9. Name few species of *Alternaria.*

10. Write asexual reproduction in *Alternaria*.

11. Give general account of Mycoplasma.

UNIT-8-OCCURRENCE, GENERAL STRUCTURE, NUTRITION, REPRODUCTION, ECONOMIC AND ECOLOGICAL IMPORTANCE OF LICHENS

8.1-OBJECTIVES

After reading this unit student will be able:

- To know about lichens and their occurrence.
- To learn the basic growth forms of lichens: crustose (crusty), fruticose(shrubby), and foliose (leafy).
- To know general parts of the lichen.
- To understand the methods of reproduction in lichens.
- To study the economic importance of lichens.

8.2-INTRODUCTION

A lichen seems like a single organism, but actually it is a combination of two plants an alga and a fungus which live together in a symbiotic relationship. The algal component in the lichen is known as phycobiont and fungal component is called as mycobiant. The 90% part of the thallus (body of the lichen) is produced by the mycobiant. In a lichen, the mycobiant produces a thallus and provides colour, shape and structure to the lichen with little contribution from algae. This fungal component mostly belongs to the group Ascomycetes, Deuteromycetes, or Basiodiomycetes of fungi. 95% of lichen species belong to the Ascomycetes. While 3% is represented by Basidiomycetes and 2% by Deuteromycetes. The Phycobiont (algal part) belong to the family Chlorophyceae or Myxophyceae. The alga supplies nutrients by photosynthesis, while the fungus protects the alga from excessive Sun rays and supplies water by absorbing water vapor from the air. It may be a type of relationship called a 'symbiosis' where both partners get benefitted.

History of Lichens: The word Lichen appeared 1600, and was drawn from the Greek 'leikhen', meaning 'what eats around itself'. The term 'Lichen' and this group of plants were introduced by Theophrastus, the father of botany. Those who studied lichen are called lichenologist. In 1867 Swiss botanist Simon Schwendener first proposed the theory of the duality of the lichen thallus. According to his theory, alga and fungus share a relationship as helotism where the Alga was slave providing nutrient to fungal master. In 1887 De-Bary used the term Symbiosis for association of lichen. Schwendener's dual theory of lichens has been accepted by every one for which experimental proof has been obtained.

8.3-OCCURRENCE

Lichens are very common and widely distributed from the arctic to Antarctic regions of the world. They can survive in extremes of hot and cold weather. They are mostly xerophytic in nature and can endure long periods of drought, tough conditions of deserts and frozen soil of the artic region. Lichens may become dormant in unfavourable environmental conditions for some time and then they become metabolically active again on return of more friendly conditions. These plants can be found most frequently on stems, bark and trunks of trees,

rocks, soil, lands, stones etc. Lichens do not have roots, thus they can grow in areas where no other vegetation is possible such as concrete, sand, stable rock surfaces etc. A few are unattached and blow about freely. Lichens are favoured by sufficient humidity, sunlight, still conditions and clear air. Most of the lichens generally depend on atmospheric sources for nutrition in feeding itself through photosynthesis in the algal cells.

8.4-GENERAL STRUCTURE

Classification of lichen:

(A) Lichens are classified on the basis of the nature of fungal part:

(i) Ascolichens: In this, the fungal component belongs to Ascomycetes. Such lichens are divided into two sub-groups-

(a) Gymnocarpeae: In which fruiting body (i.e. ascocarp) is apothecium. e.g.- *Parmelia*

(b) Pyrenocarpeae: In which the ascocarp is Perithicium type. e.g.- *Dermatocarpon*

(ii) Basidiolichens: in this, fungal component belongs to basidiomycetes. e.g. *Dictonema, Corella.*

(B) Wall Roth classified lichen into two types on the basis of Algal component:

(i) **Homiomerous:**In this, the algal component is distributed throughout the structure and the fungal part grows outside the thallus as a thin protective layer. e.g.- *Collema*

(ii) **Heteromerous:** The algal component in a heteromerous is confined to a specific region. The heteromerous thallus can be differentiated into four distinct layers, three of which are formed by the fungus and one by the alga. e.g. *Parmelia, Xanthoria.*

(C) Lichens are classified on the basis of Growth forms:

(i) Crustose: These lichens have a flattened thallus, resembling crusts, generally grown on rocks or occasionally on the bark of trees holding tightly to their substrates and it is difficult to remove them without crumbling away. e.g.- *Haematomma lecanora, Graphis, Lacidia* etc.

(ii) Foliose: These lichens have a flat, expanded, leaf like thallus which spread out in a horizontal layer over the surface. They are attached by root-like threads and can be easily dismantled without damaging the substrates. e.g. - *Physcia, Parmelia, Gyrophora* etc.

(iii) Fruticose: These lichens have a thallus that is branched and bushy and can hang from the substrate. It may be erect or pendant. They can be removed from the surface by hand. e.g.-*Cladonia rangiferina, Usnea barbata* (**Fig.8.1**).

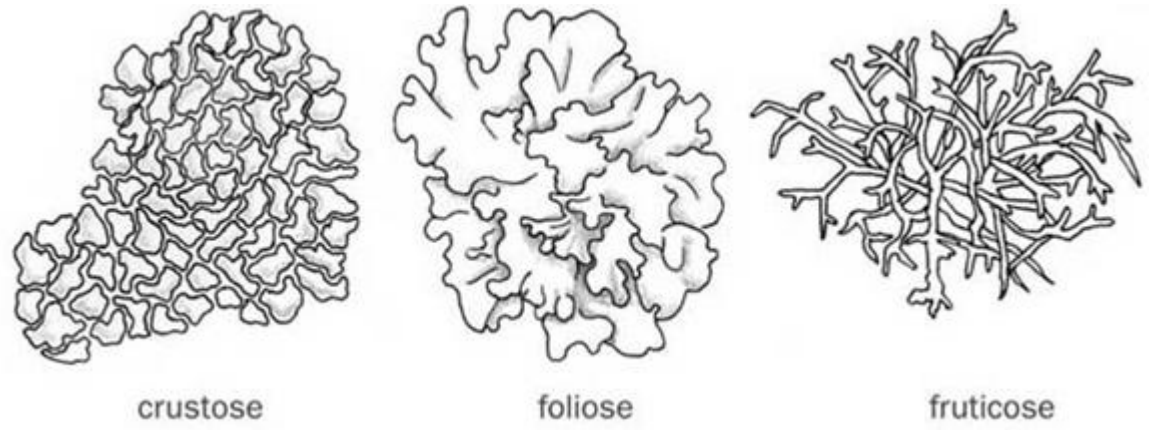


Fig.8.1- Lichens

Internal Structure of Lichens:

(a) Upper cortex: It forms the upper most layer which is generally thick and protective in nature and consists of more or less vertical fungal hyphae. The fungal hyphae are compactly interwoven without any intercellular spaces to produce a tissue-like layer which is known as Plectenchyma or Pseudoparenchyma.

(b) **Algal Zone:** This zone lies beneath the upper cortex. This zone generally consists of blue-green algae. In this layer algal cells intermingled with loosely interwoven fungal hyphae. The algal zone is the photosynthetic region of the lichen thallus and was known as gonidia.

(c) **Medulla:** It is the central core of the thallus and is composed of loosely arranged fungal hyphae with intercellular spaces. It works as a water reservoir. Usually, the wall of the fungal hyphae is thick and strong. The hyphae run in all directions.

(d) **Lower cortex:** The lower cortex is below medulla. It is formed by fungal component and made up of compact hyphae. They may be parallel to perpendicular to the surface of the thallus (**Fig.8.2**).

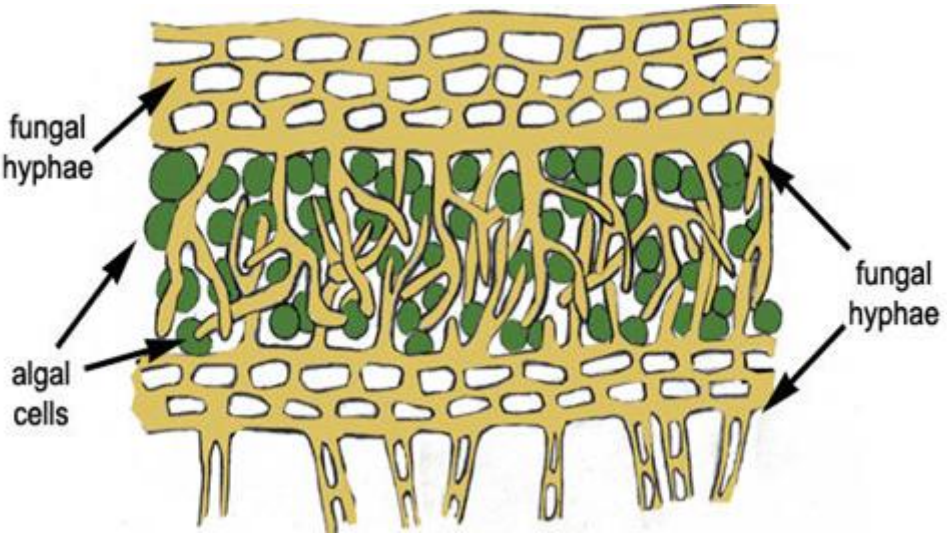


Fig.8.2: Internal structure of Lichen

Anatomy of the lichen thallus:

The vegetative structures which are associated with the lichen thallus are:

(i) **Breathing Pores:** These are localized openings which develop in the upper cortex. The breathing pores serve for aeration and helps in respiration.

(ii) **Cyphellae:** The cup-like white spots present on the lower cortex in some foliose lichens are known as cyphellae. These cup-like breaks are formed of loosely arranged medullarly fungal hyphae. Their function is aeration.

(iii) **Cephalodia:** Cephalodia are small, dark-coloured, hard, gall-like swellings found in some species of lichens that contain cyanobacterial symbionts, the cyanobacteria may be held on the upper or lower surface of lichen thalli. The cephalodium consist of fungal hyphae and a few algal components.

(iv) **Isidia:** A coralloid out growths on the surface of a lichen thallus consisting of both fungal hyphae and algal cells. The main functions of isidia is supposed to increase the photosynthetic surface of the lichen thallus. In terms of structure, isidia may vary in form in different lichen species as- cylindrical, warty, cigar shaped, clavate (club-shaped), scale-shaped, coralloid (coral-shaped), rod-shaped etc (**Fig.8.3, 8.4**).

Colour: Lichens show many colours. It can be red, oranges, yellow, green, white, grey etc. The colours vary due to presence or absence of special pigments.In the absence of special pigments, lichens are generally bright green to olive gray when it is wet and grey or grayish-green to brown when dry. This is because the cortex becomes more transparent and the underlying green photobiont layer becomes visible. Colours vary due to genetics, age and on the angle of exposure to light. Most widespread special pigments such as pale yellow usnic acid, give lichens a variety of colours as yellow, red, and orange.

8.5-NUTRITION

Lichens get mineral nutrients from whatever they are growing upon. The fungus uses the hyphae to absorb food from its surroundings. The fungus gets benefits from the symbiotic relation because algae or cyanobacteria produce food by turning sunlight into energy through photosynthesis, water and minerals in their environment. Cyanobacteria can make amino acids directly from the nitrogen gas in the atmosphere. Fog, dews, play an important role in atmospheric nutrition of lichens. It has been observed that a dry lichen thallus can take in as much as 3-35 times their weight in water.

8.6-REPRODUCTION

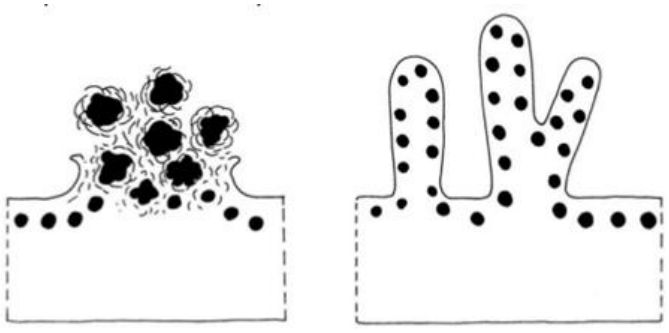
The lichens reproduce by following methods:

Vegetative Reproduction: Lichens may reproduce vegetatively by several methods.

(i) **Fragmentation:** A fragment broken off from a lichen thallus may grow into a new thallus. On maturity or accidental breakage the older portions of the thalli of lichens divide into pieces and each piece develops into a new plant. This is a means of vegetative propagation. The new thallus being genetically identical to the thallus from which the fragement came.

(ii) **Isidia:** Isidia are tiny, simple, branched, spiny, elongated out growths from the thallus that break off or scattered by animals, wind and rain to new locations. Each isidium is composed of a few algal cells surrounded by fungal cells. Each isidium grows into a new lichen thallus under favourable conditions.

(iii) **Soredia:** These are minute, powdery granules or bud-like out growth present on the upper surface or edges of the thalli of many species of lichens. At times the soredia form a grayish layer of dusty mass which is known as the soredial dust. Each soredium consisting of one or a few algal cells surrounded by fungal hyphae. Soredia can be dispersed easily by wind and contain everything needed to produce new thalli.



Soredia **Isidia**

Fig.8.3: Vegetative structure

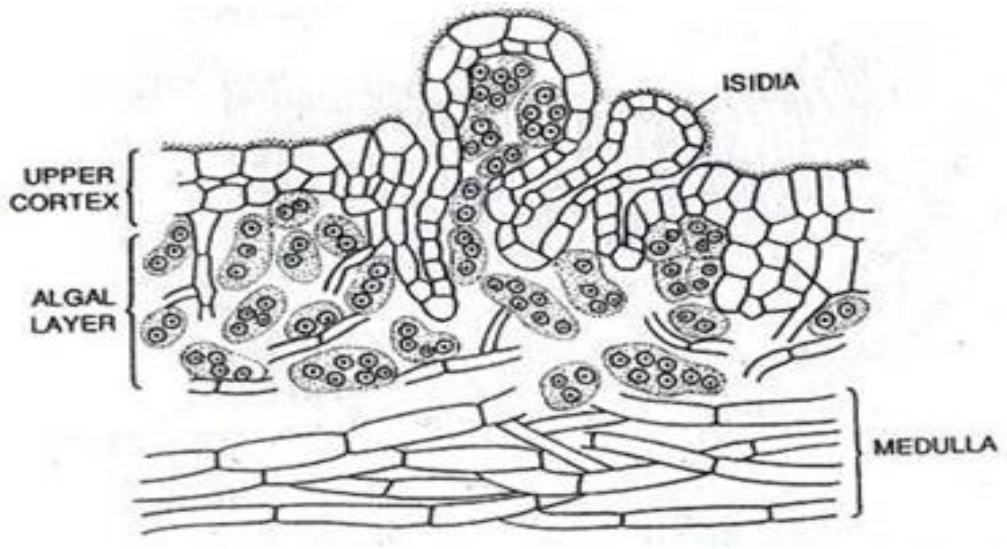


Fig. 8.4: Isidia (*Peltigera sp*.) V.S.thallus

Asexual Reproduction: Certain lichen produces large number of small non-motile spore-like structures, pycnidiospores. They are formed within conical, flask- shaped cavities known as pycnidia. The pycnidia are found sunken on the upper surface of certain lichens and each pycnidium opens to the exterior by a small pore called an ostiole. In certain species of lichens, the pycnidiospores are capable of germination. Each produces a fungal hyphae and when it comes in contact with suitable algal cell, it develops into a new lichen thallus.

Sexual Reproduction: Only the fungal partner of the association reproduces sexually. The male reproductive organ is called spermogonium and the female is called as carpogonium or Ascogonium.

i) Spermogonia: The spermogonia develop in flask-shaped cavities on the upper surface of the thallus. It opens to the exterior by small pore, an ostiole. A number of hyphae develop from the walls of the cavity. Few of them are sterile and others are fertile. The fertile ones produce the non-motile male cells called spermatia. These non-motile cells develop continuously from the tips of the fertile branches. The spermatia are set free in a slimy mass through ostiole.

ii) Carpogonia: The carpogonium develops from hypha deep in the algal layer. It consists of two portions, the upper straight portion which is known as trichogyne and the lower coiled portion which is called ascogonium (oogonium). It is multicellular and the cells are uninucleate or multinucleate in some species. The basal cell of the ascogonium is fertile.

Fertilization: A spore called conidium is released from a pycnidia structure. Pycinidia are flask-like structures embedded in the thallus of the lichen. Conidia can act as "spermatia" in sexual reproduction of the lichen. The spermatia are functional male gametes. The spermatium spore finds its way to a tiny thread (trichogyne) on a surface of lichen and attaches itself. The conidia and the trichogyne both are haploid. The growing trichogyne comes in contact with spermatia. The intervening walls between the spermatium and the trichogyne dissolve at the point of contact. The male nucleus gradually passes downward to the oogonium, where it fuses with the female nucleus. The actual migration of the male

nuclei down the trichogyne has not yet been observed, but it is assumed. Fused cell produces ascogenous hyphae within which develop 8 ascospores and asci. The hynenium is made up of Asci and Paraphysis. The fruiting body may be either apothericia. e.g. -*Parmelia* and Physcia or Perithericia e.g.-*Peltigera.*

In lichens, fruiting bodies are of following two types:

(i) Apothecia: Apothecia are variable in shape but commonly wide, open, saucer-shaped or cup shaped fruit body. Sometimes these may be plate-like and rarely it has an elaborate form. The structure of the apothecium mainly consists of three parts-Hymenium (upper convex surface), Hyopothecium, and Excipulum. The asci are present in the hymenium layer. The hymenium, composed of sac-like asci and sterile, hair-like fungal hypae known as Paraphyses. Asci and Paraphyses form a thin inner lining, which is called as hymenial layer. Each ascus contains eight ascospores. From outside apothecium generally seen to consist of two parts, the margin and the disc. Apothecia have two main types of margin: a) A proper margin consisting of fungal hyphae only; generally, the proper margin is sometimes conspicuous and of different colour as compare to the disc, but more often is similar in colour to the disc, different from the thallus.The outer margin of an apothecium is called the exciple. The lecideine type of apothecium has only a proper margin where the exciple forms the underside and outer layer of the apothecium, extending up to the rim, where it forms the proper margin consisting of fungal hyphae only. A thalline margin, which includes photobionts, different from the disc (with several exceptions e.g., in *Caloplaca*). When the excile has a color similar to colored thallus tissue the apothecium is called lecanorine. In lecanorine apothecia, the thallus tissue s extend up the outside of the apothecium to form the exciple and rim. Generally this thalline margin retains the colour of the thallus (Fig.8.5).

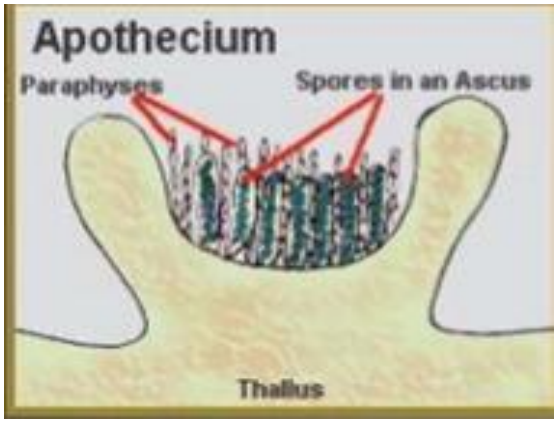


Fig.8.5: Apothecium

(ii) Perithecia: Perithecia are usually flask-shaped fruiting bodies containing the asci immersed in the lichen thallus tissue. Here the spore bearing body is much smaller in size than the apothecia and appear like black dots on the lichen surface. At maturity, a small opening at the top which is called an "ostiole", allows the ascospores to escape. Perithecium producing lichens are called Pyrenocarpic e.g., *Margacea* (Fig.8.5).

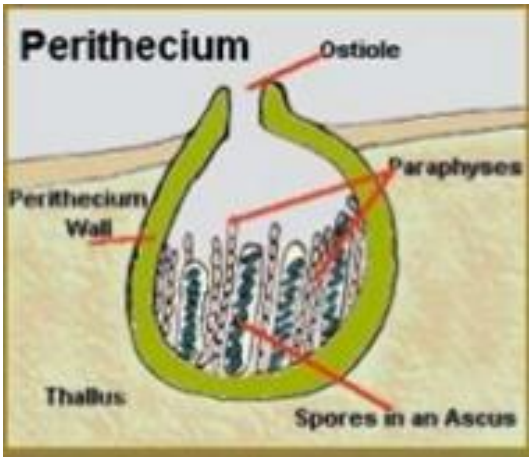


Fig.8.6: Perithecium

Basidiolichen: A very small number of lichens have the fungal part which belongs to the Basidiomycetes with fruiting bodies, basidiocarps. Basidiocarp is a sexual fruiting body produced by a member of Basidiomycota, and could take any of a diversity of shapes a club-like, sheet-like, bracket-like etc. The club-shaped basidium carries sexual spores called basidiospores. The lower surface of the thallus bears sub-hymenium. Each basidium produce four spores on the tips of minute stalks called Sterigmata.

8.7-ECONOMIC AND ECOLOGICAL IMPORTANCE

Economic importance: Lichens are very important economically. Following are some examples of there importance:

1- **Medicinal Use:** A few species of lichens have been used in folk medicines for centuries as a cure for diarrhea, fever, jaundice, skin diseases, epilepsy etc. It is known that acids from various lichen can be useful in killing bacteria. *Labaria pulmonaria*is used in asthma and lung diseases, *Xanthoria parietina* for jaundice, *Peltigera canina* for dog bite. Usnic acid secreted from *Usnea barbata*, along with streptomycin is effective in tuberculosis. Lichen mass produces mucilaginous substance obtained from *Cetraria islandica* is used as laxative. *Cladonia pyxidata* is useful in whooping cough. Researches are yet to be conducted on many other medicinal benefits of lichens.*Peltigera canina* was used as medicine for hydrophobia in ancient time. *Parmelia saxatilis* is used to cure epilepsy. The Usnea and *Evernia furfuracea*are used as astringents.

2- **Dyeing agent:** Some lichens are useful as a natural source of dyes. The fungal components of certain species of lichens produce coloured pigments specially the red, brown, orange etc which can be extracted by boiling and used to dye fabrics and wood. Many lichens such as *Pocelia tinctoria* gives purple dyes which is used for centuries. Orchil is a blue dye obtained from *Cetraria icelandica* and *Lecanora* sp.which is used to dye wollen and silken clothes. Litmus is obtained from *Rocella*

montaignei which is a commonly used dye in chemical laboratories as an acid-base indicator prior to the invention of the pH meter.

3- **Food value:** Certainspecies of lichens are consumed as food because they contain lichenin, a carbohydrate. Some lichens like *Cetraria islandica,Umbillcaria, lecnora* used are as food by human beings. *Cetraria islandica*is used as food by Eskimos and others. A species of Parmelia popularly known as rock flower (called as "rathapu" in telugu and "kallu huvu" in kannada) is used in curry preparation and is famous for its delicacy. It is prized as food in Southern India. *Evernia prunastri* was used byEgyptians as baking powder. Umbillicaria has been used as food in eastern Siberia. *Lecanora esculenta*, commonly called Manna lichen is used in desert tribes of Asia minor.

4- **As Fodder:** Lichens is important for some species of animals and small insects like snails, mites, caterpillar etc as source of food. Fruticose Lichen *Cladonia rangiferina* commonly known as Reindeer grass is used as food for horses and other animals. The other common used species for animals as fodder are *Labaria pulmonaria,Ramalina fastigiata,Ramalina fraxinea* etc. Many lichens are consumed as food for insects and their larvae.

5- **Chemical Uses:** Lichen is useful in the brewing, distilling and tanning. Some Lichen species contain tannis and used in leather tanning industry. The lung wort lichen is used in tanning and brewing.

6- **Perfumes:** Sweet-scented thalli of some lichens are used in making perfumes, scents, dhup, hawan samagris etc. It can retain the power of retaining the odor it is used. A lichen which is popularly known as an oak moss is used in perfumes as a fixative in Southern Europe. It is reported that some lichens like *Ramalina* and *Evernia* have perfumed volatile oils, which is used in manufacture of comestics.

Harmful effects of Lichens:

1- Lichens may have adverse effects on plants. Small fruit trees, Sandal wood trees, small shrubs densely covered with lichens could be damaged. Many epiphytic lichens can have harmful effects on the host plant.

2- A very few lichens are poisonous. These lichens are known to contain vulpinic acid and usnic acid, e.g. vulpicida and Letharia. These lichens are yellow due to high concentrations of bright yellow toxin vulpinic acid. The wolf lichen (*Letharia vulpine*) got its name because it was used in Europe to poison wolves. So many lichen which is yellow in colour may have possibility to be poisonous.

3- In dry season sometimes long threads of pendant lichens as *Usnea barbata* help in spreading of forest fire.

Ecological importance

Lichen is regarded as the "Pioneers of vegetation". They are capable of colonizing bare rocks. These plants play very important role in the ecological formation of soil. The organic acids secreted by lichens gradually dissolve and disintegrate the rocks into soil particles over which they grow. Mosses are the successors of the crustaceous rocks. When the lichens die and

decay they contribute organic matter to the soil, improve the soil fertility so that other plants can grow on it. Having root-like structure lichens can anchor themselves to the soil. When there is occasional rainfall, comes the shower of rain is absorbed by the lichen thalli and often slow down the flow of water. It works as a barrier between the intense down pours and the soil. Lichens can enrich the soil by trapping water to support a active life over long dry spells.

Lichen as Bio-indicators or pollution indicators: Lichens are able to survive in extreme climates but they are very sensitive to air pollution. Fruticose lichen is the most vulnerable and Crustose is the least vulnerable lichen type to air pollution. Because lichens are pollution- sensitive so they can provide the valuable information about the environment. Lichens absorb everything from the air, including chemicals like carbon, sulfur, heavy metals into their thallus. Environmental scientists can extract the toxins from lichens and monitor the intensity of air pollution. Presence of lichens in abundance in a particular area is a indicator of non-polluted environment of the area whereas the declined growth of lichen at a site is an early warning sign of air pollution.

A Source of Nitrogen: Nitrogen is a nutrient which is important for living organisms. Some lichens are able to convert nitrogen in the air into nitrates and then secrete it into the soil. When it rains, nitrate is secreted into the soil and it can be useful for plants.

8.8- SUMMARY

Lichens are the combination of two organisms, an alga and a fungus, living together in symbiotic association. Fungi provide the body of organism while the algae or cynobacteria provide the food. The lichen thalli are usually of three types- Fruticose, foliose and crustose. Internally the thallus consists of four regions, the upper cortex, the lower cortex, the algal layer and the medulla. Lichens reproduce by the three methods- sexual, asexual and vegetative reproduction. Most of the lichens depend on atmospheric sources for nutrition. These plants have had a variety of uses over the years. They are of considerable ecological and economical importance and are useful in soil formation, habitat for other organisms, bioindicators for pollution, a source of nitrogen, useful in making perfumes, medicines, dyes, and important food source.

8.9- GLOSSARY

Apothecium: A disc-shaped structure that contains the asci, especially in lichens, a type of ascocarp.
Ascocarp: mature fruiting body of an ascomycetous fungus.
Ascospore: A meiospore borne in an ascus.
Ascus: The sac or bag-like structure in which ascospores are formed.
Conidium: An asexual fungal spore.
Cynobacteria: blue-green algae.
Filamentous: Stringy or matted hair like.

Gelatinous: Jelly like.
Hyphae: Fungal filaments collectively called hyphae which form a thallus.
Leprose: Powdery.
Mycobiant: The fungal partner in a lichen.
Perithecium: A flask shaped sexual reproductive structure that produces spores.
Phycobiont: algal partner in a lichen.
Pycnidia: Flask-shaped structures which produce conidia.
Rhizines: root-like fungal structures is termed rhizines or rhizinae which bind the thallus to its Substrate.
Symbiotic: Where both the partners get the mutual benefit by living together.

8.10- SELF ASSESSMENT QUESTION

8.10.1: Multiple choices Questions-

1) The condition in which two different organismsliving together and get the mutual benefit is known as:
(a) Parasitic (b) Heterotrophic
(c) Symbiotic (d) Saprophytic

2) Generally each ascus contains:
(a) 16 ascospores (b) 4 ascospores
(c) 2 ascospores (d) 8 ascospores

3) Which of the lichen is called as the "reindeer moss":
(a) *Peltigera canina* (b) *Evernia*
(c) *Cetraria islandica* (d) *Cladonia rengiferina*

4) The female reproductive organ in lichens is called:
(a) Carpogonium (b) Paraphysis
(c) Spermagonium (d) Trichogyne

5) Lichens are described as indicators of:
(a) Water pollution (b) Xerophytic succession
(c) Air pollution (d) None

6) Vegetative reproduction in lichen takes place by:
(a) Fragmentation (b) Soredia
(c) Isidia (d) All of these

7) In sexual reproduction, the fruiting body formed is called:
(a) Perithecium (b) apothecium
(c)both a and b (d) None

8) The study of Lichen is called:
(a) Lichology (b) Mycology
(c) Lichenology (d) None of these

9) Sexual reproduction in lichens is carried out by:
(a) Fungal part (b) Algae part
(c) Both a and b (d) None

10) Litmus is obtained from:
(a) *Usnea* (b) *Cladonia*
(c) *Roccella* (d) *Peltigera*

ANSWERS KEY: (A): 1- (c), 2-(d), 3-(d), 4-(a), 5-(c), 6-(d), 7-(c), 8-(c), 9-(a), 10-(c)

8.11-REFERENCES

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8.12-SUGGESTED READINGS

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8.13-TERMINAL QUESTIONS

Q.1-Write short notes on-
a) Structure of Lichens
b) Classification of Lichens

Q.2- What is Lichen? Describe in detail the internal structure of lichen.

Q.3 Discuss the reproduction of Lichen in detail.

Q.4 Describe the economic and ecological importance of Lichen.

Q.5 Discuss lichen ‘as a pollution indicator’.

BLOCK 3 – PLANT PATHOLOGY

UNIT-9-INFECTION, DISEASE RESISTANCE AND GENERAL SYMPTOMS

9.1 OBJECTIVES

After reading this unit student will be able:

- To study the living, nonliving and environmental causes of disease and disorders inplants.
- To study the mechanisms of disease development by pathogens.
- To study the interaction between the plant and thepathogen in relation to the overall environment.
- To develop systems of management of the disease and reducing the losses caused by disease.

9.2 INTRODUCTION

Plants are primary producers of food. Man's other necessities(like fibers for clothing, timber for buildings and furnituremanmedicines for health, fuel for source of energy.

for different activities also) basically originate from plants. They represent the only machine known so far that can directly convert solar energy into food. Thus plants are the very pivot of our existence on earth and without plants the earth will be without life.

This unavoidabledependence of not only man but also other forms of life whichhelp man has its own implications which man has to reckon with. In the ancient times there was not much struggle for food since the population was low and the available per capita in plenty. In the light of above consideration of plants and man relationship and increasing demand on food sources, it is worthwhile to consider how food production can be increased by at least 75% to meet the food demand of the population to be fed in the next 20 to 25 years. At the present time, nearly 350 crore acres of land of about12% of the earth's total surface area is cultivated and used for essential crop production.

Modern technology and increase in the value of food crops have brought almost the maximum area under cultivation. Thus targets for increased food production per unit area already available for cultivation.

Limitations in management of large acreages, adverse weather andnatural calamities and most important of all, the attack of pests and disease are the main cause of reduced yields from otherwise high potential crop varieties.

What is plant disease?

When a plant is suffering, we call it diseased. However, this does not define the term 'disease'. Often the symptoms produced by a disease, the cause of the disease, and the injuries caused to the plant have been considered synonymous with the term disease. However, they signify only the condition of the plant due to disease or the cause of the disease.

Disease can be defined as a pathological process. Some of the recent definitions are –

"Disease is a malfunctioning process that is caused by continuous irritation which results in some suffering producing symptoms".

"Disease is of the normal chemical reactions that are inhibited or the abnormal chemical reactions induced inside the cells and in the tissues of the plant as a result of the irritation brought about by the causal agent".

When a plant is diseased it is at 'dis - ease'. It means that the plant is uneasy due to some condition to which its system is not accustomed. When a man is in uneasy position he usually loses his efficiency but sometimes he does not, and becomes accustomed to it and so maintains his efficiency.

Similarly, when a plant is at "unease" it may loss its efficiency or may not. For a practical plant pathologist (or for the farmer) it is the loss of efficiency of the plant that is important. Hence a practical definitionof plant disease can be as given condition of a plant when its systems are not normal and therefore it is not producing as good as it should according to normal expectations of the farmer. The cause of infection in plants may be different kinds of microorganisms (**Fig.9.1**).

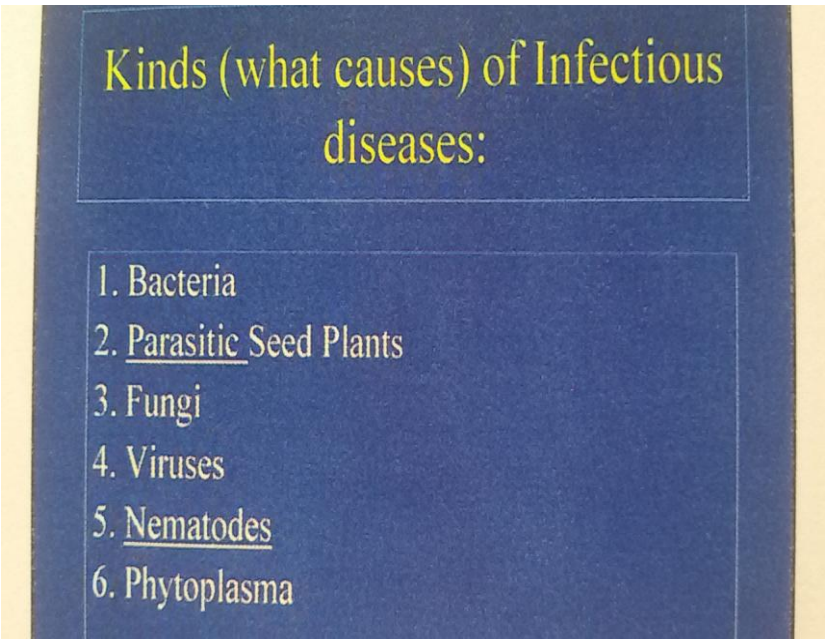

Fig.9.1: Causes of infection in plants

The Importance of Plant Diseases

Plant diseases are important because of the loss they cause. The loss can occur in the field or in the store and at any time between sowing and consumption of the harvest. Standing crop in the field is attacked by a disease and plants start drying or their capacity to yield satisfactorily is reduced. Thus farmer gets only a portion of the estimated yield.

Microorganisms destroy perishable food materials in the store. There is no doubt that a big portion of this stored food does not reach to the mouth of hungry millions.In the history

of mankind, plant diseases have been connected with a number of important events. Some of the important events are mentioned below.

The late blight of potato is a famous example of what a plant disease can do to change thecourseofhistory.In1845thisdiseasedestroyedthepotatocropofIreland. In England, IrelandandcertainpartsofcontinentalEuropepotatowasstapledietofthepopulation. Thisdisease hadstartedinthesecountriesasearlyas1830andeveryyear it wascausingsome damageresultinginfoodshortage.InEngland,freetradeandimportoffoodgrains wasnotpermitted. Whentheepiphytoticoflateblightdestroyedpotatocropin1845,therewasfamineinthesecountrieses peciallyinIreland. In this country alone, the population of 80 lacs was reduced to 60 lacs.

A large number of people died of hunger and many more become diseased due to physical weakness. This single disease forced man to realize the importance of plant disease. As a result, scientific investigations were taken up, the cause of the disease was identified and extensive use of chemicals for plant disease control came into existence.

Wheat rust has been another disease that has appeared in epiphytotic form from time to time in many countries. This disease forced the farmers in many parts of the world to change their cropping patterns. In the last years of the Second World War (1943) Bengal had to face a serious famine. One of the reasons to which this famine has been attributed is the loss in yield of rice due to attack of Helminthosporium leaf spot which had been affecting the crop for the last several years.

These instances of plant disease epidemics are worth mentioning because they left their effects not only in the country concerned but also in other countries. Ordinarily plant diseases affect every crop, every year and in all parts of the world. This results in huge loss of potential production, and money. In addition to direct loss in yield sand monetary returns, the plant diseases affect the society in many other ways. When food grains are attacked by certain fungi they may contain toxins which cause insanity, paralysis, stomach disorders etc, in human beings and animals. The money spent in the control of plant disease is also a loss which could be saved in absence of disease.

Agriculture based industries are also affected by plant diseases. Industries that consume raw materials produced in agriculture (cotton, oilseeds etc) face difficulty in utilizing their capacity when there is less production due to plant diseases. In order to make up for the loss of food production, the governments have to import food which means loss of foreign exchange at the disposal of government. Excessive use of pesticides, weedicides leads to environmental pollution which affects health of human beings.

9.3 -STAGES INDISEASE DEVELOPMENT: DISEASE CYCLE

In an infectious disease there is a series of more or less distinct events which occur in sequence and lead to development and perpetuation of the disease and the pathogen (**Fig.9.2**). This chain of events is called a Disease cycle. The disease cycle involves the changes in the plant and symptoms as well as those in the pathogens, and spans

periods within a growing season and from one growing season to the next. The main events of a disease cycle include.

[I] inoculation [II] prepenetration [III] penetration [IV] infection (also includes invasion)[V] growth and reproduction of the pathogen [VI] dissemination of the pathogen and[VII] seasonal carry-over of the pathogen.

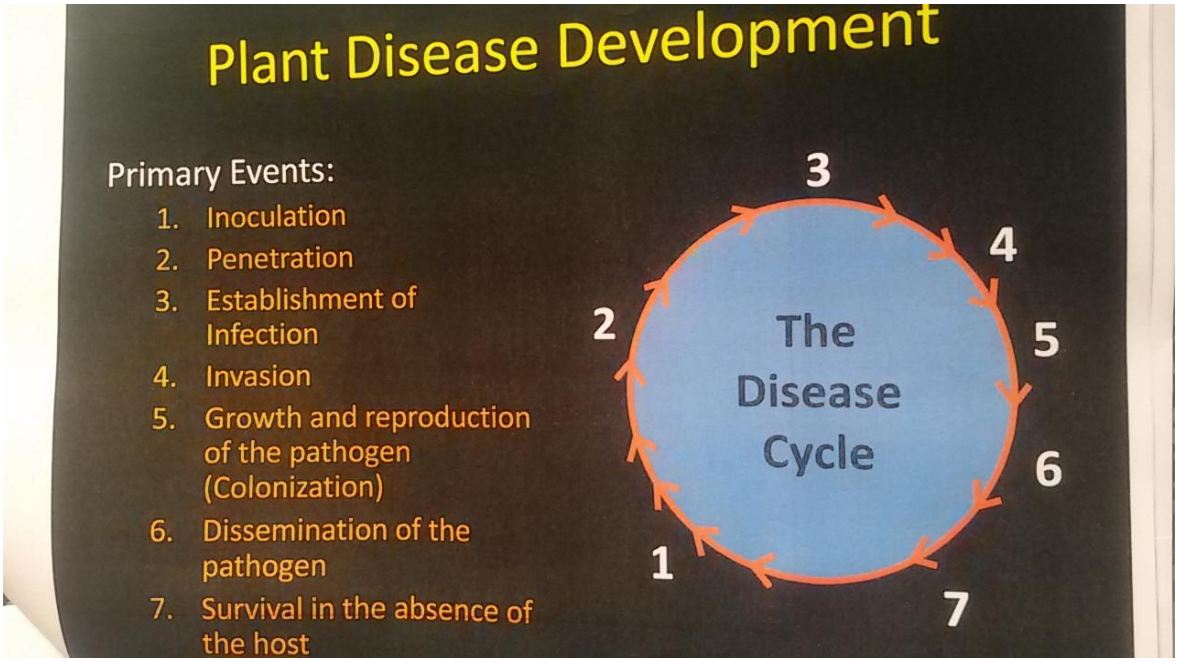

Fig.9.2: Various events of disease development

1. **Inoculation–** Inoculation is the contact of a pathogen with a plant. This is the inoculum that lands on or otherwise brought into contact with plant. Inoculum is any part of the pathogen that can cause infection. In fungi, inoculum may be spores, sclerotia or mycelial fragments. In bacteria, mycoplasmas, viruses and viroids, inoculum is always whole individuals. In nematodes, inoculum may be adults, larvae or eggs, whereas in parasitic higher plants it is plant fragment or seed. Inoculum that survives the off season periods (winter or summer) and causes the original infection in the growing seasons is called primary inoculum, and the infections as primary infections. Inoculum produced from these primary infections. It actually spreads the disease in the field under favourable conditions, and is called secondary inoculum. This brings about secondary infections. Inoculum in the absence of its host from the field survives in plant debris, field soil, seed, tubers, transplants or other plant parts, perennial weeds, alternate hosts. The inoculum is carried to host plants and this landing or arrival of inoculum is possible by wind, water, insects etc or in some cases also by active growth as in some root-infecting fungi like *Armillaria mellea*.

2. **Prepenetration-** This phase include all the events prior to actual entry of the pathogen. Such events include (i) germination of spores and seeds, (ii) hatching of eggs (nematodes), (iii) attachment of pathogen to host, and (iv) recognition between host and pathogen (early event-not still understood clearly). Lack of specific recognition factors in plant surface may not allow the attachment of pathogen to it.

Such factors in plant include lectins (proteins or glycoproteins) and some oligo-and polysaccharides.

3. **Penetration-** This is the actual entry of the pathogen into their host plants. Pathogens penetrate plant surfaces in different ways: (i) Direct penetration through intact plant surfaces. (ii) Through natural openings, and (iii) Through wounds.

4. **Infection-** Infection is the process by which a pathogen establishes contact with host cells or tissues and procures nutrients from them. Penetration does not always lead to infection and many organisms actually penetrate the cells but plants may not become diseased

These organisms are not able to proceed beyond penetration. However effective pathogens are able to invade further and cause infection. Various pathogens invade hosts in different ways and through different extents. Some fungi produce mycelium which grows only in the area between the cuticle and epidermis, other produce mycelium only on the surface of host plant sending only haustoria into epidermal cells. Most fungi spread into all the tissues (Chiefly parenchyma of mesophyle and cortex) of plant organs (leaves, stems, roots etc.) they infect. They grow directly through the cell (intracellular mycelium) or between the cells (intercellular mycelium). The fungi that cause vascular wilts invade the xylem vessels of plants.

During infection pathogens grow and multiply within the plant tissues and invade the plant to varying extents. Individual fungi and parasitic higher plants generally invade and infect tissues by growing into them from one initial point of inoculation. For a pathogen, to infect a plant, it must be able to make its way into it and through the plant and obtain nutrients from it the plant and neutralize the defense mechanisms of the plant.

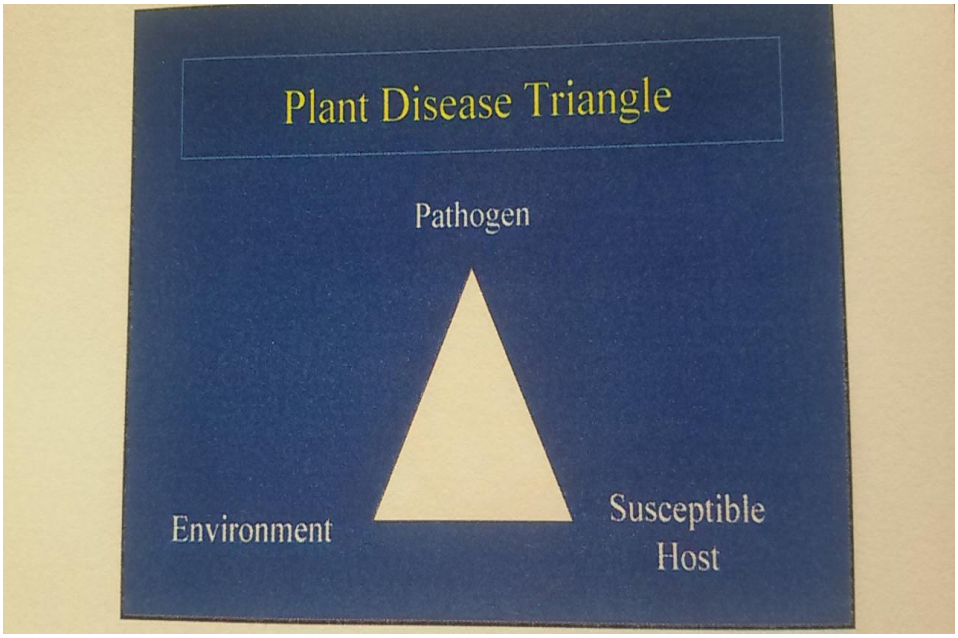

Fig.9.3: Showing three way interaction

9.4: PLANT DISEASE RESISTANCE

Plant disease resistance protects plants from pathogens in two ways: by performed mechanisms and by infection-induced responses of the immune system. For a susceptible plant, disease resistance is the reduction of pathogen growth on or in the plant, while the term disease tolerance describes plants that exhibit little disease damage despite substantial pathogen levels. Disease outcome is determined by the three way interaction of the pathogen, the plant and the environmental conditions -an interaction known as the disease triangle (**Fig.9.3**).

Defense activating compounds can move from cell to cell and systemically through the plant vascular system. However, plants do not have circulating immune cells, so most cell types exhibit a broad suite of antimicrobial defenses. Although obvious qualitative differences in disease resistance can be observed when multiple specimens are compared (allowing classification as "resistant" or "susceptible" after infection by the same pathogen strain at similar inoculum levels in similar environments), a gradation of quantitative differences in disease resistance is more typically observed between plant strains or genotypes. Plant consistently resist certain pathogens but succumb to other, as resistance is usually pathogen species- or pathogen strain-specific.

Different plants are resistant to certain diseases for various reasons. Some plants are immune to a particular pathogen even under most favorable conditions for disease development other exhibit some degree of resistance to a pathogen under most environmental conditions, still others are actually susceptible to the pathogen but under the conditions they are normally grown may appear resistant. Somevarieties show tolerance to a disease and can produce good crop in spite of infection.

Resistance:

Vertical (specific):

Resistance of a plant to a pathogen is provided by one or a few defense mechanisms controlled by one or a few genes respectively and is either monogenic (one gene) or oligogenic. Thus a given variety has resistance effective only against some races of a pathogen but not against other races.

Horizontal (general)

In horizontal type, resistance to a plant variety is provided by a combination of lesser defense mechanisms, each of which alone is rather ineffective against the pathogen.Such mechanisms are controlled by a group or groups of complementary genes. Thus, with horizontal resistance, a given variety of plant is more or less uniformly resistant to all races of a pathogen.

Plant breeding for disease resistance

The value of resistance in disease control was recognized in the early 1900s.Advances in genetics and dangers of pollution by chemicals provided additional impetus in this area.

There have been efforts throughout the world on breeding plants that combine the most useful genes for higher yields,better quality, uniform ripening and disease resistance. Thesources of genes for resistance areeither native or foreign commercial or, wild plant varieties etc.

Plant breeders emphasize selection and development of disease-resistant plant lines. Plant diseases can also be partially controlled by use of pesticides and by cultivation practices such as crop rotation, tillage, planting density disease-free seeds and cleaning of equipment, but plant varieties with inherent (genetically determined) disease resistance are generally preferred. Breeding for disease resistance began when plants were first domesticated. Breeding efforts continue because pathogen populations are under selection pressure for increased virulence, appearance ofnew pathogens, evolving cultivation practices and changing climate can reduce resistance and/or strengthen pathogens.Plant breeding for other traits can disrupt prior resistance. A plant line with acceptable resistance against one pathogen may lack resistance against others.

Breeding for resistance typically includes:

- Identification of plants that may be less desirable in other ways, but which carry a useful disease resistance trait, including wild strains that often express enhanced resistance.
- Crossing of a desirable but disease-susceptible variety to another variety that is a source of resistance.
- Growth of breeding candidates in a disease-conducive setting, possibly including pathogen inoculation. Attention must be paid to the specific pathogen isolates, to address variability within a single pathogen species.
- Selection of disease-resistant individuals that retain other desirable traits such as yield, quality and including other disease resistance traits.

Resistance is termed durable if it continues to be effective over multiple years of widespread use as pathogen populations evolve. “Vertical resistance” is specific to certain races or strains of a pathogen species, it is often controlled by single R genes and can be less durable. Horizontal or broad-spectrum resistance against an entire pathogen species is often only incompletely effective, but more durable, and is often controlled by many genes that segregate in breeding populations.

Crops such as potato, apple, banana and sugarcane are often propagated by vegetative reproduction to preserve highly desirable plant varieties, because their species outcrossing seriously disrupts the preferred traits. Vegetatively propagated crops may be among the best targets for resistance improvement by the biotechnology method of plant transformation to manage genes that affect disease resistance.

Gmor transgenic diseases resistance

The term GM (Genetically Modified) is often used as a synonym of transgenic to refer to plants modified using recombinant DNA technologies. Plants with transgenic/GM disease resistance against insect pests have been extremely successful as commercial product, especially maize and cotton, and are planted annually on over 20 million hectares in over 20

countries worldwide . Transgenic plant disease resistance against microbial pathogens was first demonstrated in 1986. Expression of viral coat protein gene sequences conferred virus resistance via small RNAs. This proved to be a widely applicable mechanism for inhibiting viral replication. Combining coat protein genes from three different viruses, scientists developed squash hybrids with field-validated, multiviral resistance. Similar levels of resistance to this variety of viruses had not been achieved by conventional breeding.

A similar strategy was deployed to combat papaya ring spot virus, which by 1994 threatened to destroy Hawaii's papaya industry. Field trials demonstrated excellent efficacy and high fruit quality. By 1998 the first transgenic virus-resistant papaya was approved for sale. Disease resistance has been durable for over 15 years. Transgenic papaya accounts for ~85% of Hawaiian production. The fruit is approved for sale in the U.S. Canada and Japan.

Potato lines expressing viral replicase sequences that confer resistance to potato leafroll virus were sold under the trade names Newleaf Y and Newleaf plus and were widely accepted in commercial production in 1999-2001, until McDonald's crop. decided not to purchase GM potatoes and Monsanto decided to closer their Nature Mark potato business. Newleaf Y Newleaf Plus potatoes carried two GM traits, as they also expressed Bt-mediated resistance to Colorado potato beetle.

No other crop with engineered disease resistance against microbial pathogens had reached the market by 2013, although more than a dozen were in some state of development and testing.

Example of transgenic disease resistance projects

Publication year	Crop	Disease resistance	Mechanism	Development status
2012	Tomato	Bacterial spot	R gene from pepper	8 years of field trails
2012	Rice	Bacterial blight and bacterial streak	Engineered E gene	Laboratory
2012	Wheat	Powdery mildew	Overexpressed R gene from wheat	2 years of field trials at time of publication
2011	Apple	Apple scab fungus	Thionin gene from barley	4 years of field trials at time of publication
2011	Potato	Potato virus Y	Pathogen-derived resistance	1 year of field trial at time of publication
2010	Apple	Fire blight	Antibacterial protein from moth	12 years of field trials at time of publication
2010	Tomato	Multibacterial resistance	PRR from Arabidopsis	Laboratory scale

2010	Banana	Xanthomonas wilt	Novel gene from pepper	Now in field trial
2009	Potato	Late blight	R genes from wild relatives	2 years of field trials
2009	Potato	Late blight	R gene from wild relative	2 years of field trials at time of publication
2008	Potato	Late blight	R gene from wild relative	2 years of field trials at time of publication
2008	Plum	Plum pox virus	Pathogen-derived resistance	Regulatory approvals, no commercial sales
2005	Rice	Bacterial streak	R gene from maize	Laboratory
2002	Barley	Stem rust	Resting lymphocyte kinase (RLK) gene from resistant barley cultivar	Laboratory
1997	Papaya	Ring spot virus	Pathogen-derived resistance	Approved and commercially sold since 1998, sold into Japan since 2012
1995	Squash	Three mosaic viruses	Pathogen-derived resistance	Approved and commercially sold since 1994
1993	Potato	Potato virus X	Mammalian interferon induced enzyme	3 years of field trials at time of publication

Defence mechanisms of plants against pathogen

The defence mechanisms of plants against pathogens fall into two categories:-

1. Morphological or structural defence mechanism.
2. Biochemical defence mechanism

1. Morphological or Structural Defence Mechanism

The first line of defence against pathogen is the surface barrier, which a pathogen must penetrate before it can cause infection. The entry of the pathogens may either be through the epidermal cell walls directly or through the natural openings in the epidermis, such as stomata, lenticels and hydathodes, or through injured areas caused by living or non-

living agencies. Certain structural features oftheepidermisor its interior may greatly affect the ability of the pathogen to penetrate or invade a host plant. Such morphological defence structures may be present before penetration or infection or may be produced afterwards as a result of the interaction of the host and the pathogen.

Defence Structures Existing Before Infection –

Waxes and Cuticles -The cuticle, which consists of cutin and waxes, forms the outermost covering of the epidermal cells and appears as a non-cellular, membranous layer. Waxes play a defensive role by forming a hydrophobic surface which acts as a water repellent and there by prevent the retention of water on sloping plant surfaces on which pathogens might be deposited and subsequently invade the host. The cuticle acts as a physical or chemical barrier to infection.

Epidermal cell wall– Lignification or the presence of silicic acid in the epidermis of some plants act as an important structural defence mechanism.

Defence Structures Formed after infection: -

Defence structures are produced inside the host plant to block or prevent the spread of the pathogen. These include the formation of cork layers, tyloses and abscission layers, deposition of gums, resins and tannin like substances, swelling of the cell walls, sheathing of hyphae and so on.

2. Bio-Chemical Defence–

Phenolic Substances - Resistance to several fungal plant pathogens has been ascribed to higher concentrations of fungitoxic phenolic substances and their oxidation products and to increased polyphenol oxidase (PPO) activity which generally, but not invariably, results from infection. The activity of poly phenol oxidase (PPO) seems to be important because it can oxidize phenolies to Quinones which may be more fungitoxic. PPO produced by the pathogen may oxidize the host polyphenols to more highly fungitoxic substances, which may prevent further development of the pathogens. It has been reported that aromatic substances such as polyphenols, phenolic glucosides, flavonoids, anthocyamins and aromatic amino acids etc tend to accumulate in and around infected plant tissue and also intissues adjacent to wounds where presumably, they might exert a fungistatic effect. Some of the most important phenolic compounds involved in the defence of plants are caffeic acid, ferulic acid, chlorogenic acid, phloretin and various phytoalexins.

Phytoalexins:-Phytoalexins can be described as low molecular weight antimicrobial compounds that accumulate in plants as a result of infection or stress. The term ‘Phytoalexins’ was introduced by Muller and Boerger (1940) to describe the substances that inhibit fungus development. These substances are formed when living plant tissues are invaded by a fungal parasite.

9.5 - GENERAL SYMPTOMS OF PLANT DISEASES

As a result of successful infection of host plant by the pathogen, a number of physiological changes occur in the plant. Respiration, photosynthesis, nitrogen metabolism, and transpiration are affected.In most cases host respiration increases after infection. There is reduced rate of photosynthesis in many diseases. Metabolites like carbohydrates, amino acids and proteins tend to accumulate in green islands. Normally, there is an increase in transpiration rates.

The physiological changes occurring in the diseased plant have their counterparts in the anatomical and morphological changes (morbid anatomy). Anatomical and morphological changes are brought about by affecting individual cells and growth of entire plant or of an organ, vegetative as well as floral. Thus, changes in physiology of plants due to infection result into several anatomical and morphological changes which are expressed externally in the form of visible symptoms. Symptoms are characteristic of a disease by which it can be recognized in the field.

In some cases, however two different pathogens can produce identical symptoms and a single pathogen can develop more than one kinds of symptoms i.e. disease syndrome. Types of symptoms are as varied as the pathogens. For instance different groups of pathogens cause a variety of symptoms. These are mentioned below:

Myxomycetes and phycomycetes- Galls, scab, warts, rots, blights, damping off, white rusts, downy mildews etc.

Ascomycetes and Fungi Imperfectii- Leaf curls, powdery mildews,cankers, leaf spots,anthracnose,root and stem rots,vascular wilts,scab,fruit and vegetable rots, early blight etc.

Basidiomycetes- Rusts,smuts,root and stem rot,wood rots etc.

Bacteria- Galls,rots,blights,spots,cankers,scab,vascular wilts etc.

Mycoplasma- Aster yellows,proliferation,general yellowing,stunting,phloem necrosis etc.

Viruses-Mosaics and mottles, ring-spots, dwarfing, rolls, yellows, galls and tumors, pitting etc.

Nematodes-Root knots [galls], root lesions, excessive root branching, root rots. Galls, lesions and rots on leaf, stem, galls in grains etc.

General Symptoms caused by different groups of pathogens are shown in (**Fig.9.4- 9.9**).

These enormous variety of symptoms, basically belong to any of the three main categories; (A)-Necrosis (B)-Hypertrophy and Hyperplasia, and (C)-Hypoplasia.

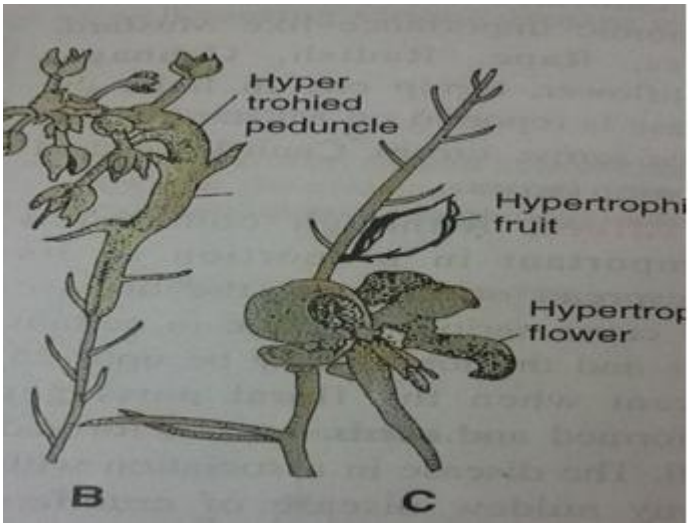

Fig.9. 4: Hypertrophy and Hyperplasia symptoms

[A]Necrosis-

Necrosis is the commonest and most destructive type of effect.This kind of symptoms is produced by rather unspecialized parasites. Among these pathogens, the most destructive ones, *Pythium, Botrytis, Fusarium* showing wilts and many other symptoms. These pathogenscause immediate and severe damage to host tissues and obtain their nutrients from cells killed in advance by secretion of enzymes and toxins.They are thus called necrotrophs. Some other necrotrophs, somewhat intermediate group between the above mentioned ones (and the specialized parasites as rusts,mildews etc),do not kill cells in advance but invade and thenkill the host cells. Such necrotrophs include fungi like *Phytophthora, Venturia, Taphrina and Claviceps.*

Necrosis is caused by specialized parasites like rusts,mildews,smuts but only in later stages of development when they sporulate. Thus, during early stages of infection there is only slight adverse effect.Necrosis is also caused by bacteria and viruses.

The symptoms of necrotic nature are as follows:

1-Leaf spots: The cells are killed in a limited area to form lesion or spots.The spots are of various size and shape as zonate,target board, angular,frog eye etc.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. Example *Rhytisma and Phyllachora*.In some cases, the dead tissue shrinks and separates from the healthy tissue. This condition is known as Shot Hole.

Fig.9. 5: Leaf spots symptoms

2-Streaks or Strips-: These are elongated, narrow lesions on leaves, which are usually of brown shade. Example-Bareley stripe by Helminthosporium.

3-Blights: This gives a burnt appearance.There is sudden death of plant parts as leaves, blossoms, stamens, or twigs or even entire plant.The dead part usually turns brown or black and soon disintergrates. Example-early and late blight of potatoes. Some bacteria e.g. xanthomonas also cause blight symptoms in may plants.

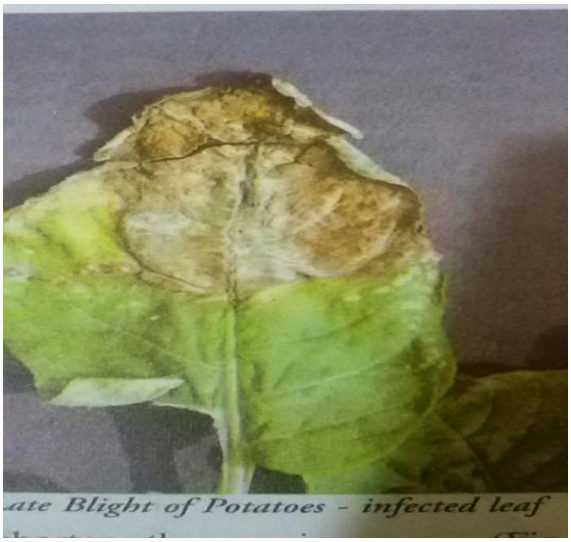

Fig.9.6: Blight symptoms

4-Damping off: This is the condition where 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.Damping off of vegetable seedlings is common and caused by *Pythium and Phytophthora.*

5-Burn, Scald or Scorch: In succulent plant parts, like fruits, limited areas necrose, die and turn brown.

6-Rots:The infected tissue dies,decomposes rapidly and turns brown.Fungi and bacteria which are able to dissolve cell walls cause this symptom.The examples include root-rot,leaf rot,stem rot,bud rot and fruit rot.

7-Wilting: Wilting of infected leaves occurs perhaps due to plugging of xylem vessels by fungus or mucilaginous substances. It is caused by some fungi like *Fusarium* spp. and *Trichometasphaeria turcica* (in maize). Later, the whole plant wilts and dies.

8-Die-back: Death of plant part,such as stem or branches the from tip back-wards.

9-Cankers:A canker is a dead area in the bark or cortex of woody stems.There are often large areas with definite margin.Dead bark splits and falls away. Example-Citrus canker (bacterial).

10-Chlorosis: Discolouration from normal colour is common in some cases.The green pigment may be destroyed and the tissue becomes yellow.This condition is known as chlorosis, caused by viruses, bacteria and some fungi.

11-Blotch: Some fruits,due to infection,develop superficial growth appearing as a blotch area.It occurs in sooty blotch and fly speck disease of apple.

12-Scab: This is a rough,crust-like lesion or a freckled organ.It occurs mostly in fruits and vegetables.Examples- apple scab by *Venturia inaequalis.*

13-White blisters or pustules: On leaves of some plants there numerous shining white blister-like pustulesdevelop which break open at maturity exposing powdery mass of spores.Such diseases are commonly called white rusts caused by *Albugo* spp.

14-Smuts: This refers to a sooty or charcoal-like powdery mass.In plants infected by smuts (*Ustilago, Tilletia*), this is very common. Smut spores develop mostly in floral organs, particularly ovaries; other affected organs include leaf, stem and roots.

Fig.9.7: Smut symptoms

15-Rusts: The rusts appear as small pustules of spores forming a powdery mass of brownish, red, yellow or dark brown colour. These break through epidermis. Example- members of uredinales. Rusts disease of wheat is quite common.

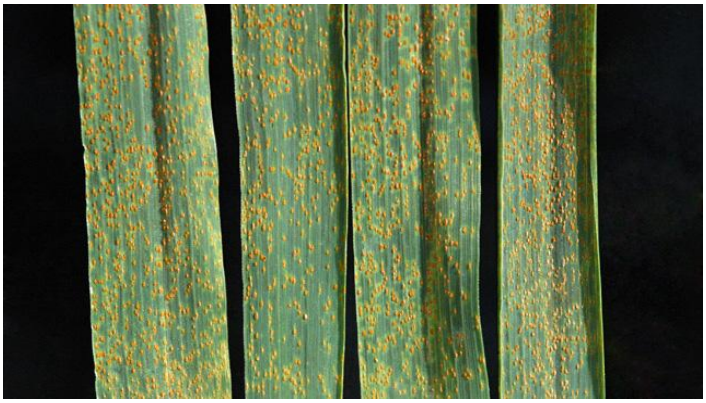

Fig.9.8. Rust symptoms

16-Mildews: In this case the pathogens grow mostly superficially on host surface.They appear as white,grey, brownish or purple patches of various sizes on leaves.In case of Downy mildew(Peronosporaceae),the superficial growth is a tangled cottony or downy layer consisting of sporangiophores and sporangia of the pathogen. In Powdery mildew (Erysiphaceae), there is superficial, white powdery growth of mycelium, numerous conidiphores and conidia. These give a dusty appearance.

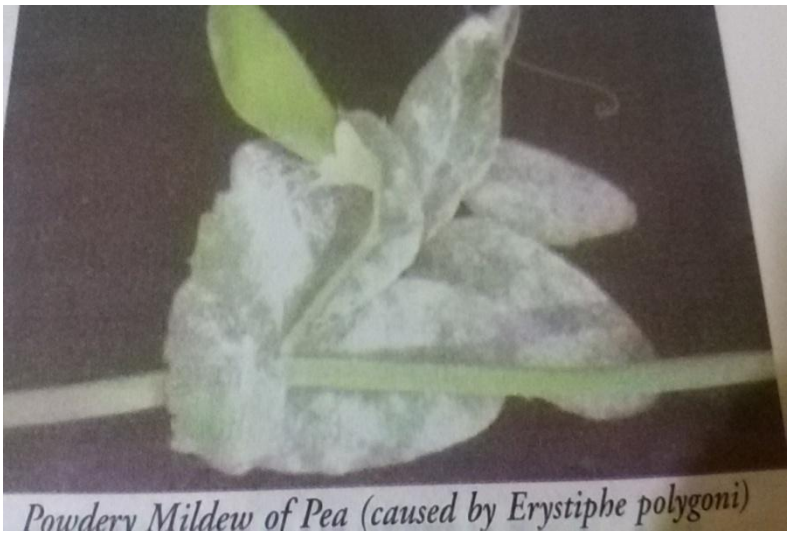

Fig.9.9. Powdery mildew symptoms

[B] Hypertrophy and Hyperplasia (over-development)

In some diseases,there is abnormal increase in size of an organ or of the entire plant.This increase may be due to increase in size of individual cells of affected tissue, i.e.,hyperplasia.Various overgrowths developed are of the following types.

1-Elongated internodes: some infected plants develop elongated internotes and become abnormally tall. Example-rice infected with *Giberella fujikuroi*, *Euphorbia cyperissias* with *Uromycespisi,* sugarcane with *Sclerospora sacchari*.

2-Galls and tumors: These are globose, elongated or irregular large sized out-growths formed on attacked part.Smaller galls are warts tubercles. Examples are club-root of crucifers, some species of Albugo, crown-gall tumors, root knot etc.

3-Witche's broom: They are formed by fungi (*Taphrina*, some rusts), bacteria (*Coryne bacterium fasciens*), and certain viruses as well as insects and mites. They arise basically from stimulation of structures which normally remain dormant, such as buds. The broom very often appears as an upright cluster of small shoots,contrasting with horizontal growth habit of normal shoots.

4- Curls: In some diseases the leaves are arched,puckered, twisted,curled and distorted. Example-peach leaf curl (*Taphrima deformans*), papaya leaf curl (Virus).

5-Floral abnormalities: some fungi, such as *Albugo candida* cause the infected inflorescence toenlarge; it becomes green and fleshly with stamens converted into leafly structures. The inflorescence becomes distorted.

[C] Hypoplasia (under-development)

In these instances,there is reduced development of the whole plant, plantparts,certain flowers or fruits (sterility),or of chlorophy II (chlorosis). In extreme cases,it leads to atrophy in which the organ or tissue does not develop at all.

1.Chlorosis: Reduced development of chlorophyll results into various kinds of chlorosis, mosaic and mottling in many viral and deficiency diseases. Chlorosis may take the form of streaking. There may be Vein clearing-a translucent appearance of the veins or vein banding in which dark green bands bound the veins or a general chlorosis of the whole leaves turning them yellows.

2. Reductionof Individual Organ: Individual leaves and flowers may be reduced in size and/or altered in shape. Reduced leaves develop in viral infections and in some fungi such as *Exobasidium*. Examples are "fern leaf",where lamina disappears, "little leaf" etc. Inter modes are reduced in dwarf bunt of wheat caused by *Tilletia contraversa*, and in several viral diseases.

3.Floral Abnormalities: In smut of Caryophyllaceae members, stamens become sterile (caused by *Ustilago violacea*). One very common and characteristic floral abnormality is Phyllody, i.e., transformation of floral parts into green leafy twisted structures. These are caused by mites, insects, fungi and viruses. Example- green ear disease of bajra.

9.6 - SUMMARY

Disease is a complex phenomenon and thus difficult to define in a few words.According to modern concept, disease results from an interaction among the host, parasite and environment. Diseased plants are distinguished by changes in their morphological structures or physiological processes,which are brought about by unfavorable environmental conditions or by parasitic agents.

Sometimes variation in the environment also makes a plantsusceptible to infection. The microorganisms are thus not the sole causal factor. After a microorganism has

successfully infected a susceptible plant, the subsequent interaction between the plant and the microorganism is also affected by environmental influences.

Most casual organisms are parasites. A parasite is not synonymous with the pathogen. The mycorrhizal fungus is certainly parasitic on the roots of trees but is not pathogenic.In a literal sense, a pathogen is any agent that causes damage. Most, but not all pathogens, are also parasites in that they derive the materials needed for existence from a living plant. The sequence of processes in disease development starts from the initial contact between a pathogen and its host until to the completion of thesyndrome.

A large number of plant diseases and sometimes the scale of destruction they cause, suggests that diseases can even change the course of history and the economy of a country. They of course are a limiting factor in crop production. The advances made in food production in developing countries due to green revolution could be offset if proper attention is not given to plant diseases and pests.

9.7 - GLOSSARY

Appresorium: An enlarged, usually disc shaped structure formed at the tip of hypha of some parasitic fungi from which entry is made into the host.

Autotrophic: An organism capable of utilizing energy from light for metabolic synthesis.

Aetiology(Etiology) – The science of the cause of diseases.

Autoecious: Completing the lifecycle on one host.

Biotroph: Living on living cells.

Biological control: Control of a disease by the activity of other organisms.

Blight: A necrotic symptom on leaves which is characterized by sudden and serious leaf damage.

Centrifugal: From the centre outwards.

Centripetal: Towards the centre.

Ceraceous: Wax – like.

Coenocytic: A multinucleate mass of protoplasm, noncellular, in the sense of being non septate.

Canker: A necrotic symptom of woody parts. The wound is surrounded by a raised tumor like margin.

Chlorosis: Symptom of chlorophyll deficiency.

Disease: A malfunctioning process due to continuous irritation from the presence or absence of some agent.

Dioecious: The organism in which the male and female sex structures are on different thalli.

Diplanetism: The condition in which there are two motile stages with a resting stage in between.

Diplobiontic: Having haploid and diploid generations as free living individuals.

Epidemic: Widespread and destructive development of a disease in a large human population.

Epidemiology: The study of epidemics.

Etiology: The study of the causal agents of a disease and its relation to the susceptible plant.

Fungicide: A chemical that reversibly inhibits the growth of a fungus as long as it is in contact with the fungus.

Gall: A tumor, formed by hyperplastic growth of unorganized cells.

Heteroecious: Undergoing different parasitic stages on two unrelated hosts.

Haustorium: A special hyphal branch within the living cell of the host, for absorption of food.

Heterothallism: Condition of sexual reproduction in which conjugation is possible only through the interaction of different thalli.

Heterokaryosis: The state of a fungus having genetically different nuclei in its cells.

Heterotrophic: Organism deserving food from other living or dead organisms; unable to synthesize organic material from inorganic sources.

Host: A living organism providing sustenance to a parasite.

Hyperplasia: Outgrowth due to abnormal increase in the division of cells.

Hypertrophy: Outgrowth due to abnormal increase in size of the cells.

Inoculum: Portion of pathogen capable of dissemination and initiation of disease.

Immune: Resistant from infection.

Innate: Immersed.

Karyogamy: The fusion of two sex nuclei after cell fusion, i.e., after plasmogamy.

Metula: A conidiophore branch having phialides.

Monoecious: Having genetically identical haploidnuclei.

Mould: A micro fungus having a well-marked mycelium or spore mass.

Mycelium: A mass of hyphae; the thallus of a fungus.

Mycorrhiza: A symbiotic, non- pathogenic or feebly pathogenic association of a fungus with the roots of a plant.

Mycosis: A fungal disease of humans and animals, rarely of plants.

Necrosis: Death of cells.

Parasite: An organism that derives its food from a different species of plant.

Pathogen: Causal agent of a disease.

Pathogenic: Capable of causing disease.

Protectant: A substance, usually a fungicide that prevents infection.

Pustule: A pimple like eruption of host tissue formed by fruiting body, such as uredium or telium, in case of rust disease.

Resistance: The inherent capacity of a plant to prevent, restrict or withstand disease.

Rogue: To remove unwanted plants.

Sign: Visible structures of pathogens appearing on or in diseased tissue.

Symptom: A visible expression of disease in the host plant.

Syndrome: The pattern of symptoms and signs of a disease.

Systemic: Which spreads throughout the plant body.

Tolerance: The ability of a host to survive and give satisfactory yield when other varieties of the same species suffer great loss.

Variety: Subdivision of a species that differs from the type species in certain characteristics.

Vector: An agent that causes dissemination of a pathogen.

Virulence: Relative ability to cause disease, a measure of pathogenicity.

9.8- SELF ASSESSMENT QUESTION

9.8.1: Fill in the blanks –

1. The word fungus is derived from the latin word……..
2. The fungi which grow on dead or decaying organic matter are called……..
3. Monoccious fungus is that which bears male and female sex organs on the ……thallus.
4. The Study of plant disease is known as ……..
5. The chemicals used for killing fungal pathogen are called…….
6. Loose smut of wheat is caused by……..

9.8.1: Answers Key: 1. Fungous, 2. Saprophytes, 3. Same, 4.Plant pathology, 5.Fungicides, 6. *Ustilago tritici*

9.8.2: Differentiate between –

1. Hypertrophy and hyperplasia
2. Parasitic and non-parasitic diseases.
3. Black rust and white rust.
4. Epidemic and endemic diseases.
5. Obligate parasite and obligate saprophytes.

9.8.3: Write short notes on-

a. Disease
b. Fungicides
c. Infection
d. Prepenetration
e. Haustoria
f. Quaranine
g. Phytoalexins

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9.11 TERMINAL QUESTIONS

Q.1-What is disease? Discuss the components of disease and the kinds of diseases in plants.

Q.2- Discuss the general process of infection in plant pathogens.

Q.3- Give an accountof symptoms of plantdisease with suitable examples.

Q.4- Give an account of the techniques used in plant breeding for disease resistance.

Q.5-What is parasitism? Give a broad outline of physiology of parasitism.

Q.6- Discuss theroleofenzymesandtoxinsindevelopmentofplantdisease.

Q.7- Discuss thestructuralandbiochemicallinesofdefense inplantsagainst pathogens.

Q.8-The cuticle acts as a physical or chemical barrier to infection. Comment upon the statement.

Q.9-Distinguish between penetration and infection.

Q.10-Write notes on:

(i)-Biological inhibition of germination of spores of fungal pathogens.
(ii)- Tissue culture techniques in disease resistance.
(iii)- Phytoalexins.
(iv)- Disease resistance.

UNIT-10-SYMPTOMS, MORPHOLOGY OF THE CAUSAL ORGANISM, DISEASES CYCLE AND CONTROL MEASURES-I

10.1- OBJECTIVES

After reading this unit student will be able:

- To study the morphology of the causal organism of the diseases.
- To get an idea of etiology or disease cycle of the causal organism of the disease and
- To know the control measures or the management of disease.

10.2-INTRODUCTION

Globally, enormous losses of the crops are caused by the plant diseases. The loss can occur from the time of seed sowing in the field to harvesting and storage. Important historical evidences of plant disease epidemics are Irish Famine due to late blight of potato (Ireland, 1845), Bengal famine due to brown spot of rice (India, 1942) and Coffee rust (Sri Lanka, 1967). Such epidemics had left their effect on the economy of the affected countries. The normal physiological functions of plants are disturbed when they are affected by pathogenic living organisms or by some environmental factors. Initially plants react to the disease causing agents, particularly in the site of infection. Later, the reaction becomes more widespread and histological changes take place. Such changes are expressed as different types of symptoms of the disease which can be visualized macroscopically. As a result of the disease, plant growth in reduced, deformed or even the plant dies. When a plant is suffering, we call it diseased. Thus a disease is a condition that occurs in consequence of abnormal changes in the form, physiology, integrity or behaviour of the plant.

Plant diseases are caused by pathogens. Hence a pathogen is always associated with a disease. In other way, disease is a symptom caused by the invasion of a pathogen that is able to survive, perpetuate and spread. Further, the word “pathogen” can be broadly defined as any agent or factor that incites 'pathos or disease in an organism or host. In strict sense, the causes of plant diseases are grouped under following categories:

1. Animate or biotic causes: when disease is caused by a pathogens i.e. a living organism.Pathogens of living nature are categorized into the following groups, (i) Fungi (ii) Algae (iii) Bacteria (iv) Phanerogams (v) Phytoplasma (vi) Protozoa (vii) Rickettsia-like organisms (viii) Nematodes

2. Mesobiotic causes: These disease incitants are neither living nor non-living, e.g. (i) Viruses (ii) Viroides

3. Inanimate or abiotic causes: In true sense these factors cause damages (any reduction in the quality or quantity of yield or loss of revenue) to the plants rather than causing disease. The causes are: (i) Deficiencies or excess of nutrients (e.g. ‘Khaira’ disease of rice due to Zn deficiency) (ii) Light (iii) Moisture (iv) Temperature (v) Air pollutants (e.g. black tip of mango) (vi) Lack of oxygen (e.g. hollow and black heart of potato) (vii) Toxicity of pesticides (viii) Improper cultural practices (ix) Abnormality in soil conditions (acidity, alkalinity).

Classification of plant disease

To facilitate the study of plant diseases they are needed to be grouped in some orderly fashion. Plant diseases can be grouped in various ways based on the symptoms or signs (rust, smut, blight etc.), nature of infection (systemic or localized), habitat of the pathogens, mode of perpetuation and spread (soil-, seed- and air-borne etc.), affected parts of the host (aerial, root disease etc.), types of the plants (cereals, pulses, oilseed, ornamental, vegetable, forest diseases etc.). But the most useful classification has been made is based on the type of pathogens that cause plant diseases. Since this type of classification indicates not only the cause of the disease, but also the knowledge and information that suggest the probable development and spread of disease alongwith their possible control measures. The classification is as follows:

1. Infectious plant diseases: a. Disease caused by parasitic organisms: These organisms are included under animate or biotic causes incite diseases in plants. b. Diseases caused by viruses and viroid (mesobiotic causes).

2. Non-infectious or non-parasitic or physiological diseases: The factors included in inanimate or abiotic causes incite such diseases in plants under a set of suitable environmental conditions.

Symptoms of plant diseases

A visible or detectable abnormality expressed on the plant as a result of disease or disorder is called symptom. The totality of symptoms is collectively called as syndrome while the pathogen or its parts or products seen on the affected parts of a host plant is called sign. Different types of disease symptoms are cited below:

Necrosis: It indicates the death of cells, tissues and organs resulting from infection by pathogen. Necrotic symptoms include spots, blights, burn, canker, streaks, stripes, damping-off, rot etc.

Wilt: Withering and drooping of a plant starting from some leaves to growing tip occurs suddenly or gradually. Wilting takes place due to blockage in the translocation system caused by the pathogen.

Die-back: Drying of plant organs such as stem or branches which starts from the tip and progresses gradually towards the main stem or trunk is called die-back or wither tip.

Mildew: White, grey or brown coloured superficial growth of the pathogen on the host surface.

Rusts: Numerous small pustules growing out through host epidermis which gives rusty (rust formation) appearance of the affected parts.

Smuts: Charcoal-like and black or purplish-black dust like masses developed on the affected plant parts, mostly on floral organs and inflorescence.

Blotch: A large area of discolouration of a leaf, fruit etc. giving a blotchy appearance.

White blisters: Numerous white coloured blister-like ruptures are surfaced on the host epidermis that forms powdery masses of spores of fungi.

Colour change: Conversion of green pigment of leaves into other colours mostly to yellow colour, in patches or covering the entire leaves. It happens because of the following symptoms.

Etiolation: Yellowing due to lack of light, **(ii) Chlorosis**: Yellowing due to infection viruses, bacteria, fungi, low temperature, lack of iron etc. **(iii) Albino**: Lack of any pigment which causes white or bleached symptoms **(iv) Chromosis**: Red, purple or orange pigmentation due to physiological reasons.

Exudation: Such symptom is commonly found in bacterial diseases when masses of bacterial cells ooze out to the surface of affected plant parts and form some drops or smear. The exudation forms a crust on the host surface after drying.

Overgrowth: Excessive growth of the plant parts due to infection by pathogens. Overgrowth takes place by two processes **(i)Hyperplasia**: abnormal increase in size due to excessively more cell division **(ii)Hypertrophy**: abnormal increase in size or shape due to excessive enlargement of the size of cell of a particular tissue.

Atrophy: It is known as or dwarfing which is caused by the inhibition of growth due to reduction in cell division or cell size.

Sclerotia: These are dark and hard structures of various shaped composed of dormant mycelia of some fungi. Sometimes, sclerotia are developed on the affected parts of the plant. Presence of sclerotia on the host surface is specifically called a sign of disease rather than symptom.

Plant disease management

The word ‘control’ is a complete term and a permanent ‘control’ of a disease is rarely achieved whereas, ‘management’ of a disease is a continuous process and is more practical in influencing adverse effect caused by a disease. Disease management requires a detail understanding of all aspects of crop production, economics, environmental, cultural, genetics and epidemiological information upon which the management decisions are made.

A.Principles of plant disease management:

There are six basic concepts or principles or objectives lying under plant disease management.

1. Avoidance of the pathogen: Occurrence of a disease can be avoided by planting/sowing a crop at times when, or in areas where, inoculum remain ineffective/ inactive due to environmental conditions, or is rare or absent.

2. Exclusion of the pathogen: This can be achieved by preventing the inoculum from entering or establishing in a field or area when it does not exist. Legislative measures like quarantine regulations are needed to be strictly applied to prevent spread of a disease.

3. Eradication of the pathogen: It includes reducing, inactivating, eliminating or destroying inoculum at the source, either from a region or from an individual plant (rouging) in which it is already established.

4. Protection of the host: Host plants can be protected by creating a toxin barrier on the host surface by the application of chemicals.

5. Disease resistance: Preventing infection or reducing the effect of infection of the pathogen through the use of resistance host which is developed by genetic manipulation or by chemotherapy.

6. Therapy: Reducing severity of a disease in an infected individual. The first five principles are prophylactic (preventive) procedure and the last one is curative.

B. Methods of plant disease management

1. Avoidance of the pathogen:

i. Choice of geographical area
ii. Selection of a field
iii. Adjustment of time of sowing
iv. Use of disease escaping varieties
v. Use of pathogen-free seed and planting material
vi. Modification of cultural practices

2. Exclusion of inoculum of the pathogen

i. Treatment of seed and plating materials
ii. Inspection and certification
iii. Quarantine regulations
iv. Eradication of insect vector

3. Eradication of the pathogen

i. Biological control of plant pathogens
ii. Eradication of alternate and collateral hosts
iii. Cultural methods:
    a. Crop rotation
    b. Sanitation of field by destroying/burning crop debris
    c. Removal and destruction of diseased plants or plant parts
    d. Rouging
iv. Heat and chemical treatment of diseased plants
v. Soil treatment: by use of chemicals, heat energy, flooding and fallowing

4. Protection of the host

i. Chemical control: application of chemicals (fungicides, antibiotics) by seed treatment, dusting and spraying
ii. Chemical control of insect vectors
iii. Modifications of environment
iv. Modification of host nutrition

5. Disease resistance/Use of resistant varieties: Development of resistance in host is done by

i. Selection and hybridization for disease resistance
ii. Chemotherapy
iii. Host nutrition
iv. Genetic engineering, tissue culture

6. Therapy: Therapy of diseased plants can be done by

i. Chemotherapy
ii. Heat therapy
iii. Tree-surgery

10.3-MOSAIC DISEASE ON TOBACCO

This plant disease is caused by Tobacco Mosaic Virus (TMV). it is known to occur in all tobacco growing countriesof the world. It was first described in detail by Adolph Mayerin 1886. Tobacco Mosaic Virus speciallyinfectstobacco and members of family Solanaceae. The virus may affect more than 150 genera of herbaceous dicotyledonous plants including many vegetables, flowers and weeds.

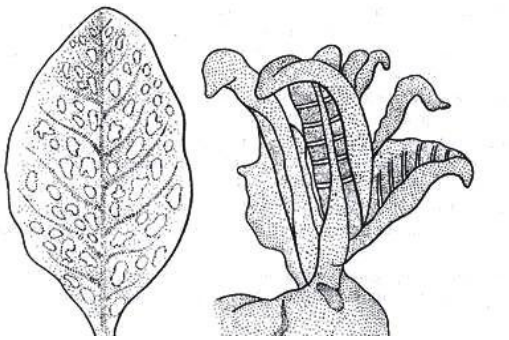

Fig. 10.1 Tobacco leaf showing the mosaic symptoms

10.3.1-Symptoms

It produce mosaic* like symptoms on plants. The symptoms on the healthy plant appear after ten days of infection. The first symptom is light green coloration between the veins of the young leaves. This is quickly followed by the development of mosaic or mottled pattern of light abd dark green areas in the leaves (**Fig.10.1**). In infected plants, the leaves become small, curled and puckered. Symptoms on plants includechlorosis, curling, mottling, dwarfing, distortion and blistering of leaves. Mosaic does not result in plant death, but if infection occurs early in the season, plants are stunted.

10.3.2-Morphology of the causal organism

It is the first virus to be discovered by **Iwanowski** in 1892. It is a rod shaped virus. The virion measures 18×300 nanometers and weighs 39 million Daltons. The capsid is made

from 2130 protein subunits (each subunit consists of 158 aminoacids) and one molecule of genomic single stranded RNA. The ssRNA consists of 6,400 nucleotides.

10.3.3-Disease cycle

It is the most persistent plant virus. It has been known to survive 50 years in dried plant parts. The most common sources of virus inoculum for TMV are the debris of infected plants that remain in the soil and certain infected tobacco products which contaminate workers hand. It is also transmitted mechanically by vegetative propagation of plants, grafting, seeds, pollens and by being carried on the mouth parts of chewing insects.

Once the virus enters the host, it begins to multiply by inducing cells to form new virus. It takes over the metabolic cell processes resulting in abnormal cell function. Its RNA directs the synthesis of viral protein. After the RNA and the protein is produced, they undergo self assembly. After the assembly, they are released outside the cell after its lysis.

10.3.4-Control measures

There are no known chemicaltreatments used under field conditions that eliminate viral infection from plant tissues once it occurs. However, some important control measures to check the infection are:

- Discarding infected plants.
- Growing virus free plants.
- Propagate plants via seeds rather than vegetatively.
- Crop rotation.
- Growing of resistant strains.
- Removing of all weeds during and after the growing season.
- Disinfecting tools by placing them in boiling water for 5 minutes and washing with a strong soap or detergent solution.
- Discouraging use of tobacco by workers.
- Encouraging the practice of washing hands by workers with soap and water before and after handling plants.

*__Mosaic__: A viral disease in plants that causes yellow patches to develop on the leaves, giving these a variegated appearance. Variegation is the occurrence of different coloured patches, spots or streaks in plant leaves, petals or other parts, due to absence of pigments or different combination of pigments in the affected area of the leaf. Variegation may be brought about by infection for *e.g.*, TMV infection.

10.4 CITRUS CANKER

Fawcett and **Jenkins** (1933) reported that citrus canker disease is originated in India and Java because they had detected canker lesions on the oldest citrus herbaria kept at Royal Botanical Gardens in Kew, England (*Citrus medica* collected in India between 1827-1831, *Citrus aurantifolia* collected in Java between 1842-1844). Now the disease known to occurs almost in all citrus growing countries of the world. This disease affects cultivars and hybrids

of citrus and citrus relatives including orange, grapewine,mandarin, lemon, lime, tangerine, sour orange and roughlemon. Because of its rapid spread, high potential damage and impact on export and domestic sales, the disease is a significant threat to all citrus growing countries.

Fig.10.2. Fruits leaves and stem showing Citrus canker

10.4.1- Symptoms

Plants infected with citrus canker disease have characteristic lesions on leaves, stems and fruits with raised brown, water soaked margins which develop around the necrotic tissues (**Fig.10.2**). A characteristic symptom of the disease on the leaves is the yellow halo that surrounds lesion. These lesions start as pin point spots and attain a maximum size of 2-10mm diameter. Lesions become visible about 7-10 days after infection on the lower surface. The lesion persists on twigs and branches for several years and support long term survival of the bacterium. Severely infected fruits can drop prematurely, leading to reduced yield. The internal quality of mature fruit with lesions is unaffected and is still edible and usable for juice.

10.4.2- Morphology of the causal organism

(***Xanthomonas axonopodispv. Citri andXanthomonas axonopoidspv.aurantiofolli.)***

*Xanthomonas*is small aerobic, rod shaped motile bacterium. It is 5.20×0.5-0.75 micron in size and has single polar flagellum. It formschains and capsules but no spores.

10.4.3-Disease cycle

Infected twigs having old lesions are the main source of infection. These lesions ooze bacteria which are blownaway by the wind or dispersed by rain.Healthy plants are infected by these bacteria which entered through stomata or wounds (caused by citrus leaf minor*phyllocnistiscitrella*in feeding activity).The bacteria after penetration in to host multiply in the intercellular spaces, dissolve the middle lamella and get established in to the cortex.Lesions on healthy plants become visible about 7 to 10 days after infection on the underside of the leaves and soon thereafter on the upper surface. Man is also an important agent of dissemination through infected nursery stock. High mean temperature (20-30)coincide with high rain fall favours the development of disease.

10.4.4-Control measures

- Eradication of infected trees and burning them.
- Applying preventive sprays of copper based **Kocide 3000.**
- Pruningof infected twigs and leaves during the dry seasonand then spraying the trees with 1% **Bordeaux mixture.**
- Spraying the antibiotics e.g. streptomycin sulphate and phonomycin.
- Strictly applying quarantine methods.
- Replacing susceptible citrus cultivars with resistant cultivars.
- Growing winds breaks to hinder inoculum dispersal.
- Devloping transgenic plants for e.g.*Xanthomonas*resistance gene from the rice has been transferred in to sweet orange.

10.5 WART DISEASES OF POTATO

Potato wart is an important and serious disease of cultivated potato (*Solanum tuberosum*) with numerous accounts of disease detections occurring worldwide. Potato wart is known by various names, including black scab, black wart, cauliflower disease, potato tumor, potato cancer, potato canker, wart, warty disease, and certainly many other descriptive names in several languages. The disease is caused by the fungus *Synchytrium endobioticum*. This organism is considered to be the most important world-wide plant pathogen of cultivated potato. *S. endobiotocum* is a primitive fungus characterized by the lack of hyphae, the formation of zoospores and thedevelopment of long-lived resting sporangia. This fungus invades certain meristematic tissues of the potato plant. This can preclude plant emergence when sprout tips are infected. After plant emergence, when stolon tips or tuber eyes become infected, resultant disease processes can render the potato tuber unrecognizable and unfit for human consumption. The potato wart fungus is not a human pathogen and poses no threat to human health. This pathogen is readily distributed via movement of infested soil and by infected seed tubers. Because of the great potential for disease loss, any degree of infestation or indication of potato wart universally (world-wide) results in strict quarantine and other regulatory measures designed to confine the known infestation and to preclude pathogen re-distribution. Although direct losses from potato wart may be insignificant when first detected, indirect economic losses resulting from zero-tolerance regulations for potato wart can be devastating to the people. Indirect economic losses become especially evident in situations where seed tuber production areas become subject to quarantine measures, as well as when the movement of commercial potatoes is restricted. For example, a well-documented detection of potato wart on Prince Edward Island (Canada) late in the year 2000 growing season and the ramifications of subsequent regulatory actions resulted in an estimated $30 million loss to the Island's economy in that first year alone.

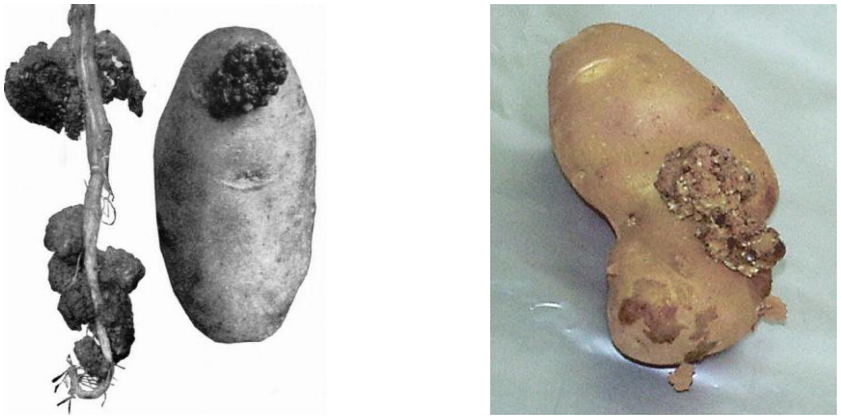

Fig.10.3: Potato tubers showing wart disease

10.5.1- Symptoms

The diagnostic symptoms of potato wart are galls produced on several plant parts. These galls are primarily parenchymatous and may form on stem tissue, including the stem base, stolon buds, and tuber eyes (**Fig.10.3**). Galls most often form on below-ground portions of the plant, and the presence of the disease is typically not noticed until tubers are lifted during harvest. However, galls also occasionally form on the upper stem, leaf, or flower. Galls vary in shape but are mostly spherical outgrowths, ranging from 1 to 8 cm (0.4 to 3.2 inches) or more in diameter, but may become fist-sized in some instances. Aboveground galls are green to brown, turning black at maturity, and are prone to decay. Belowground galls are white to brown and turn black as they decay. Tubers may be disfigured or become almost unrecognizable when they are infected early in development and replaced by galls. Galls present on tubers at harvest may become desiccated and barely noticeable or, alternatively, the galls may decay. Because the disease can continue developing during storage, desiccated galls barely noticed at harvest may become increasingly evident during prolonged storage. Although potato wart does not kill the host, the meristematic tissue of sprouts may be so severely attacked that plants ultimately fail to emerge from seed tubers early in the growing season. Also, infected plants may develop general symptoms of reduced vigor, especially in association with the formation of small greenish-yellow warty growths at the stem base. The true potato roots are not known to be infected.

10.5.2-Morphology of the causal organism

The fungus (*Synchytrium endobioticum* Schilberszky) does not produce hyphae and is an obligate, holocarpic, endobiotic parasite. It is a long-cycled chytrid characterized by a short-lived swarm (summer) sporangial stage resulting from host infection by uniflagellate zoospores and a resting (winter) sporangial stage. Both sporangial types germinate to release 200-300 zoospores, which are pear-shaped infective units (1.5 to 2.2 µm in diameter) and motile by means of a posterior flagellum. Resting sporangia are golden brown and spheroidal (35 to 80 µm in diameter). The resting sporangium wall has prominent exterior ridges and contains chitin fibrils.

10.5.3-Disease cycle

In the spring, resting sporangia in decaying warts and soil germinate to release haploid (uninucleate) zoospores. These zoospores migrate in soil water for a limited distance (50 mm or less) with the help of single flagellum to arrive at epidermal cells of meristematic tissues of growing points, (buds, stolon tips, or young leaf primordial). Zoospores are short-lived. They loose flagellum, become encysted and infect susceptible host tissue within 1-2 hr after their formation. Potato host cells become greatly enlarged. Haploid soriare formed inside the host cells while neighboring host cells begin to proliferate, resulting in the characteristic warty galls and the increased presence of the meristematic tissue. This provides new infection courts for the fungus. Each sorus contains one to nine summer sporangia, which in turn germinate to produce new haploid zoospores. These zoospores again infect susceptible tissue (i.e., a secondary disease cycle). These rapidly repeating secondary disease cycles ultimately result in an extensive invasion of host cells and rapid onset of gall formation. Young galls are nutrient sinks and expand rapidly at the expense of other plant tissue. For example, gall volume has been observed to increase more than 1,800-fold in 16 days.

Under conditions of stress, such as water shortage, zoospores may also conjugate (fuse) in pairs to form uninucleate, diploid, biflagellate zygotes which infect the host tissue to form resting sporangia. Following infection by zygotes, the host cell in which resting sporangia form does not swell but divides to form galls. The host cell wall remains closely attached and forms an outer layer to the resistant, thick-walled resting (winter) sporangium. As these galls decay and disintegrate, they release the thick-walled resting sporangia into the soil environment. Resting sporangia are endogenously dormant and can remain viable for 40 to 50 years at depths of up to 50 cm (20 inches) in the soil profile.

The resting sporangia are primarily spread in infected seed tubers, which may have incipient warts that pass undetected, or in infested soil adhering to tubers, equipment, and by other carriers of contaminated soil. Resting sporangia survive even in passage through the digestive system of animals which fed on infected potatoes, and contaminated manure also can disperse inoculum. Earthworms have also been found to serve as means of inoculum dispersal. Resting sporangia can also be dispersed by wind-blown soil or by flowing surface water. The zoospores are too short-lived to significantly contribute to inter-field dispersal of the potato wart pathogen. Most potato wart diseases have been recorded in small garden plots where there has been repeated culture of susceptible potato hosts.

Under ideal conditions, potato wart can develop when the inoculum density is less than one resting spore per gram of soil. Potato wart is favored by cool, wet soils during tuber development. A soil temperature of at least 8°C (46°F) and water is required for the germination of both winter and summer sporangia and for the dispersal of zoospores. Cool summers with average temperatures of 18°C (64°F) or less, and annual precipitation of 70 cm (28 inches) are important for the development of the disease. Soil pH is of less importance; the disease has been found to occur in plants growing in soils ranging from pH 3.9 to pH 8.5. Temperatures of 12 to 24°C (54 to 75°F) favor infection.

10.5.4-Control measures

- Wart affected tubers should not be planted.
- Wart resistant varieties like KufriKanchan, KufriJyoti should be grown.

10.6-EARLY BLIGHT OF POTATO

Alternaria solani is a fungal pathogen, which produces a disease in potato plants called **early blight (Fig.10.4)**. The pathogen produces distinctive "bullseye" patterned leaf spots and can also cause stem lesions and fruit rot on tomato and tuber blight on potato. Despite the name "early," foliar symptoms usually occur on older leaves. Distinguishing symptoms of *A. solani* include leaf spot and defoliation, which are most pronounced in the lower canopy.

Fig. 10.4: Leaf showing blight disease

10.6.1- Symptoms

In potato, primary damage by *A. solani* is attributed to premature defoliation of potato plants, which results in tuber yield reduction. Initial infection occurs on older leaves, with concentric dark brown spots developing mainly in the leaf center. The disease progresses during the period of potato vegetation, and infected leaves turn yellow and either dry out or fall off the stem. On stems, spots are gaunt with no clear contours (as compared to leaf spots). Tuber lesions are dry, dark and pressed into the tuber surface, with the underlying flesh turning dry, leathery and brown. During storage, tuber lesions may enlarge and tubers may become shriveled. Disease severity due to *A. solani* is highest when potato plants are injured, under stress if they lack proper nutrition.

10.6.2-Morphology of the causal organism

A. solani belongs to Fungi-imperfectii. Colony morphology of *A. solani* varies widely, but is generally effuse, greyish brown to black, with a cotton-felt, or velvet-like texture. Growth is rapid on many growth media, but special conditions are required for sporulation. Orange to dark red pigments are produced by the fungus which colour the medium. Cells of *A. solani* are multinucleate, but different organs vary in the number of nuclei. Nuclear division in hyphal cells is followed by multiple septation, which results in the division of elongated tip cells into several multinucleate cells. Conidiophores are dark or olivaceous brown, thick-walled, straight to flexuous, septate, and arise singly or in small groups, up to

110 µm in length and 6–10 µm in diameter. Conidia are usually pale to olivaceous-brown, produced singly or seldom in short chains, straight or slightly flexuous, obclavate to elongate, double walled with 0–8 longitudinal or oblique and 6–19 transverse septa, 75–350 µm in length and 20–30 µm in diameter in the broadest part. Beaks are about one-half to one-third the length of the conidium, filiform, straight,septate, hyaline to pale brown and 5–9 µm in diameter. Because of the variability in spore dimensions, they overlap with dimensions of other large sporedAlternaria species. In routine work, identification is assisted by leaf symptoms, host range and cultural characteristics.

10.6.3-Disease cycle

Alternaria solani belongs to Deuteromycetes and shows a polycyclic life cycle. The fungus reproduces aesexually by means of conidia. The life cycle starts with the fungus overwintering in crop residues or wild members of the Solanaceae family, such as Black nightshade. In the spring, conidia are produced. Multicellular conidia are splashed by water or by wind onto an uninfected plant. They infect the plant by entering through small wounds, stomata, or direct penetration. Infections usually start on older leaves close to the ground. The fungus takes time to grow and eventually forms a lesion. From this lesion, more conidia are created and released. These conidia infect other plants or other parts of the same plant in the same growing season. Every part of the plant can be infected and form lesions. This is especially important when fruit or tubers are infected as they can be used to spread the disease.

In general, development of the pathogen can be aggravated by an increase in inoculum from alternative hosts such as weeds or other solanaceous species. Disease severity and prevalence is highest when plants are mature.

10.6.4-Control measures

Cultural control

- Clear infected debris from field to reduce inoculum for the next year.
- Water the plants in the morning that so plants are wet for the shortest amount of time.
- Use a drip irrigation system to minimize leaf wetness which provides optimal conditions for fungal growth.
- Use mulch so that spores in soil cannot splash onto leaves from the soil.
- Rotate to a non-Solanaceous crop for at least three years.
- If possible, control wild population of *Solanaceae*. This will decrease the amount of inoculum to infect new plants.
- Closely monitor field, especially in warm damp weather when it grows fastest, to reduce loss of crop and spray fungicide in time.
- Use plant resistant cultivars.

- Increase air circulation in rows. Damp conditions allow for optimal growth of *A. Solani* and the disease spreads more rapidly. This can be achieved by planting farther apart or by trimming leaves.

Chemical control

There are numerous fungicides on the market for controlling early blight. Some of the fungicides on the market are azoxystrobin, pyraclostrobin, Bacillus subtilis, chlorothalonil, copper products, hydrogen dioxide (Hydroperoxyl), mancozeb, potassium bicarbonate, and ziram. Specific spraying regiments are found on the label. Labels for these products should be read carefully before applying.

10.7 SUMMARY

Disease is a condition that occurs in consequence of abnormal changes in the form, physiology, integrity or behaviour of the plant. Plant diseases are caused by pathogens. Hence a pathogen is always associated with a disease. The disease is caused by bacteria, fungi, viruses and nematodes e.g., Citrus canker, Blight and wart disease of potato, mosaic disease of tobacco etc. They show various types of symptoms or sign on the plant leaves, twigs, flower and fruits. These diseases can be controlled biologically or chemically.

10.8 GLOSSARY

Biotroph: A plant pathogenic fungus that requires living host cells i.e. an obligate parasite. Pathogenicity: The relative capability of a pathogen to cause disease.
Deficiency: Abnormality or disease caused by the lack or subnormal level of availability of one or more essential nutrient elements.
Disease cycle: The chain of events involved in disease development.
Disease: Any deviation in the general health, or physiology or function of plant or plant parts, is recognized as a disease.
Infection: The initiation and establishment of a parasite within a host plant.
Inoculum: The portion of pathogen which is transferred to the host plant and cause disease.
Incubation period: The period between penetration of a pathogen to the host and the first appearance of symptoms on the plant.
Parasite: An organism living upon or in another living organism (the host) and obtaining the food from the invading host.
Pathogen: An entity, usually a micro-organism that can cause the disease.
Pathogenesis: It is a process caused by an infectious agent (pathogen) when it comes in contact with a susceptible host.
Primary infection: The first infection of a plant by the over wintering or over summering of the pathogen.
Symptoms: The external and internal reaction or alterations of a plant as a result of disease.
Virulence: The degree of infectivity of a given pathogen.

10.9 SELF ASSESSMENT QUESTION

10.9.1: Fill in the blanks-

1. TMV was first discovered by……

2. Inhibition of growth due to reduction in cell division or cell size is called…….

3. Wart disease of Potato is caused by…….

4……is a condition that occurs in consequence of abnormal changes in the form, physiology, integrity or behaviour of the plant.

5. Plant pathology is the study and control of……. and……that cause diseases.

6. The chain of events involved in disease development…….

10.9.2: Choose the correct answer from the given below:

1. The Irish potato famine occurred in North Europe during 1845 was caused by:
(a) Alternaria solani *(b) Phytophthora infestans*
(c) Synchytrium endobioticum *(d) Erwinia carotovora*

2. Which of the following combination of factors are responsible for a disease, its study and control?
(a) Abiotic and environmental factors. (b) Biotic and abiotic factors.
(c) Pathogens and environmental factors. (d) Answers b and c are true.

3. Early blight of Potato is caused by:
(a) Alternariasolani *(b)Phytophthorainfestans*
(c) Synchytriumendobioticum *(d) Solanum virus-14*

4. Symptoms (but not signs) of a disease include:
(a) Galls produced by parasitic wasps.
(b) The powdery substance on lilac leaves infected with powdery mildew.
(c) Wilt.
(d) Both a and c are correct.

ANSWER Keys:
10.9.1: Fill in the blanks: 1. Iwanowski, 2. Atrophy, 3. *Synchytriumendobioticum,* 4. Disease, 5. disease and pathogen, 6. disease cycle

10.9.2: Choose the correct answer from the given below:

1. (b), 2. (b), 3. (a), 4, (d)

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10.12 TERMINAL QUESTIONS

Q.1-What do you mean by a disease? Write symptoms, causal organism and control measures of any fungal disease studied by you.

Q.2-Write causal organism, symptoms, disease cycle and control measures of Early blight of potato.

Q.3-Write short notes on:

1-Wilt symptoms.
2-Hypertrophy.
3-Hyperplasia.
4-Necrosis.

UNIT-11- SYMPTOMS, MORPHOLOGY OF THE CAUSAL ORGANISM, DISEASES CYCLE AND CONTROL MEASURES-II

11.1 OBJECTIVES

After reading this unit student will be able:

- To know the morphology of the causal organism of the diseases of Potato, Sugarcane and Wheat.
- To understand the signs or symptoms associated with the disease.
- To learn etiology or disease cycle of the causal organism of the disease.
- To study the control measures or the management of disease.

11.2 INTRODUCTION

Fungi are a group of such plants which lack root system and chlorophyll. Hence, they lack the capacity of mineral absorption and photosynthesis. They obtain their nutrition from other living or dead plants and animals. Most fungi are saprophytic, i.e., they obtain their nutrition from dead organic matter. There are certain fungi which obtain their nutrition from living cells. These are known as parasitic fungi. These fungi also affect the physiological processes of the host. The toxins produced by certain fungi cause morphological deformity in the host. Such effects of the parasite appear in the host plant in the form of disease symptoms.The late blight epidemics of the 1840s triggered the Irish potato famine, but the history of the potato as a food crop is much older. The potato (*Solanum tuberosum*) originated in the highlands of the Andes in the Lake Titicaca area between Bolivia and Peru, where native people had selected hundreds of different cultivars for centuries. Most scientists agree that *Phytophtora infestans* originated in Mexico where both mating types of the fungus are commonplace. Where and how the plant and the pathogen first came together is not certain, but late blight epidemics seem to be described in the northeastern U.S. in about 1843 and Europe in 1845. Potato crops failed for a number of years during the cool and rainy "hungry '40s." Although poor people who were dependent on potatoes for food suffered in many areas, the disaster was greatest in Ireland. One and one-half million people starved and a similar number emigrated during the famine, resulting in a large Irish diaspora in many parts of North America. As with many famines, politics enhanced the suffering. Many Irish peasants grew cereal crops to pay their rent. Although the grain was harvested, it could not be eaten, and was exported to the English landlords throughout the famine. In the 1990s, many exhibits and gatherings in North America and Ireland commemorated the 150th anniversary of the famine. One reason that the early history of late blight is unclear is that the germ theory of disease had not yet been accepted. Many preliminary studies of various plant diseases had been conducted, but Anton de Bary's (the "father of plant pathology") conclusive studies finally convinced the scientific community that the white sporulation of *P. infestans* on infected plants was the causal agent of the disease and not the result of spontaneous generation from the decaying vegetation. Thus, late blight signifies the official beginning of the science of plant pathology. These early studies also contributed to Louis Pasteur's germ theory which was proposed 15 years later. Red rot is one of the major constraints in the profitable cultivation of sugarcane in many states of India. Except Maharashtra, the disease has been

recorded in all the states. This disease drastically retards the yield and considerably deteriorates the juice quantity and quality thus hitting both the cane growers and millers. Many good varieties have gone out of cultivation due to red rot. Loose smut of wheat is a common disease throughout the wheat-growing regions of the world. The mycelium remains dormant in the embryo, and developing kernels are replaced by black teliospores. No seeds develop in infected heads. The disease is spread by windblown teliospores. Cool, humid weather favors the development of this disease. Some of the important fungal diseases of plants are discussed in this chapter.

11.3 LATE BLIGHT OF POTATO

The late blight is one of the destructive diseases of potato. It is the most serious of all the potato diseases when conditions are favourable for its spread. The famous Irish famine of 1845-46 was due largely to the failure of potato crop due to late blight infection. In India, the disease was first introduced into the Nilgiri hills between 1870 and 1880 and very soon, it spread to Darjeeling in the Himalayan ranges. The first severe outbreaks of the disease were reported between 1912 and 1928 from Assam, Bengal and Bihar. In northern India, the disease was first reported from the plains of western U.P. Since then, the disease appears as a regular feature in the plains causing severe losses to the potato crop.

Fig.11.1. Leaves, stems and tuber showing late blight symptoms

11.3.1- Symptoms

The symptoms of the disease can be seen on any part of the plant, viz., leaves, petioles, stems and tubers (**Fig.11.1)**. On the leaves, the symptoms appear in the form of dark brown, oval or irregular water soaked areas. In the early stages, the symptoms develop at the tips or the margins of the old leaves. The infection spreads vigorously when temperature is low and atmosphere is humid, and soon appears in the form of blight. In case of severe infection,practically all the parts of the host become brown and then degenerate. After the tops have been blighted, the infection reaches to the underground tubers. In the infected tubers, the skin becomes slightly sunken and dark in colour. If conditions are humid, the cells of the tuber become soft and dark brown. This symptom is known as **wet rot**. In dry

atmosphere, however, the potato pulp does not rot but its anterior part becomes black. This condition is called **dry rot.**

11.3.2-Morphology of the causal organism

The disease is caused by *Phytophthora infestans*. The mycelium is endophytic consisting of hyaline, profusely branched coenocytic hyphae. The hyphae develop intercellularly and form haustoria. Ovoid or lemon-shaped sporangia are produced on sporangiophores. The sporangia are at first terminal but become lateral due to sympodial branching of the sporangiophore. The sporangium may germinate directly, forming a germ tube at the apex or its protoplasmic contents divide to form a number of biflagellate zoospores which emerge through the papilla. The method of germination is much governed by temperature; low temperature favours zoospores formation, whereas higher temperature is responsible for the germ tube development. Sexual reproduction is oogamous type. The antheridium is somewhat elongated and amphigynous, whereas the oogonium is pear-shaped to almost spherical, smooth and reddish brown in colour. The fusion of egg and male nucleus results in the formation of a diploid oospore. The oospore germinates by producing a germ tube and the tip of which tube ultimately develops in to a sporangium.

11.3.3-Disease cycle

The infected tubers are the main source of infection. Thedormant mycelium in the tubers becomes active and grows upward in the stem and sporulates on small shoots. Epiphytotics of the disease are likely to occur when unusually cool weather combined with abundant moisture prevails at the time the sporangia are being produced. The optimum temperature for the sporulation is 21˚C. The sporangia germinate giving rise to zoospores at 12˚C and by germ tube, 21˚C. 100 percent relative humidity causes abundant production of sporangia. Sporangia are easily detached and disseminated by rain or air. On reaching a suitable host, the sporangia germinate either by germ tubes or by zoospores depending upon the environmental conditions. The spores from the blighted leaves are washed down into the soil where they penetrate to different depths reaching the healthy tubers. Contact of healthy tubers with diseased leaves at the harvesting time is another source of tuber infection **(Fig.11.2)**.

Several theories have been put forth to explain the yearly occurrence of late blight; but (i) persistence of mycelium in the affected tubers, and (ii) survival of fungus in the fruiting stage or as dormant mycelium in the tubers left in the field from the preceding crop, constituting the primary inoculum are the most accepted ones. In Indian climatic conditions, the possibility of survival of the pathogen through soil in any form is very remote. Thus, it seems the infected seed tubers are responsible for the spread of the disease.

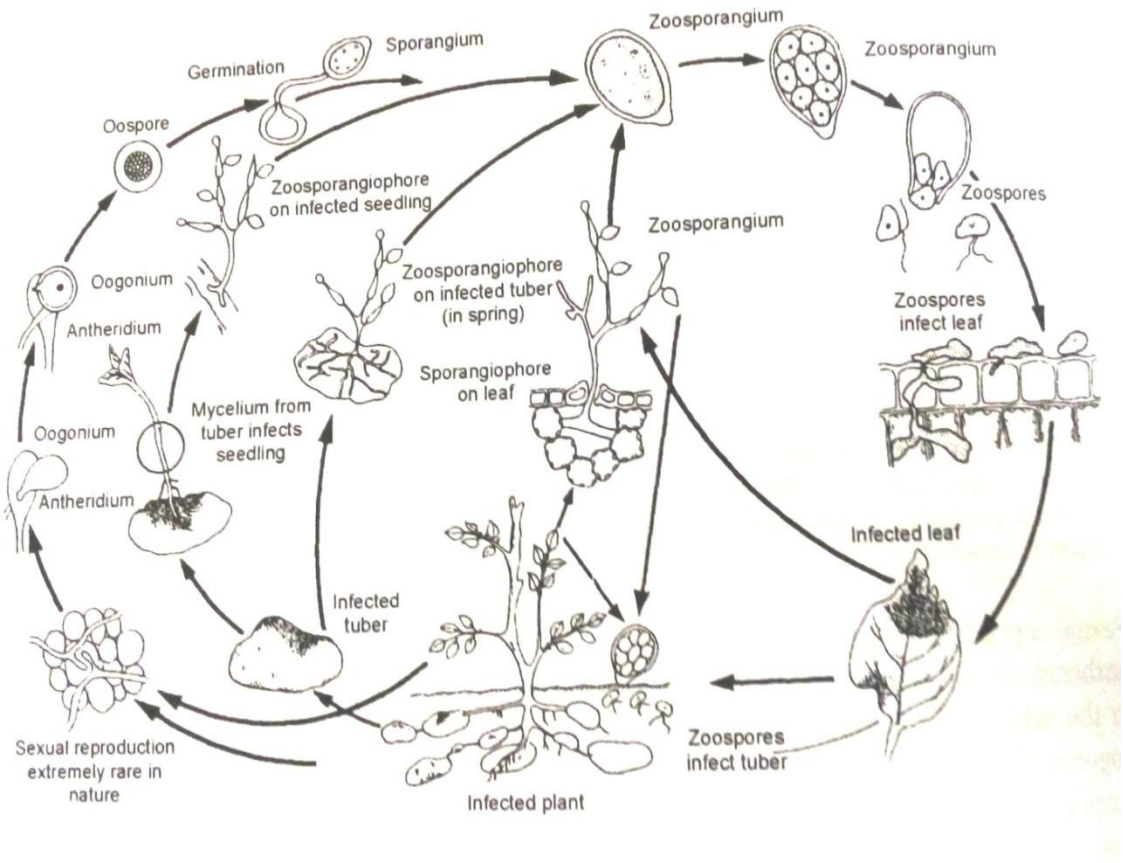

Fig.11.2: *Phytophthorainfestans* causing late blight disease of Potato (Disease cycle)

11.3.4-Control measures

The late blight can be effectively controlled by the following methods:

- Healthy seed tubers.
- Field sanitation.
- Delayed harvesting and sorting potatoes from a blighted field.
- Tuber treatment before storage.
- Storage in a cool, dry and well aerated store house.
- Use of disease-resistant varieties, like Kufri red, Kufrineela, Kufrikundan, etc.
- Foliar spray of fungicides like Bordeaux mixture, Dithane Z-78, Fytolan, Blitox, etc.

Good late blight management practices include disease prevention, sanitation, cultural practices, field monitoring, an effective fungicide spray program and postharvest protection.

Sanitation, Cultural Practices and Field Monitoring

1. Plant disease-free seed. Inspect seed potatoes within 24 hours of delivery. Cut a sample of tubers and look for the reddish brown dry rot characteristic of late blight tuber rot.

2. Test the seed lots for late blight before planting. When purchasing seed, it is recommended to have them tested for the absence of blighted seed by an authorized provincial service in your region.

3. Grade seed potatoes after being cut to remove any late blight infected tubers. Infected tubers can be a potential source of early infections in the field.

4. Frequently disinfect seed cutting equipment.

5. Immediately after cutting, treat seed with a recommended mancozeb-based seed piece fungicide.

6.Bury cull piles before crop emergence. Infected tubers in cull and rock dump piles are a major source of infection for the new crop. Buried tubers may germinate and grow. Rogue or treat volunteer plants with a herbicide. Slivers and pieces of potato remaining from cutting operations should also be buried.

7. Volunteer potato plants can be a source of infection. Any volunteer potato plants in a field should be removed by rouging or using herbicides. For non-seed fields where late blight is found, consider applying a sprout inhibitor to control volunteers in the following year.

8. Controlling late blight susceptible Solanacaeous weeds such as hairy nightshade, in potato as well as in non-potato crops is an important measure of controlling late blight incidence in potato.

9. Immediately report any suspected incidence of late blight to your extension specialist or to the nearest agricultural center. If late blight is identified, roguers and other workers should wear pants and boots which can be disinfected (e.g. Bleach solution diluted 1:9 with water; or other disinfectants) between different fields. Equipment should also be washed and disinfected before entering adjoining fields.

10. Construction of a deep hill may help restrict spores from washing down through the soil and infecting the developing tubers.

11. Weather conditions favorable for late blight development can be determined using late blight forecasting models that use relative humidity, rainfall and temperature data. The weather data is converted into units called "severity values" for the purpose of predicting late blight outbreaks. Consult your extension specialist for information on late blight forecasts for your area.

12. Monitor your crop. Scout fields with special attention to low spots and along tree edges where moisture persists after rains or dews. Have a good look at stems and leaves for late blight symptoms. Stem infections will be diminished during dry periods but will be re-activated in humid weather.

13. When late blight is first identified, top kill or rogue an area twice the size of the infected area. All rogued infected plants should be put in plastic bags and then taken out of the field.

14. Rolling or rotobeating a crop before top killing would expose the soil and lower canopy to drying. Rolling also seals cracks in the soil and may reduce tuber infections.

15. Top kill at least 2 weeks prior to harvest to allow time for infected tubers to rot and to promote tuber maturity and thicker skins at harvest. Vines should be completely dead at harvest. 16. Late blight causing spores survive longer in wet soils. Harvest when the soil surface is dry or wind row the potatoes and allow the surface of tubers to dry before harvest.

17. Dig potential problem areas such as sprayer rows and low areas last and store these potatoes where they can be easily moved out in case of a problem.

18. Wet or bruised tubers are more likely to get infected with late blight. Skinned or cut and bruised areas are direct entry points for late blight and other diseases. However, wound is not always a requirement to occur an infection on wet tubers.

19. Grade out any obviously diseased potatoes before they are put into storage.

20. If late blight is seen on the foliage, there will also likely be tuber infections. Immediately following harvest, these tubers should be ventilated with a high volume of air at low humidity until the surface of the potatoes is dry. This may lead to higher shrinkage than normal, but losses due to storage rots will be reduced.

21. Potato lots with 5% or more late blight infections (by weight) should be stored in the front of the storage or in separate bins, so they can be easily removed in a high risk situation.

22. Postharvest treatment with fungicides containing phosphorous acid will protect healthy tubers from pink rot or late blight infections occurring at harvest. Ensure even coverage with the fungicide. Follow label rate and recommendations.

Fungicide Spray Programme:

A preventive spray program is always recommended. Effective control by fungicides requires good coverage of the foliage, proper rates and timing of applications. Generally, fungicides are most effective in the early stages of infections before symptoms appear. However, no fungicide can cure an established infection. Fungicides against late blight are essentially protectants and not particularly persistent. They must be used to protect plants as prophylactic sprays in routine programmes, in an overall strategy designed to prevent the disease infecting the crop.

Contact fungicides retain on the surface of the plant where these are applied and only protect the plant where the spray is deposited or subsequently re-distributed by moisture. Contact fungicides are not taken into the plant and therefore are vulnerable to erosion by wind, rain and degradation by sunlight. They do not protect new plant growth formed after the spray has been applied. These fungicides have no effect against already established late blight infections.

Translaminar fungicides are absorbed by the leaves and show limited redistribution from upper sprayed surface to lower unsprayed surface. They are generally more rainfast than contact fungicides, but do not move within the plant to protect the new growth.

Systemic fungicides are absorbed into plant tissue and may offer some after-infection activity. Very few fungicides are truly systemic (i.e., move freely throughout the plant); however, some are upwardly systemic (i.e., move only upward in the plant through xylem tissue), and some are locally systemic (i.e., move into treated leaves and redistribute to some degree within the treated area of the plant.

11.4 RED ROT OF SUGARCANE

Red rot of sugarcane is a serious disease prevalent wherever sugarcane is grown in the world. The disease was first described in Java in 1893 under the name red smut. In 1906, Butler reported the disease symptoms in several cane varieties in India and renamed it as red rot. Serious Epiphytotics of this disease have occurred in Northern India (U.P., and Bihar during 1939-40 and 1946-47). However, localized epidemics occur almost every year.

11.4.1- Symptoms

The disease appears on all aerial parts of the plant. The early symptoms of the disease are yellowing and drooping of leaves. The stems show little indication of the disease in early stages but as severity of the disease increases the cane splits lengthwise and subsequently red blotchesappear throughout the length of the cane. These blotches emit a peculiar smell of alcohol fermentation. The reddening appears mainly in the vascular region. Ultimately, the cane becomes dull in appearance, get rotten and shrinks at the internodes. Besides, the conspicuous symptoms also appear on the leaves. On the mid-ribs of leaves, infection originates as a dark reddish area which elongates rapidly, forming blood-red lesions with dark margins. In later conditions, the centre of the lesions becomes straw coloured (**Fig. 3)**.

Fig. 11.3: Yellowing and drooping of leaves and reddening of stems shows the Red rot disease in Sugarcane

11.4.2-Morphology of the causal organism

The red rot of sugarcane is caused by *Colletotricum falcatum.* The perfect stage of this fungus has been described in Ascomycetes as *Glomerella tucumanensis*. The mycelium is inter-or intracellular, profusely branched, septate and contains characteristic oil droplets. In later stages the hyphae closely interwine with one another and form small stromata under the host epidermis. The fungus reproduces asexually by the formation of conidia developed in acervuli. The conidiophores are usually aseptate, unbranched and are arranged compactly like palisade tissue. Usually a single conidium develops at the tip of each conidiophore but occasionally the conidia are produced in acrogenous chains. The conidia are hyaline, crescent or sickle-shaped. An oil drop is present in the centre of each conidium. The conidia germinate by producing 1 to 4 germ tubes and form the new mycelium.

Perithecia are globose and are produced on the various part of the host, and measure 150-300µm in diameter. Asci are numerous, hyaline, clavate and measure 50-60 × 7-10.5µm. Each ascus contains eight ascospores arranged biserially. Intermixed with asci are present numerous delicate paraphysis. New physiological races differing in their pathogenicity to sugarcane cultivars have been frequently reported from different parts of the world.

11.4.3-Disease cycle

Seed sets from diseased canes are the chief means of survival and annual occurrence of the disease. Once, the pathogen establishes, secondary spread occurs by conidia. Ratoon crops also serve as a source of perennation and inoculum multiplication. High humidity, water-logging conditions, lack of proper cultural operations and continuous cultivation of same variety year after year are the main factors responsible for the development of the disease.

11.4.4-Control measures

The following measures may be adopted for the control of red rot of sugarcane:

- Healthy seed selection.
- Cultural practices-ratooning results in the multiplication of the disease, hence it must be discouraged.
- Field sanitation and crop rotation.
- Use of resistant varieties like Co 846, 951, 975, 1007, 1148, 62101, 62399, S109, BO22, 22, COLK 7702, 7710, etc.
- Treatment of seed setts with organo-mercurials like aretan or agallol (0.25% suspension) helps in eradication of superficial inoculum. Besides, treatment of setts in 0.5% bavistin solution reduces the incidence of red rot from infected setts.

11.5 LOOSE SMUT OF WHEAT

Loose smut, a very serious disease of wheat, is world-wide in occurrence and is a serious problem in the humid and semi-humid wheat growing regions. In India, the disease occurs in all wheat growing areas, but its incidence is higher in the cooler and moist northern parts than in the south. According to an estimate, the disease causes about 40% loss in wheat yield every year.

11.5.1 Symptoms

It is very difficult to detect infected plants in the field until heading. At this time, infected heads emerge earlier than normal heads. The entire inflorescence is commonly affected and appears as a mass of olive-black spores, initially covered by a thin gray membrane (**Fig.4).** Once the membrane ruptures, the head appears powdery. Spores are dislodged, leaving only the rachis intact. In some cases remnants of glumes and awns may be present on the exposed rachis. Smutted headsare shorter than healthy heads due to a reduction in the length of the rachis and peduncle. All or a portion of the heads on an infected plant may exhibit these

symptoms. While infected heads are shorter, the rest of the plant is slightly taller than healthy plants. Prior to heading affected plants have dark green erect leaves. Chlorotic streaks may also be visible on the leaves.

Fig.11.4. Loose smut of wheat: infected inflorescence

11.5.2-Morphology of the causal organism

The fungus responsible for the disease is *Ustilago nuda*var *tritici.* The mycelium of this fungus consists of multicelluar hyphae which are hyaline but turn brown at maturity. The primary mycelium is septate and uninucleate, whereas the secondary mycelium is branched, septate and bi-nucleate. In vegetative phase the secondary mycelium is profusely branched and spreads in intercellular spaces of the host tissues. The pathogen reproduces by the formation of chlamydospores and basidiospores. The hyphal cells are transformed into olivaceous brown, spherical and echinulate chlamydospores which germinate readily and produce basidia and basidiospores. The haploid basidiospores produce uninucleate primary mycelium on germination.

11.5.3-Disease cycle

Ears of infected plants emerge early. The spores released from the infected heads land on the later emerging florets and infect the developing seed. Infection during flowering is favored by frequent rain showers, high humidity and temperature. The disease is internally seed borne, where pathogen infects the embryo in the seed (**Fig.11.5).**

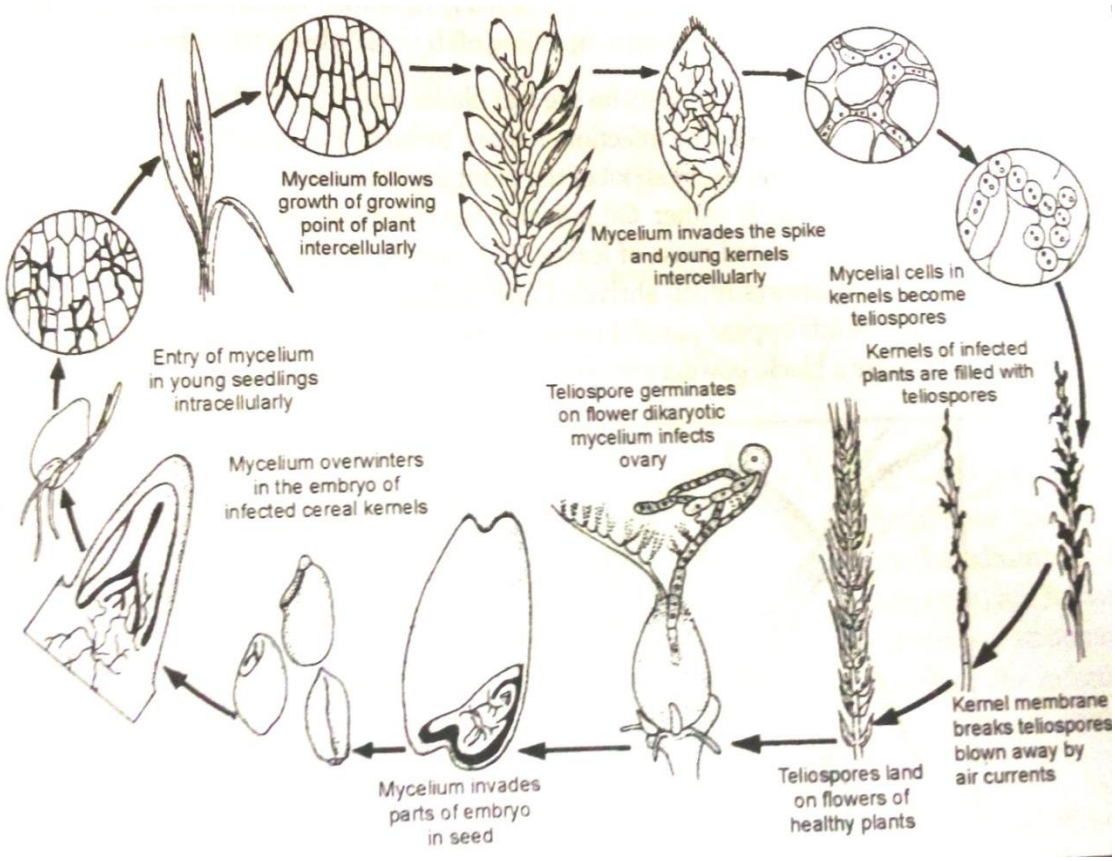

Fig.11.5: *Ustilago nuda* causing Loose smut of wheat disease showing disease cycle

11.5.4-Control measures

1. Sow certified seed of wheat varieties that are resistant to loose smut and recommended for your area by your nearest Extension adviser. None of the wheat varieties are resistant to all the physiologic races of the loose smut fungus, however some are moderately to highly resistant.

2. If you grow a variety susceptible to loose smut, be sure to plant certified seed purchased from a reliable dealer. Certified seed carries a minimum amount of infection. Only wheat fields that meet rigid specifications with respect to disease will pass certification requirements. Competent inspectors closely examine fields of all growers who apply for seed certification to make sure that no loose smut, or other serious seedborne wheat diseases are present.

3. The best insurance against loose smut is seed treatment with a fungicide containing carboxin or triadimenol systemic fungicides applied to the seed. These fungicides have the unique ability of being taken up by the germinating seed. They check or kill the loose smut fungus within the seed while controlling surface-borne bunt or covered smut and a number of fungi that cause weedling blights (damping-off). Carboxin is sold under various trade names

often in combination with another fungicide. These mixtures give excellent smut control and also provide protection against a wide range of fungi that attack the germinating seed and young seedling.

4. The hot-water soak technique for ridding wheat seed of the loose smut fungus, while highly effective, is difficult to use and often reduces the germination percentage and vigor of the wheat seed. This procedure should be attempted only by experienced personnel with the necessary equipment.

11.6 SUMMARY

Fungi are a group of such plants which lack root system and chlorophyll. Hence, they lack the capacity of mineral absorption and photosynthesis. They obtain their nutrition from other living or dead plants and animals. Most fungi are saprophytic, i.e., they obtain their nutrition from dead organic matter. There are certain fungi which obtain their nutrition from living cells. These are known as parasitic fungi. These fiungi also affect the physiological processes of the host. The toxins produced by certain fungi cause morphological deformity in the host. Such effects of the parasite appear in the host plant in the form of disease symptoms. The disease caused by these fungi include late blight of potato, Red rot of sugarcane, Loose smut of wheat etc., which can be controlled by number of methods, i.e. chemically or biologically.

11.7 GLOSSARY

Acervulus: A mat of hyphae giving rise to short conidiophores closely packed together forming a bed-like mass.
Ascus: The cell in which nuclear fusion occurs in ascomycetes and in which ascospores subsequently develop.
Chlamydospore: A thick-walled, resistant spore formed by the direct differentiation of the cells of the mycelium.
Chlorosis: Yellowing due to lack of chlorophyll.
Epiphytotic: A widespread occurrence of a plant disease.
Fungus: The achlorophyllous thalloid microorganisms with filamentous and branched somatic structure, cell wall demonstable nuclei and capable of reproducing typically by sexual and asexual methods.
Host: A living organism harboring a parasite.
Parasite: An organism that derives its nourishment from a living organism (host).
Rot: Fungal infection resulting in softening, discolouration and disintegration (in some types) of succulent plant tissues.
Smut: The diseases in which infected plants show masses of dark, powdery spores caused by members of Ustilaginales.

11.8 SELF ASSESSMENT QUESTION

11.8.1-Fill in the blanks:

1. The disease of sugarcane is caused by……..

2. Causal organism of early blight of potato is ……..

3. CO846 and BO22 is the resistant varieties of…….crop.

4. A widespread occurrence of a plant disease is called……

5. Carboxin is a ……..fungicide.

6. *Glomerellatucumanensis* is the perfect stage of the fungus……

11.8.2- Choose the correct answer from the given below:

1. An organism that derives its nourishment from a living organism is called

(a) Pathogen (b) Saprophyte

(c) Parasite (d) Host

2. Late blight of potato is caused by

(a) Alternariasolani (b) *Albugo candida*

(c) Phytophthorainfestans (d) *Colletotrichumfalcatum*

11.8.3- True or False (T/F):

1. Red Rot of sugarcane is caused by *Colleotrichum falcatum.*

2. Loose smut of wheat is caused by *Xanthomonas oryzae.*

Answers keys:

11.8.1: 1**.** *Colleotrichumfalcatum*, 2. *Alternariasolani*, 3. Sugarcane, 4. Epiphytotic, 5. Systemic, 6. *Colleotrichumfalcatum,*

11.8.2: 1. (c), 2. (c),

11.8.3: 1.True, 2. False

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11.11-TERMINAL QUESTIONS

Q.1-Write causal organism, disease cycle and control measures of any two fungal diseases.

Q.2-Write short notes on Red Rot of Sugarcane and Loose smut of wheat.

Q.3- Differentiate Ascospores and Basidiospores.

Q.4-Write short note:

a) Define Ascospores
b) What is a pathogen and parasite?
c) Define the term Epiphytotic.

UNIT-12- PLANT PROTECTION AND CONTROL MEASURES PLANT DISEASES

12.1- OBJECTIVES

After reading this unit student will be able:

- To understand the meaning of plant protection and its aims.
- To study the various procedures of plant protection and their impact on the environment.
- To understand the disease cycle – disease control mechanism.
- To study the various control measures of plant diseases.

12.2- INTRODUCTION

There are many infectitious organisms (pathogens) present in the environment that cause different types of diseases in plants. Plant disease epidemics can cause famines, destroying a thriving industry or can poison animals and humans. In order to control and eradicate the pathogen, Plant protection plays a very important role in the management of plant diseases.

Successful disease control requires thorough knowledge of the causal agent and the disease cycle, host-pathogen interactions in relation to environmental factors, and cost. Disease control starts with the best variety, seed, or planting stock available and continues throughout the life of the plant.

Agro-technical method was used for the first time for plant protection early in the 20th century by the Russian entomologist N. V. Kurdiumov. The German scientists P. Zorauer and G. Gassner; the American scientists G. Keitt and R. Sprague; and the Swiss scientist E. Gäumann worked further for the development of this technique

Correct crop rotations were considered a very important measure because the continuous cultivation of any annual plant results in the concentration of pests and causative agents of diseases. Their numbers can also frequently be reduced by appropriate methods of cultivating the soil.

Protection

Preventing infection by creating a chemical toxic barrier between the plant surface and pathogens.

- Chemical treatment
- Chemical control of insect vector
- Modification of environment or environment condition

12.3-PLANT PROTECTION

Plant Protection focuses on keeping plants healthy---from diagnosing diseases to implementing environmentally friendly pest-management practices. With an ever-expanding

population and increasing pressure on food and fiber supplies, Plant Protection plays a vital role in improving our quality of life.

Plant protection is a branch of agricultural science that devises ways and means of controlling diseases, pests, and weeds of crops and trees, as well as a set of measures used in agriculture and forestry to prevent and eliminate the damage done to plants by harmful organisms.

The goal of plant protection is not only to destroy harmful organisms or limit their activity but also to forecast the time they appear and the possible extent to which they might spread, as well as to prevent especially harmful organisms from moving from one country and region to other.

Plant protection is based on the data obtained by several agronomic, zoological, and botanical disciplines; genetics; biochemistry; plant and animal biochemistry and physiology. Plant protection is closely related to such sciences as meteorology and climatology; chemistry and physics, which provide the scientific basis of chemical and biophysical control methods; and hygiene and toxicology, which study the direct and indirect effects of pesticides on plants and animals.

Ever since humans have relied on planted crops as the main source of food, plant diseases, insects, rodents, weeds and other pest organisms have been a constant threat to food supply, therefore plant protection plays a very important role in controlling loss and damage to the important food crops and plants.

Process of plant protection

A fundamentally important starting point for plant protection is the ability to anticipate the emergence and spread of noxious organisms and to prevent their introduction and spread before they become agricultural pests in specific crops and regions.

The various steps in plant protection are quite similar to those of human medicine.

1. To diagnose the problem which involves proper identification of the organisms responsible for the damage symptoms observed.
2. To assess the extent of the damage and the yield or revenue loss likely to result from this damage, which helps grower to make decisions on whether to invest resources in combating the pest.
3. To consider the various options available for controlling pests, including host plant resistance, cropping system and cultivation practices that reduce pest populations.

Plant protection measures

Plant protection measures are carried out to limit performance and yield losses in crop production during the growing season and afterwards (storage protection) as well as for quarantine purposes. They serve primarily to safeguard yields, although in combination with other cultivation measures they can also help to raise yields.

A wide variety of individual measures - with varying ecological, economic and socio-economic impacts - are available for keeping harmful organisms (diseases, pests, weeds) below the economic threshold. To reduce the probability of damage, preventive measures are taken in the areas listed below:

- site design (hedges, border strips, tree guards etc.)

- site and variety selection
- sowing, planting
- healthy seed and planting stock
- crop rotation, intercropping
- tillage, land improvement
- crop tending
- harvesting
- storage

Measures in these areas are backed up by the following direct forms of plant protection:

- physical methods
- chemical methods
- biotechnical methods
- biological methods
- integrated methods

Physical methods

Physical methods directly destroy harmful organisms, aim to retard their development or prevent them from spreading. They can be divided into mechanical and thermal measures.

The mechanical measures include:

- Tillage to control weeds and pests (hoeing, removal of affected parts of plants and intermediate hosts),
- Flooding of fields to combat soil-borne harmful organisms (e.g. Fusariumoxysporum, which causes banana wilt),
- Laying of sticky belts to trap flightless insect pests and other measures for catching pests or keeping them away from crops, such as fences, trenches (locust control), traps and picking-off of pests.

Thermal methods utilise the harmful organisms' sensitivity to high or low temperatures and includes:

- Hot-water treatment of seed and planting stock (e.g. to combat viruses and bacteria in sugar cane cuttings)
- Solarisation (covering the surface of the ground with plastic sheeting produces phytosanitary effects by virtue of the greenhouse effect resulting from insolation, e.g. for controlling parasitic seed plants, soil-borne harmful organisms etc.),
- Burning-over to control weeds and burning of crop residues. Low temperatures inhibit the spread of certain storage pests.

Chemical methods

Eradicative, protective and curative methods are used in chemical plant protection to destroy harmful organisms or keep them away from plants, to protect plants against attack and penetration by harmful organisms and to cure plants (or parts of plants) that have already become infested or diseased.

Chemical methods can be used in three ways:

1. Soil treatments

2. Seed treatments
3. Foliar sprays

Soil treatments are designed to kill soil-inhabiting nematodes, fungi and bacteria. This can be achieved by using steam or chemical fumigants. Soil borne nematodes can be killed by applying granular or liquid nematicides. Most soil is treated well before planting; however, certain fungicides can be mixed with the soil at planting time.

Fig.12. 1. Application of protective sprays

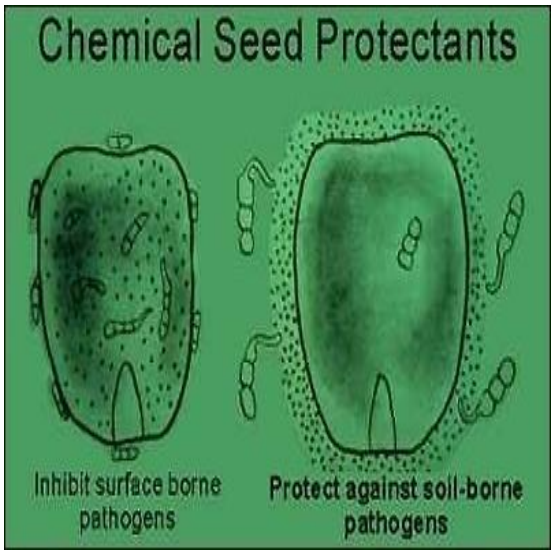


Fig.12.2. Benefits of seed protectant

Seed treatments consist of dusts o slurries applied to seed to protect it primarily against decay and damping-off caused by soil borne pathogenic fungi. Seeds, bulbs, corms and tubers are frequently treated with chemicals to eradicate pathogenic bacteria, fungi and nematodes.

Protective sprays and dusts applied to the foliage and fruit crops and ornamentals include a wide range of organic chemicals designed to prevent infection. Protectants are not absorbed by or translocated through the plant, thus they protect only those parts of the plant treated before invasion by the pathogen (Fig.12.1, 12.2).

Fig.12.3. A farmer applying pesticides to the crops

Biotechnical methods

Biotechnical methods utilise the natural reactions of the harmful organisms to physical and

chemical stimuli in order to bring about changes in their behaviour for the purpose of plant protection (e.g. light and colour traps, chemical attractants, antibodies, pheromones, hormones, growth regulators). The emphasis is on measures which aim not to directly kill the harmful organisms, but rather to permit population monitoring for the purpose of forecasting, defensive action and deterrence. The harmful organisms can be killed by combining biotechnical methods with chemical measures. Disease resistant varieties could be developed through biotechnological methods.

Biological methods

Biological plant protection involves using organisms and their activity to protect plants and enhance their resistance to biotic (harmful organisms) and abiotic limiting factors. For the purpose of pest and disease control, beneficial organisms are specifically preserved and fostered, released in large numbers or introduced into habitats where they have not been found hitherto. Biological control of weeds has to date primarily involved introducing beneficial organisms into new habitats.

Another biological method is that of inducing resistance to disease. This can be done, for example, by infecting plants with pathogens having low virulence.

Biological insecticides, derived from naturally occurring microorganisms (*e.g.*: *bacillus thuringiensis*, entomopathogenic fungi and entomopathogenic nematodes), also fit in this category.

Biological control also involves introduction of exotic species, or it can be a matter of harnessing whatever form of biological control exists naturally in the ecosystem in question. The induction of plant resistance using non-pathogenic or incompatible micro-organisms is also a form of biological control. Some diseases that can be successfully controlled using biological agents are pathogens of pruning wounds and other cut surfaces, crown gall, diseases of leaves and flowers, such as powdery mildew, diseases of fruits and vegetables, such as Botrytis, and fungal pathogens in the soil (disease suppressive soils).

Integrated methods

Integrated plant protection is a concept which involves coordinated use of all ecologically and economically justifiable methods in order to keep harmful organisms below the economic threshold. The emphasis is on utilising natural limiting factors. The main aim is to preserve the balance of nature as far as possible; this is to be achieved by reducing use of chemical plant protection methods and simultaneously employing a variety of measures from the other categories. It is here that the links with the plant production sector are particularly close.

Use of pesticides is to be reduced to the essential minimum by abandoning the practice of routine or calendar-based spraying, gearing pesticide dosage to actual conditions, refraining from the use of broad-spectrum persistent agents (liable to harm beneficial organisms) and selecting the time of application such that beneficial organisms suffer no adverse effects.

Integrated plant protection methods generally prove more successful in permanent crops than in short-lived crops. The limits and risks attaching to these methods become clear if the work is performed by untrained personnel. Use of integrated plant protection methods generally calls for detailed knowledge of biological, ecological and economic factors.

Environmental impact of plant protection

The environmental impacts of plant protection are caused by the influence of substances and/or forms of energy on organisms and their functioning as well as on soil, water and air. The extent to which a plant protection measure is harmful, and in particular the degree to which it is liable to cause lasting harm, is determined by its varied influences on the functioning of the ecosystem.

Harmful effects of environmental impacts are observed if plant protection measures fail to take adequate account of ecological considerations. Repeated, one-sided application of a particular active ingredient (like pesticides) will cause the harmful organism to develop resistance to it.

Although non-specific control methods control the spread of a harmful organism, they also unintentionally affect numerous useful organisms. They, thus adversely influence the diversity of species and biological regulation mechanisms, creating a risk that harmful organisms may multiply more rapidly and it consequently needs additional plant protection measures.

It also has bad effects on the abiotic environment (e.g. soil erosion caused by tillage carried out for the purpose of plant protection).

Plant protection is linked to other plant production measures and is thus subordinate to the goals of plant production (cf. environmental brief Plant Production). Measures in the field of plant production also have a bearing on the goals and environmental impacts of the following sectors:

- Livestock farming (fodder, quality control)
- Fisheries (prevention of water pollution)
- Agro-industry (quality standards)
- Health and nutrition, including drinking-water supplies (toxicology, residues)
- Analysis, diagnosis, testing (quality control, development, analytical techniques)
- Chemical industry (pesticide production)

Decisions on plant protection measures may therefore be influenced by measures in these areas. When assessments are being made, attention must be paid to the possibility that impacts generated by the various sectors could have a cumulative effect and thereby increase the amount of damage done.

12.4-CONTROL MEASURES OF PLANT DISEASES

In controlling diseases, plants are generally treated as populations rather than individuals, although some plants, especially trees, ornamentals and sometimes virus-infected plants are treated individually.

In control of diseases of plants, the measures are mostly preventive i.e. Prophylactic, and there are only a few situations where diseased plants are cured individually by treatment i.e. therapeutic, or curative. Thus, except cases as diseased tree, fruit or some ornamentals, where curative methods are used, in general, care of healthy plants is taken in advance to prevent their infection.

Factors in the control of plant diseases:
1. Soil management.
2. Selection of disease-resistant plants (cultivars).
3. Proper watering of plants.
4. Protection of plants from extreme weather conditions.
5. Rotation of crops.

The soil selected for cultivation should be good. The best soil for most plants is loamy, with good drainage and aeration. This minimizes diseases that attack the roots and allows the roots to feed nutrients from the soil to the rest of the plant. Organic methods, such as the addition of compost, can improve soil quality, and fertilizers can be added to the soil to enrich the nutrient base.
Disposal of infected plants is important in the control of diseases, as is the careful maintenance of tools and equipment used in farming and gardening.
Crop rotation is an important part of reducing plant diseases. Pathogens that favor a specific crop are deprived of their preferred host when crops are rotated. This reduces the virulence of the pathogen and is a natural way to reduce plant disease.

Methods of control

The main methods of plant disease control have been categorized in different ways. Basically there are three main control measures:
Prophylactic measures
Curative measures
Biological measures

12.4.1- Prophylactic measures

Prophylaxis aims protection of host plant from infection in following three major ways:
1. Avoidance of the pathogen: Occurrence of a disease can be avoided by planting/sowing a crop at times when, or in areas where, inoculum remain ineffective/inactive due to environmental conditions, or is rare or absent. Avoidance can be carried out by:

(i) Choice of geographical area
(ii) Selection of a field
(iii) Adjustment of time of sowing
(iv) Use of disease escaping varieties
(v) Use of pathogen-free seed and planting material

(i) Choice of geographical area: Selection of geographical area for any crop is made on the basis of suitability of climate for the crop. The same climate may be suitable for the activities of the pathogen also. Many fungi and bacterial disease are more severe in wet areas than in dry areas.
Crops susceptible to such diseases can be grown in dry areas with the help of irrigation. Bean anthracnose disease is common in wet areas where seeds produced are generally infected. For seed production of bean dry areas are always preferred. Smut and ergot of pearl millet are

serious in areas where rains occur for long duration; therefore cultivation of this crop in such areas is not profitable.

(ii) Selection of a field: For successful cultivation of a crop, a proper field is necessary. If a disease caused by a soil-borne pathogen has been located in a field, it is not used to the same crop for some time. In such diseases, as bacterial wilt of potato, wilt of pigeonpea, smut of pearl
millet, root knot of nematodes the infested field can be avoided.In selecting a field, drainage management is also important. Poor drainage can lead to disease.

(iii) Adjustment of time of sowing: In many diseases the incidence or disease severity is most serious when susceptible stage of the plant growth coincides with favourble conditions for the pathogen. This coincidence can be avoided by alteration in date of planting. It helps in avoiding critical period. Thus, late sown winter crops escape incidence of root rot and wilt favoured by high temperature and moisture that usually occur after the summer rainy season.

(iv) Use of disease escaping varieties: In different crops, certain varieties escape damage by disease because of their growth characters, not due to their genetic resistance to the disease. In India, varieties of peas that mature early generally escape much damage from powdery mildew which becomes serious in January or later.

(v) Use of pathogen-free seed and planting material: Diseases which are carried by seed or vegetative planting material and spread the infection in the field require proper selection of seed to avoid multiplication of the pathogen in the field. This can avoid contamination of healthy crop. Therefore planting of disease free seeds in pathogen free soil is often the most effective measure of control of certain diseases.

2. Exclusion of the pathogen: This can be achieved by preventing the inoculum from entering or establishing in a field or area when it does not exist. Legislative measures like quarantine regulations are needed to be strictly applied to prevent spread of a disease. Exclusion can be carried out by:

(i) Treatment of seed and planting materials
(ii) Inspection and certification
(iii) Quarantine regulations
(iv) Eradication of insect vector

(i) Treatment of seed and planting materials: Seed tubers, grafts, bulbs and other propagation materials can be given heat, gas or chemical treatments to exclude the pathogen present in or on them. Seed treatment methods are mandatory for seed agencies supplying certified seeds. Generally seed treatments are prescribed for imports also and the exporting agency has to provide a certificate regarding this for quarantine purposes.

(ii) Inspection and certification: The crops grown exclusively for seed are periodically inspected for presence of diseases that are disseminated by seed. Necessary precautions are taken to remove the diseased plants. The produce is certified as seed. The badly affected plots and seed lots are usually rejected. The method is supposed to prevent regional and inter-regional spread of seed-borne pathogens.

(iii) Quarantine regulation: Plant quarantine aims at preventing entry of pathogens from infested areas into no-infested areas at international or national level. If in a particular area, some disease is present in serious form and is likely to be disseminated by propagating materials, the government passes necessary regulations. For implementation of these regulations at the international level, proper check is maintained at the points of entry (airports and seaports). Suspected material is kept under quarantine for a specific period and if found contaminated it is either destroyed or effectively treated.

(iv) Eradication of insect vector: For effective exclusion of pathogens that can gain entry into a new area through insect vectors or carriers particularly insects having long flight range, a check on these vectors is necessary. Since the flight of insects cannot be checked, the crop should be given insecticidal cover before arrival of the vectors on the plant surface.

3. Eradication of the pathogen: It includes reducing, inactivating, eliminating or destroying inoculum at the source, either form a region or from an individual plant (rouging) in which it is already established. Eradication can be achieved by:

i. Biological control of plant pathogens
ii. Crop rotation
iii. Removal and destruction of diseased plants or plant parts
iv. Eradication of alternate and collateral hosts
v. Heat and chemical treatment of diseased plants
vi. Soil treatment: by use of chemicals, heat energy, flooding and fallowing

(i) Biological control of pathogens: This type of control aims at eradication and reduction of inoculums and protection of plant surfaces through the activity of other microorganisms. It includes activities that enhance microbial numbers and quality on plant surfaces in soil or on the leaf.

(ii) Crop rotations: When the same crop is raised year after year on the same land the soil-borne pathogens of that crop easily perennate in the soil becomes so heavily infected that it becomes unfit for cultivation of that crop. On the other hand, when immune, resistant or non-host crops are grown for a definite duration after a susceptile crop in the field it is expected that the pathogen will be weakened, starved and killed. It is also possible that different crops modify the chemical and biotic environment of the soil against the pathogens. Crop rotation is the oldest methods of fighting soil against the pathogens.

(iii) Removal and destruction of diseased plants or plant organs: the presence of diseased plants in the field is a source of continuous release of inoculums. Therefore, as far as possible, such plants or their affected organs should be removed and destroyed to reduce the amount of inoculum.

(iv)Eradication of alternate and collateral hosts: The removal of alternate or collateral hosts is also recommended. Plant organs bearing dispersible pathogens propagules and their vectors should be removed carefully so that they are not dispensed while the plant or its organs is being physically removed.

(a) Roguing: This practice involves removal of diseased plants or their affected organs from the field. This method can be applied to diseases caused by fungi, bacteria, viruses as well as nematodes.

(**b) Sanitation**: Field sanitation is essential for control of soil-borne and facultative parasites or saprophytes. Many obligate parasites also perennate through dormant structures in plant organs lying in or on the soil. Destruction of crop debris by burning in the field decreases this type of survival of pathogen in the field. Sanitation is very important when diseased crop residue is left on the field as a general practice by the farmers.

(v) Heat and chemical treatment of the diseased plants: The pathogen present in the plant or in its special organs can be inactivated or killed by heat or chemical treatments. This approach has been found useful mostly in virus diseases of fruit trees. Heat therapy inactivates viruses in fruit tree seedlings and grafts and destroys the exposed fungal and bacterial propagules. Bare root dip in nematicides or fungicides is a method of sanitizing the seedlings before transplanting.

(vi) Soil Treatments: The aim of soil treatment is to inactivate or eradicate the pathogens present in the soil. It involves the use of chemicals, heat and such cultural practices as flooding. In chemical treatment of soil, fungicides and fumigant or granular nematicides are generally used. Most of the fungicides are selective in action and destroy specific fungi. Therefore, by their use the development of other non-target pathogens may increase due to reduced microbial competition.

Soil solarization is a novel method of soil treatment to destroy most fungal, bacterial and nematode propagules as well as weed seeds.

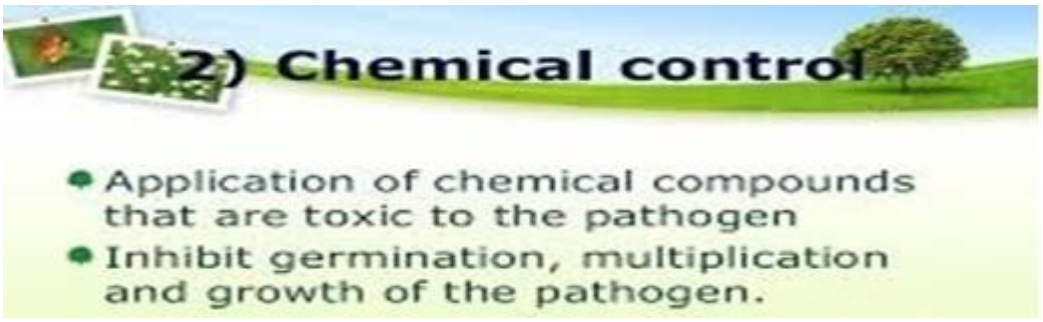
Flooding of the field is a method of eradicating fungal and nematodal pathogens from the field. If about 30 cm deep water is allowed to stand in the field for several weeks, the anaerobic or low oxygen conditions and toxins produced by the anaerobic bacteria destroy fungal scleratia and plant parasitic nematodes.

Fig.12.4. Different chemicals used in chemical control of diseases

4. Protection of the host: Host plants can be protected by creating a toxin barrier on the host surface by the application of chemicals. Various methods can be used in order to achieve protection:

i. Chemical control: application of chemicals (fungicides, antibiotics) by seed treatment, dusting and spraying
ii. Chemical control of insect vectors
iii. Modifications of environment

iv. Modification of host nutrition



(i) Chemical control: The aim of most chemical sprays, dusts and seed treatment is to form a protective toxiclayer on the host surface so that when the pathogen comes in contact with the surface it is killed or prevented from growth. The chemicals used for this type of protection are called protective chemicals. The same chemical can be protectant as well as eradicant. The use of systemic fungicides or naematicide is protectant as well as eradicant. It destroys the pathogen present within the plants (**Fig.12. 4**).

I. Direct protection by chemical controls

Chemical compounds which are toxic to the pathogens can be used for controlling disease in the fields and in greenhouses.

Such chemicals either inhibit germination, growth and multiplication of the pathogen or are lethal to the pathogen. Sulphur, copper, zinc and mercury compounds have been used for last more than 150 years.

Chemicals used in plant protection are termed as pesticides, and depending on the kind of pathogens they affect, the chemicals are called fungicides, bactericides, viricides, nematicides, insecticides, herbicides and so on.

Most of the chemicals are used to control diseases of the foliage and other aboveground parts of plants.

Some are used to disinfect and protect seeds, tubers and bulbs from infection. Some are used to disinfest the soil, others to protect stored fruits, vegetables from infection.

Different groups of chemicals are in use as fungicides, different terminologies are used for fungicides, they are as follows:

(a) **Protectants:** These kill the pathogen before it attacks the plants. They are thus designed to be present at the infection court in advance of the pathogen in order to prevent infection. While acting in this way, they may also have a direct effect on organisms which have already invaded the host. In this case they act as **eradicants**. As a rule protectants are of relatively little value as eradicants.

(b) **Therapeutic (Direct or eradicants):** These fungicides are applied after attack and kill fungi after they have invaded the plant. They may be fungistatic preventing the growth or spore suppressants preventing sporulation. They are further categorized as : contact fungicides and residual fungicides.

The former is applied before or after it has found the host plant, while the later is applied before the fungus reaches to it so that chemicals forms a protective layer over the plant surface.

II Chemical methods to eradicate or reduce the inoculums

A few chemicals are curative in nature and used to eradicate a pathogen that has already infected the plant. In addition to these, there are a few chemical treatments that act as eradicating or greatly reducing the inoculums before it comes in contact with the plant. These are as follows:

(a) Soil treatment : soil to be planted with vegetables, fruits, ornamentals or high value crops like tobacco is frequently treated with chemicals for control mainly of nematodes, but occasionally also of soilborne fungi, such as *Fusarium, Verticillium,* weeds and bacteria. Fungicides used for soil treatment include: metalaxyl, diazoben, PCNB, captan, chloroneb. Nematodes are controlled by soil fumigation with one of the nematicides applied before planting. Commonly used nematicides are: chlorpicrin, methyl bromide, dazomet and metam sodium.

(b) Disinfestation of warehouses: in order to remove any pathogens left over from previous years, the storage rooms are first cleaned thoroughly, debris are removed and burnt. The walls and floors are washed with bleach (a CuSO4 sloution 1 lb in 5 gall. Of water). In the room, the relative humidity maintained at 100% and temperature between 25 and 30^{o}C, and fumigated with chloropicrin (tear gas).

(c) Insecticides: for controlling insect vectors of fungal and bacterial pathogens, insecticides are to be used. Virus transmission by insects can also be reduced by spraying the plants several times with mineral oil. This method has been found effective in control of cucumber mosaic virus on cucumber.

(ii) Chemical control of insect vectors: many diseases are due to insect vectors. Some viruses are transmitted only by insect vectors. If a plant which has been sprayed by an insecticide, many of them escaped instant death and may infect healthy plant. The success of chemical control of insect vectors depends to a great extent on the stage of plant growth and nature of the pathogen. It also depends on the speed with which the chemical can kill the insect. Those chemicals which kill the insects within few seconds are most effective in control of insect-transmitted diseases.

(iii)Modification of environment: Improvement of aeration under crop canopy reduces humidity on leaves and other aerial parts and thereby checks growth of fungi which flourish in humid atmosphere. Reducing the number of irrigations also helps in modification of environment against certain diseases. Mixed cultivation of crops, one of which provides ground cover, often provides low temperature and high soil moisture. These conditions are not favourable for some root pathogens. Root diseases favoured by high temperature are often controlled by irrigation. Aeration through proper ventilation of the store house provides proper environment for storage of plant products. Cold storage can provide protection to fruits and vegetables by post-harvest decay.

(iv) Modification of host nutrition: The disease of a plant is often influenced by host nutrition. It generally acts through strengthening of the tissues. Many leaf diseases are fovoured by high level of nitrogen in the soil. Lowering nitrogen in soil in such disease is a method of checking such disease. In rice application of 100 kg N/ha instead of 120 kg is recommended to prevent from leaf spots, blast and sheath blight. Deficiency of potash in plants renders the tissue susceptible to water soaking and many more diseases. High calcium increases resistance to wilt and soft rot diseases. Intensity of several diseases is decreased by such micronutrients as zinc, boron and manganese etc.

Antibiotics: are metabolites of microorganisms which in very dilute concentration, and have the capacity to inhibit growth of or destroy other microorganisms. Antibiotics have systemic action in plants moving in both directions, from leaves to roots and from roots to the foliage. They are not only eradicants but also protectants providing temporary resistance in the host. The antibiotics used in plant disease control mainly belong to groups known as streptomycin, tetracyclines, polyenescyclo hexilmide and griseofulvin.

Streptomycin was the first antibiotic used in plant disease control, it was used against fire blight of pear (*Erwinia amylovora*) in 1953.

Antibiotics in the tetracycline group are tetracycline (Acromycin), oxytetra cycline (Teramycin) and chloretetracycline (Aureoycin). These antibiotics are bacteriastatic and bactericidal.

Aureogungin, a heptoene antifungal antibiotic, is recommended for the control of rice leaf spot, rice blast, barley stripe disease and covered smut of barley through seed treatment.

Nematicide: Nematicide belongs to two groups: volatile soil fumigants and non-fumigants (contact and systemic fungicide). The fumigants consist of compounds belonging to halogenated hydrocarbons and isothiocyanate groups while the non-fumigants are mostly organo-phosphorus and carbamates. The former directly kill the nematode larvae while the latter do not cause direct kill. Eggs are generally not affected by nematicieds being protected in cysts or in crop debris.

The soil fumigants such as methyl bromide, ethylene dibromide, D-D mixture, methyl isothiocyanateetc pose high vapor pressure, get dissolved in soil moisture in concentrations high enough to kill nematode larvae and disperse through the soil pores.

The halogenated hydrocarbon group includes methyl bromide, ethylene dibromide, Toluene, Nemagon and chlorpicrin.

The isothiocyanate group includes fumigants that release methyl cyanate such as Vapam, Dazomet, Mylone or Basamid.

The non-fumigant nematicides have gradually replaced the fumigants. They are available in granular form and can be applied to rows of crops at the time of planting or to soil around the standing trees.

The contact non-fumigants include fenulphathion (Dasanit or Terracur P), thionazin (Nemafas, zinophos), diazinon (Basudin) and ethoprop.

Non- conventional chemicals in disease control: minerals and vegetable oils have been used as plant control measures. Large scale use of mineral oil for control of Shigatoka disease of banana was prevalent in the past.

Oil of sunflower, olive, maize and rapseed have antifungal properties and have shown efficacy against powdery mildew of apple.
Significant control of grapevine powdery mildew with rape oil derivative is reported from Australia. Groundnut oil coating prevents papaya fruit decay. Similarly, aqueous extracts of many plants are also reported to provide control of fungi, bacteria and viruses.
Some herbicides are known to directly affect a disease. The herbicide propanil has antifungal activity against *Drechsleraoryzoe*.

5. Disease resistance: It is a method of preventing infection or reducing the effect of infection of the pathogen through the use of resistance host which is developed by genetic manipulation or by chemotherapy.

Use of resistant varieties: Development of resistance in host is done by

i. Selection and hybridization for disease resistance
ii. Chemotherapy
iii. Host nutrition
iv. Genetic engineering, tissue culture

(i) Selection and hybridization for disease resistance: Selection of resistant individual with poor commercial qualities and hybridizing them with susceptible plants of high commercial qualities is the aim of developing resistance through hybridization. This technique can help to produce disease resistant varieties and use of such varieties not only eliminates loss due to disease but also saves money to be spent on chemicals and other methods of control.

(ii) Chemotherapy: Temporary physiological resistance in plants can be developed through chemotherapy. Systemic fungicides and antibiotics when applied to the foliage or through the roots persist in the plant for some time and while their toxic level is maintained the pathogen cannot invade the tissue. Systemic nematicieds applied to soil and taken up by the plant keep away not only the nematodes but also aphids and leafhoppers for several weeks. This protects the plant against viruses too.

(iii) Host nutrition: Nutrition cannot change a susceptible variety to a resistant variety. But making available major and micronutrients through foliar sprays, seed treatment or soil application is reported to strengthen the tissues that can ward off invasion by the pathogen. Although the effectiveness of this approach is doubtful, a vigorous growth of the plant is always desirable. Vigorous plants with capacity to form new roots and shoots to replace the damaged once tolerate the attack of many diseases. Application of Nitrogen more than required for normal development of the plant causes new succulent growth of vegetative parts and also delays maturity.

(iv) Genetic manipulation through biotechnology: Plant tissue culture and genetic engineering are being used for genetic manipulation a multiplication of plants. This has

enabledhybridization between species which normally do not hybridize or those plants which normally do not produce viable seeds. Creation of transgenic plants in which resistance genes from sources other than the particular plant species or in which avirulence genes of the pathogen are introduced to impart resistance is now possible.

Immunization or improvement of host resistance

Though there is no antibody producing system in plants, treatment with some pathogens sometimes leads to temporary or permanent resistance. Besides this the genetic resistance of the host can also be improved. Following are some examples of immunization and improvement of host resistance methods:

i. Cross protection. This is protection of plants by mild strain of a virus from infection by a virulent strain of the same virus. This is general phenomenon among viral pathogen.

ii. Induced resistance. In some cases, a plant infected by one pathogen becomes resistant to subsequent infection by another pathogen. Bean and sugarbeet inoculated with virus show greater resistance to some obligate fungal pathogens (rusts, powdery mildew). TMV in tobacco induces resistance to *Phytophthoraparasitica*var.*nicotianae*and to bacteria *Pseudomonas tabaci*.

iii. Improving the growth conditions of plants. This is done by using some cultural practices that improve the plant vigour and help increase its resistance. Proper fertilization, field drainage, irrigation, proper spacing between plants and weed control are useful in this direction.

iv. Use of resistant varieties. This is the safest and most effective method of disease control. Once successful, it eliminates expenses of chemicals or time spent in other methods. Farmers could get maximum gain through the use of resistant varieties of field crops, particularly cereals. Several techniques are used for enhancing the useful life span of a resistant variety.

12.4.2- Curative measures

These are the measures applied after the plant is infected (i.e. the plant is treated for the disease). This can be illustrated by heat or chemical treatment of vegetative parts such as bulbs, corms and woody cuttings to eliminate pathogens that are established within the plant material. Although cure of the diseased plant or its organ in most crops is not possible or extremely difficult in many crops and fruit trees chemical and physical therapy has been applied to cure the plant by eradicating the pathogen.

Curative or therapeutic measures include:

i. Heat or thermotherapy
ii. Chemotherapy
iii. Tree surgery

(i) Heat or Thermotherapy: Plants which can tolerate the thermal inactivation or death point of the pathogen can be treated by heat to destroy the pathogen. These treatments are especially used for seed, tubers, bulbs and grafts. Grafts of fruit trees are exposed to high temperatures for inactivation of many viruses. For inactivation of nematodes from roots of

grafts heat therapy has been suggested. Ratoon stunting bacterium and many viruses of sugarcane are eradicated by hot water, air or moist hot air treatment of the seed conses.

(ii) Chemotherapy: Chemical treatments applied to eradicate the pathogen from the tissues of the diseased plant and thus curing it is called are included in chemotherapy. Such chemicals are called chemotherapeutants. They are mainly systemic fungicides and antibiotics. The principle underlying chemotherapy is site where the pathogen is present. It either kills the pathogen or incapacitate it by preventing sporulation, growth or both. So long as they remain at a toxic level they provide temporary resistance also. The chemotherapeutants can also act by detoxifying the toxins produced by the pathogen. In this way, the tissues not invaded by the pathogen are saved and the plant is cured.

(iii) Tree surgery: In this technique the large size fruit trees are cleaned of infection by cutting or scrapping of the diseased part and covering the wound with a fungicidal paste. This removes the infection and saves the tree.it is concerned with the health of the trees and treatment of diseases with various techniques including crown reduction, crown trimming, pruning or tree felling.

Trees in urban or populated areas require a higher level of care to maintain their safety and aesthetics. Pruning should be done with an understanding of how the tree responds to each cut, this can only be performed by a qualified arborist. Improper pruning can cause damage. This damage lasts for the life of the tree, and it shortens the life of the tree.

12.4.3- Biological measures

Biological control of disease employs natural enemies of pests or pathogens to eradicate or control their population. This can involve the introduction of exotic species, or it can be a matter of harnessing whatever form of biological control exists naturally in the ecosystem in question. The induction of plant resistance using non-pathogenic or incompatible micro-organisms is also a form of biological control. Some diseases that can be successfully controlled using biological agents are pathogens of pruning wounds and other cut surfaces, crown gall, diseases of leaves and flowers, such as powdery mildew, diseases of fruits and vegetables, e.g. *Botrytis*, and fungal pathogens in the soil (disease suppressive soils).

The most common mechanisms for microbial antagonism of plant pathogens are parasitism, predation, competition, induced resistance and the production of antimicrobial substances. Often, several mechanisms act together.

Biological control involves following methods:

1. Eradication or reduction of the pathogen inoculums by antagonists
2. Direct protection of plants by antagonists
3. Improvement of plant resistance (Immunisation)

1. Eradication or reduction of the pathogen inoculums by antagonists: Under some situations biological methods can be used to eradicate or reduce the pathogen inoculums. These are as follows:

i. Suppressive soils. Several soil-borne pathogens, such as *Fusarium oxysporum, Phytophthora cinnammomi, Pythium sp.* thrive and cause severe diseases in some soils called

conducive soils, whereas they develop much less and cause much milder diseases in other soils, known as suppressive soils. This is due to the presence of several microorganisms, antagonistic to the pathogens in these soils. These antagonists do not allow the pathogen to reach high population by producing antibiotics, lytic enzymes and competition for food. Suppressive soil added to conducive soil can reduce the extent of disease by introducing microbes antagonistic to the pathogen.

ii. Biocontrol through hyperparasites. Hyperparasites prove ideal organisms for several soilborne as well as aerial plant pathogens. Soilborne pathogens can be eliminated / reduced by using appropriate hyperparasites, both fungi and bacteria. These fungi and bacteria infect the resting spores of several pathogenic fungi. Among the most common mycoparasitic fungi are *Trichoderma* spp. Which parasites mycelia of *Rhizoctonia* and *Sclerotium*, inhibits the growth of many other fungi like *Pythium, Phytpthora, Fusarium* and reduce the disease caused by most of these pathogens.

Besides fungi, bacteria of the genera *Bacillus, Enterobacter* and *Pseudomonas* are known as parasitic and inhibit the pathogenic fungi.

iii. Trap plants. The pest is attracted from the main crop to a preferred plant the trap plant, which is planted deliberately. Rows of rye, corn or other taller plants planted around a field of bean, pepper or squash stop many incoming aphids carrying viruses that attack the bean, pepper and squash. Since most aphid-borne viruses are non-persistent in the aphid, many of the aphids loose bean, pepper or squash by the time they move into these crops. In this way trap crops reduce the amount of inoculums reaching a crop. Trap plants are also used against nematodes. Some plants, not susceptible to sedentary plant-parasitic nematodes produce exudates that stimulate their eggs to hatch. The juveniles though enter these plants but unable to develop into adults, and thus eventually die. Such plants are also called trap crops.

iv. Antagonistic plants. Plants like asparagus and marigold are antagonistic to nematodes because they release substance in the soil that are toxic to several plant parasitic nematodes. Like trap plants, however, antagonistic plants are also not being used so far on commercial scale. Planting of marigold or tobacco around tomato has been found promising.

2. Direct protection of plants by antagonists: As with chemicals, strategies have also been evolved during recent past for direct protection of plants through the use of biological, mainly microbial antagonists (fungal as well as bacterial). Biological control practices for direct protection involve the deployment of antagonistic microorganisms at the infection court before or after infection. Fungal as well as bacterial antagonists have been used.

i. Fungal antagonists. Several fungi have been used in the biocontrol of both, soilborne as well as airborne disease of plants, and also of postharvest diseases. For control of post-harvest diseases of several fruits, spores of saprophytic yeasts and filamentous fungi are sprayed at different stages of fruit development. Yeasts reduced postharvest rotting of peach and apple.

ii. Bacterial antagonists. Like fungi, several bacteria also have been successfully used for the biocontrol of soilborne and postharvest diseases of plants. Crown gall of stone fruits, pome and grapes caused by bacterium *Agrobacterialtumefaciens* is being controlled commercially by treating the seeds, seedlings and cuttings with Galltrol, a suspension of

strain K84 of the related but nonpathogenic bacterium *Agrobacterium rudiobacter*. This antagonist produces an antibiotic, bacteriocin, called **agrocin 84** which inhibits agrobacteria that arrives at the surfaces occupied by strain K84.

iii. Viral parasites of plant pathogens. Viruses are known to attack all plant pathogens-fungi bacteria, mollicutes and nematodes. Bacteriophages when mixed with bacterial pathogens, successful control could be achieved in case of several bacterial diseases of plants. However, this tactic is yet to be developed at commercial scale.

3. Improvement of plant resistance (Immunisation): Since plants do not have antibodies like humans and animals, they cannot be immunized by vaccination. Through genetic engineering however, scientists have successfully introduced and expressed in plants genes from mice coding for the production of antibodies against viruses with which the mice had been injected artificially.

Inoculation of plants with some pathogens often results to temporary or nearly permanent induced resistance (immunization) to a pathogen to which plants are normally susceptible. Induced or systemic acquired resistance (SAR) can be induced by treatment with chemicals, such as salicylic acid, and dichloroisonicotinic acid and certain benzothiazoles.

The resistance of plants can also be improved by genetic engineering technique. In this method genes obtained from other plants, pathogens or other organisms (that code for the production of enzymes, peptides or toxins for interfering with infection by the pathogen) are incorporated in the desired plant. Plants developed in this way are called transgenic plants resistant to a particular or more diseases.

12.5- SUMMARY

Different types of harmful pathogens, weeds, pests and microorganisms are responsible for plant diseases. Loss of crops from plant diseases may result in hunger and starvation, especially in less developed countries where access to disease-control methods is limited and annual losses are extremely high. To help decrease the loss done by various pests, weed and pathogens, different methods of protections are being used. The principle of protection involves placing a barrier between the pathogen and the susceptible part of the host to shield the host from the harmful organisms.

Plant protection measures are carried out to limit performance and yield losses in crop production during the growing season and afterwards (storage protection) as well as for quarantine purposes.

Different types of plant protection methods like: physical, chemical, biological, biotechnical and integrated methods help in protecting the plants from pathogens.

Physical plant protection methods use both mechanical (tillage, flooding of fields, using traps, fences etc.) and thermal means (hot water treatment, solarization) to control plant pathogens.

Chemical methods of plant protections involve mainly the treatment of soil, seeds, and foliar sprays with fungicides, nematicides and other chemical products.

Biological methods of protection involve using organisms and their activity to protect plants and enhance their resistance to biotic (harmful organisms) and abiotic limiting factors.
Biological control of weeds primarily involved introducing beneficial organisms into new habitats and introducing new pathogens which develop resistance in the host.

Biotechnical methods include the use of light and colour traps, chemical attractants, antibodies, pheromones, hormones, growth regulators for controlling harmful pathogens.
Integrated methods aim at using natural limiting factors which can be achieved by reducing use of chemical plant protection methods and simultaneously employing a variety of measures from the other categories.
Plant diseases can be controlled using different measures like: prophylactic, curative and biological measures.
Prophylactic measures involve avoidance, exclusion eradication, protection and therapy to achieve protection of host plant from infection.
Curative measures are used for treating diseased plants. Infections are cured using thermotherapy, chemotherapy or tree surgery methods.

Biological measures involve the use of different methods like: using antagonists for eradication or reduction of the pathogen, direct protection of plants by antagonists and immunization. Transgenic plants resistant to a particular disease can also help in controlling many harmful diseases of crops.

12.6- GLOSSARY

Abaxial directed away from the stem of a plant; pertaining to the lower surface of a leaf (see adaxial).
Acute toxicityability of a single dose of a compound to poison (see chronic toxicity).
Aerification the act of infusing or forcing air into, for example, soil.
Aerobic living only in the presence of oxygen.
Antagonism a general term for interference between organisms that may include antibiosis or competition for nutrients or space; action of two or more pesticides that reduces the effectiveness of one or all (see synergism).
Antagonist an organism or substance that limits or counteracts the action of another
Antibiotic a chemical compound produced by one microorganism that inhibits growth or kills other living organisms.
Aphidsmall, sucking insect of the family Aphididae (order Homoptera) that produces honeydew and injures plants when in large populations.
Avirulence (*avr*) genegene in a pathogen that usually causes a hypersensitive reaction, is associated with active plant defense reactions in a resistant plant, and causes disease in a susceptible plant.
Avirulent (syn. nonpathogenic) unable to cause disease (see virulent).
Avoidanceprinciple of plant disease control in which plants are grown at times or locations where the pathogen is inactive or not present.
Bactericide a chemical or physical agent that kills bacteria.

Bacteriocina protein antibiotic, one or more types of which can be produced and excreted by certain strains of bacteria.
Biocidea compound toxic to all forms of life.
Biocontrol(syn. biological control) exploitation by humans of the natural competition, parasitism and/or antagonism of organisms for management of pests and pathogens.
Biological control(syn. biocontrol) exploitation by humans of the natural competition, parasitism and/or antagonism of organisms for management of pests and pathogens.
BiotechnologyTechnology based on biology by exploitation of biological processes to develop technologies and products for the improvement of the life of mankind and our planet.
Biotic relating to life, as disease caused by living organisms.
Borer insect or insect larva that forms tunnels or cavities in the bark or within the wood of trees.
Certification seeds, propagative plant material, or nursery stock produced and sold under inspection to maintain genetic identity and purity, freedom from harmful pathogens, insect pests, and weed seeds. It is approved and certified by an official certifying agency.
Chemical attractants insects or other arthrooods are attracted to animal and plant host through chemicals either on contact or in air.
Chemotherapy treatment of plant disease with chemicals (e.g. antibiotics or fungicides) absorbed and translocated internally.
Crop rotationthe successive planting of different crop species; often used to improve soil fertility or to reduce disease and pest problems.
Cultural practicesthe manner in which plants are grown, such as: application of nutrients, irrigation practices, type of cultivation; may be used for disease management.
Disinfect to eliminate a pathogen from infected plant tissues.
Disinfest to kill pathogens that have not yet initiated disease, or other contaminating microoganisms, that occur in or on inanimate objects as such soil or tools, or that occur on the surface of plant parts such as seed.
Epidemicsspread of infectious disease to a large number of people in a given population within a short period of time.
Eradicationcontrol of plant disease by eliminating the pathogen after it is established or by eliminating the plants that carry the pathogen.
Fallow cultivated land kept free from a crop or weeds during the normal growing season
Fisheries a place where fish are reared for commericial purposes.
Fungistat (adj. fungistatic) a chemical or physical agent that inhibits fungal growth, sporulation, or spore geminiation, but does not cause death.
Foliarpertaining to leaves.
Fungistasisinhibition of fungal growth, sporulation, or spore germination but not death; used to describe the nonspecific phenomenon in natural soils where spore germination is inhibited and often overcome by rhizosphere nutrients.
Growth regulator(syn. hormone) a chemical substance produced in one part of an organism and transported in minute quantities to induce a growth response in another part, e.g. in plants, auxins, cytokinins, and gibberellins.
Herbicide a chemical used for killing plants or inhibiting plant growth, e.g. a weed or grass killer.

Hormone(syn. growth regulator) a chemical substance produced in one part of a an organism and transported in minute quantities to induce a growth response in another part, e.g. in plants, auxins, cytokinins, and gibberellins.
Host plantliving plant attacked by or harboring a parasite or pathogen and from which the invader obtains part or all of its nourishment.
Initial inoculum (syn. primary inoculum) inoculum, usually from an overwintering source, that initiates disease in the field, as opposed to inoculum that spreads disease during the season.
Inoculum(pl. inocula) pathogen or its parts, capable of causing infection when transferred to a favorable location.

Mildewthin coating of mycelial growth and spores on the surfaces of infected plant parts.
Nematicideagent, usually a chemical, that kills nematodes.
Nematode nonsegmented roundworm (animal), parasitic on plants or animals, or free living in soil or water.
Prophylaxis treatment given or action taken to prevent disease.
Protectant fungicide(syn. contact fungicide) a fungicide that remains on the surface where it is applied; no after-infection activity (see systemic fungicide).
Protectant agent, usually a chemical, applied to a plant surface in advance of a pathogen to prevent infection.
Protection a principle of plant disease control in which a barrier is placed between the susceptible plant and pathogen (e.g. the use of protective chemical dusts or sprays)
Quarantine legislative control of the transport of plants or plant parts to prevent the spread of pests or pathogens.
Sanitation destruction or removal of infected and infested plants or plant parts; decontamination of tools, equipment, containers, work space, hands, etc.
Secondary inoculumsinoculum produced by infections that took place during the same growing season.
Seed treatmentapplication of a biological agent, chemical substance, or physical treatment to seed, to protect the seed or plant from pathogens or to stimulate germination or plant growth.
Susceptible (n. susceptibility) prone to develop disease when infected by a pathogen (see resistance).
Systemic fungicidea fungicide that is absorbed into plant tissue and may offer some curative or after-infection activity; includes fungicides that are locally systemic, xylem-mobile (upward moving), and amphimobile (move in phloem upward as well as downward in the plant) (see contact or protectant fungicide).
Systemic pertaining to a disease in which the pathogen (or a single infection) spreads generally throughout the plant; pertaining to chemicals that spread internally through the plant.
Thermotherapy use of heat to reduce or eliminate pathogens in plant tissue; often used on plants prior to meristem culture to produce pathogen-free plants.
Tillagethe process of turning or stirring the soil.
Toxicity capacity of a substance to interfere with the vital processes of an organism
Toxin poisonous substance of biological origin.

Transgenic (syn. genetically modified organism; GMO) possessing a gene from another species; used to describe the organisms that have been the subject of genetic engineering.
Trap cropcrop planted around a field to protect the inner crop from diseases transmitted by aerial vectors; host crop of a parasitic plant, such as witchweed (*Striga* spp.), that is planted to stimulate seed germination, and later sacrified by plowing under before the parasitic plant produces new seeds.
Trenchingphysical separation of soil in a vertical plane to sever grafted roots between trees.
Virulence degree or measure of pathogenicity; relative capacity to cause disease.
Virulenthighly pathogenic; having the capacity to cause severe disease (see avirulent).
Virusa submicroscopic,intracellular, obligate parasite consisting of a core of infectious nucleic acid (either RNA or DNA) usually surrounded by a protein coat.

12.7- SELF ASSESSMENT QUESTIONS

12.7.1 Objective questions:

1. Prophylactic measures means-
(i) protection of host plant from infection (ii) protection of host plant from pollution
(iii) protection of plant from draught (iv) none of the above

2. The principle of avoidance involves-
(i) Choice of time of planting (ii) Choice of geographic area
(iii) Both (i) and (ii) (iv) None of the above

3.Application of which nutrient in the soil is required for new vegetative growth, and delayed maturity-
(i) Nitrogen (ii) Zinc
(iii) Boron (iv) Iron

4. The antibiotic becteriocin that inhibits the growth of *Agrobacterium tumifaciens* in Crown gall disease is-
(i) viridian (ii) agrocin 84
(iii) bt 43 (iv) none of the above

5. SAR means-
(i) Systemic Advance Reaction (ii) Systemic Adverse Reaction
(iii) Systemic Acquired Reaction (iv) None of the above

6. The antagonistic plant that is found useful in controlling nematodes in Tomato-
(i) Bouganvillia (ii) Marigold
(iii) Asparagus (iv) None of the above

7. A hyperparasite that controls soil borne diseases caused by fungi-
(i) *Trichoderma* spp. (ii) *Bacillus* spp.

(iii) *Rhizopus* spp. (iv) None of the above

8. Individual preventive methods taken for plant protection-
(i) Site design (ii) Crop rotation
(iii) Both (i) an (ii) (iv) None of the above

9. Factors that control plant disease are-
(i) Soil management (ii) Selection of disease resistant plants
(iii) Rotation of crops (iv) All of the above

10. Soil solarization is a noval method of soil treatment to destroy-
(i) Fungal spores (ii) Nematode propagules
(iii) Both (i) and (ii) (iv) None of the above

12.7.1 Answer Key

1-(i), 2-(iii), 3-(i), 4-(ii),5-(iii),6-(ii),7-(i), 8-(iii), 9-(iv),10-(iii)

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12.10- TERMINAL QUESTIONS

Q1. What do you understand by Plant protection? Explain briefly the various methods employed in plant protection.

Q2. How can we control a spread of disease in plants? Give brief notes on Prophylactic measures of control.

Q3. Explain briefly the various techniques of Biocontrol of plant diseases.

Q4. What are the different types of chemical measures taken for control of plant pests, pathogen and insects?

Q5. How can plant protection help in reducing crop loss done by pathogens?Briefly describe the process and measures of plant protection.

Q6What are the Curative measures used in control of plant diseases?

Q7. How can antagonists help in eradication or reduction of the pathogen inoculums?

Q8. What is Chemotherapy? How can it help in controlling plant disease?

Q9. What is Immunisation? How can it help in treatment of pathogens?

Q10. Answer the following-

(i) Name two Nematicides.

(ii) Name two non-conventional chemicals used for disease control.

(iii) Name two antibiotics used in disease control.

(iv) Name two chemicals used for seed treatment.

Q11. Write short notes on:

(i) Trap plants

(ii) Antagonistic plants

(iii) Quarantine regulations

(iv) Environmental impact