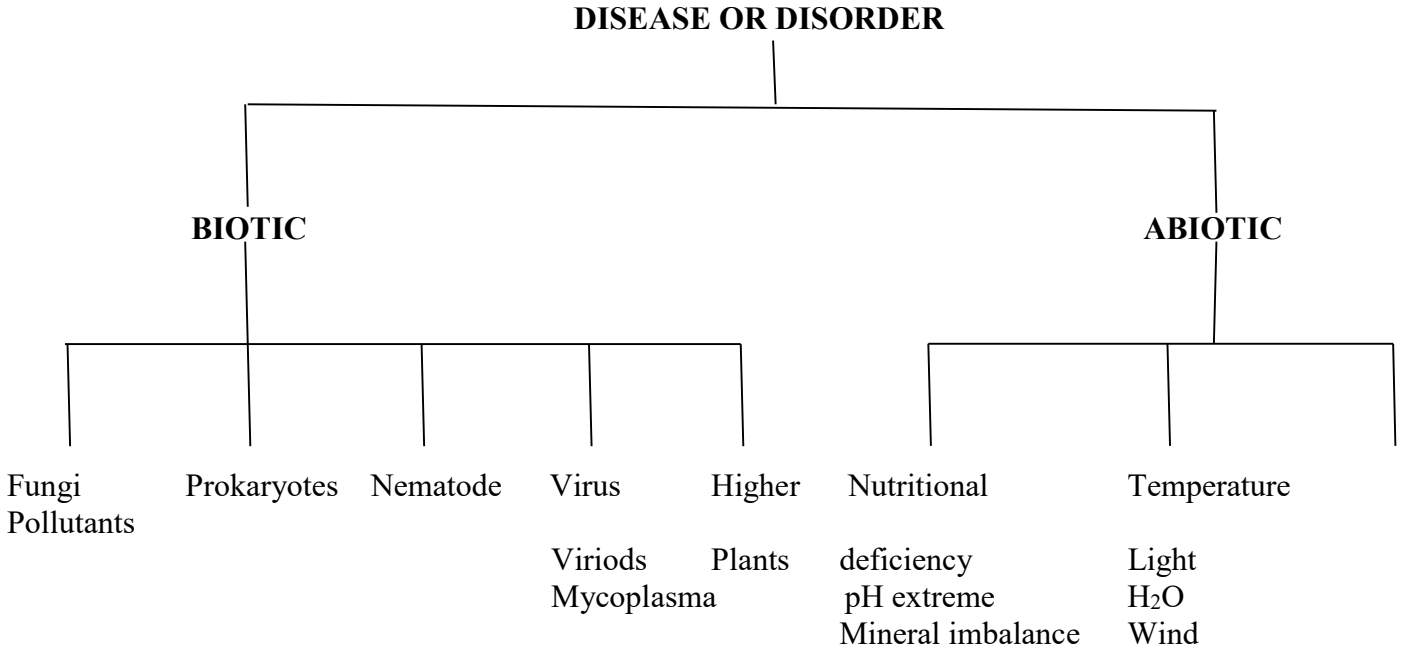
PLANT PATHOLOGY

INTRODUCTION

Plant pathology is a science which covers all the aspects related to diseased plants such as causal organisms, their life cycle, diagnosis, physiology, population dynamics and management.

Any abnormal functioning in the physiology of plants is designated as "disease". This may be caused by biotic factors or "disorder" due to Abiotic factors. Generally, these two words can be used alternatively. Biotic and Abiotic factors have been classified as follows:



All the biotic factors include microscopic and submicroscopic organisms. Followings are some terms related to study of microorganisms:

PARASITES (BIOTROPHS):

These are the microorganisms which take their nutrition from living organisms.

PATHOGENS:

These are the parasites which cause diseases in humans, animals and plants.

OBLIGATE PARASITES:

These are parasites which strictly live only on living organism for their nutrition and multiplication.

SAPROPHYTES:

These are microorganisms which get their nutrition from dead organic matter. These are also known as saprotrophs.

OBLIGATE SAPROPHYTES:

These are microorganisms which live only on dead organic matter to obtain their nutrition.

FACULTATIVE SAPROPHYTES:

The organisms which are originally parasites but can live saprophytically i.e. on dead organic matter in the absence of living host.

FACULTATIVE PARASITE:

The organisms which are originally saprophyte but can get nutrition from living host in the absence of dead organic matter.

SYMBIOSIS:

Mutual beneficial relation of host and parasite.

ANTAGONISM:

Counter action of two microorganisms against each other e.g. fungus *Trichoderma harzianum* is antagonist to many fungi.

SYNERGISM:

It is the combined effect of two microorganisms on host plant.

PATHOGENESIS:

All the disease events in host plant starting from infection to development of symptoms.

INFESTATION:

It is just the presence of microorganism on the surface of the host.

INFECTION:

When pathogens get entry, start getting nutrition and disturb the normal physiology of the plant.

SYMPTOM:

Physiological expression of the host as result of infection is called symptom.

BRIEF DESCRIPTION OF PLANT PATHOGENS

PATHOGEN	MORPHOLOGY	SIZE	SYMPTOMS
Fungi	Microscopic to Macroscopic, Branched, Filamentous, Somatic structure	Few micrometers to several meters in length. 0.5 µm-10 µm in diameters	Leaf spots, Gummosis, Powdery Mildew, Downy mildew, Rust, Smut, Blight, root rot of different crops.
Bacteria	Microscopic, Rod shaped (*Bacillus*), Spherical (*Coccus*), Comma shaped (*Vibrio*), Spiral (*Spirillum*)	0.6-3.5 µm in length 0.5-1.0 µm in diameter	Leaf spots, Blight, Canker, soft rot, Scab and Gall formation in vegetable and fruit crops.
Virus	Sub-microscopic, rod shaped, Spherical or polyhederal, flexous	480-2000 nm in length 10-13 nm wide Polyhedral viruses 17-60 nm in diameter	Yellowing, Mosaic, Mottling, Ring spot, Necrotic lesion, Curling, Rolling and Enation of leaves. Dwarfing, Stunting of Plants
Nematode	Microscopic, eel shaped	300-1000 µm 4 mm in length 15-35 in width	Nutritional deficiency like symptoms, Gall Formation, Stunting, Rottening of roots, Bushy appearance of roots.

EXERCISE # 1

LABORATORY BIOSAFETY PROTOCOLS

The protocols emphasize the use of good microbiological work practices, appropriate containment equipment, proper facility design, operation and maintenance, and administrative considerations to minimize the risk of worker injury or illness.

"Laboratory biosafety" is the term used to describe the containment principles, technologies and practices that are implemented to prevent unintentional exposure to pathogens and toxins, or their accidental release. "Laboratory biosecurity" refers to institutional and personal security measures designed to prevent the loss, theft, misuse, diversion or intentional release of pathogens and toxins.

MICROBIOLOGICAL RISKS

1. Pathogenicity of the agent and infectious dose.
2. Potential outcome of exposure.
3. Natural route of infection.
4. Other routes of infection, resulting from laboratory manipulations (parenteral, airborne, ingestion).
5. Stability of the agent in the environment.
6. Concentration of the agent and volume of concentrated material to be manipulated.
7. Presence of a suitable host (human or animal).
8. Laboratory activity planned (sonication, aerosolization, centrifugation, etc.).
9. Any genetic manipulation of the organism that may extend the host range of the agent or alter the agent's sensitivity to known, effective treatment.

PERSONAL PROTECTION

1. Laboratory overalls, gowns or uniforms must be worn at all times for work in the laboratory.
2. Appropriate gloves must be worn for all procedures that may involve direct or accidental contact with blood, body fluids and other potentially infectious materials or infected animals. After use, gloves should be removed aseptically and hands must then be washed.
3. Personnel must wash their hands after handling infectious materials and animals, and before they leave the laboratory working areas.

4. Safety glasses, face shields (visors) or other protective devices must be worn when it is necessary to protect the eyes and face from splashes, impacting objects and sources of artificial ultraviolet radiation.
5. It is prohibited to wear protective laboratory clothing outside the laboratory, e.g. in canteens, coffee rooms, offices, libraries, staff rooms and toilets.
6. Open-toed footwear must not be worn in laboratories.
7. Eating, drinking, smoking, applying cosmetics and handling contact lenses is prohibited in the laboratory working areas.
8. Storing human foods or drinks anywhere in the laboratory working areas is prohibited.
9. Protective laboratory clothing that has been used in the laboratory must not be stored in the same lockers or cupboards as street clothing.

LABORATORY WORKING AREAS

1. The laboratory should be kept neat, clean and free of materials that are not pertinent to the work.
2. Work surfaces must be decontaminated after any spill of potentially dangerous material and at the end of the working day.
3. All contaminated materials, specimens and cultures must be decontaminated before disposal or cleaning for reuse.
4. Packing and transportation must follow applicable national and/or international regulations.
5. When windows can be opened, they should be fitted with arthropod-proof screens.

CONTINGENCY PLAN

The contingency plan should provide operational procedures for:

1. Precautions against natural disasters, e.g. fire, flood, earthquake and explosion
2. Biohazard risk assessment
3. Incident-exposure management and decontamination
4. Emergency evacuation of people and animals from the premises
5. Emergency medical treatment of exposed and injured persons
6. Medical surveillance of exposed persons
7. Clinical management of exposed persons
8. Epidemiological investigation
9. Post-incident continuation of operations

In the development of this plan the following items should be considered for inclusion:

1. Identification of high-risk organisms
2. Location of high-risk areas, e.g. laboratories, storage areas, animal facilities
3. Identification of at-risk personnel and populations
4. Identification of responsible personnel and their duties, e.g. biosafety officer, safety personnel, local health authority, clinicians, microbiologists, veterinarians, epidemiologists, and fire and police services.
5. Lists of treatment and isolation facilities that can receive exposed or infected persons.
6. Transport of exposed or infected persons.
7. Lists of sources of immune serum, vaccines, drugs, special equipment and supplies.
8. Provision of emergency equipment, e.g. protective clothing, disinfectants, chemical and biological spill kits, decontamination equipment and supplies.

EMERGENCY EQUIPMENT

The following emergency equipment must be available:

1. First-aid kit, including universal and special antidotes.
2. Appropriate fire extinguishers, fire blankets.

The following are also suggested but may be varied according to local circumstances:

1. Full protective clothing (one-piece overalls, gloves and head covering for incidents involving microorganisms in risk).
2. Full-face respirators with appropriate chemical and particulate filter canisters.
3. Room disinfection apparatus, e.g. sprays and formaldehyde vaporizers.
4. Stretcher.
5. Tools, e.g. hammers, axes, spanners, screwdrivers, ladders, ropes.
6. Hazard area demarcation equipment and notices.

DISINFECTION AND STERILIZATION

The basic knowledge of disinfection and sterilization is important for biosafety in the laboratory to handle the contaminated materials.

DEFINITIONS:

The following are the commonly used terms for disinfection and sterilization.

ANTIMICROBIAL:

An agent that kills microorganisms or suppresses their growth and multiplication.

ANTISEPTIC:

A substance that inhibits the growth and development of microorganisms without necessarily killing them. Antiseptics are usually applied to body surfaces.

BIOCIDE:

A general term for any agent that kills organisms.

CHEMICAL GERMICIDE:

A chemical or a mixture of chemicals used to kill microorganisms.

DECONTAMINATION:

Any process for removing and/or killing microorganisms. The same term is also used for removing or neutralizing hazardous chemicals and radioactive materials.

DISINFECTANT:

A chemical or mixture of chemicals used to kill microorganisms, but not necessarily spores. Disinfectants are usually applied to inanimate surfaces or objects.

DISINFECTION:

A physical or chemical means of killing microorganisms, but not necessarily spores.

MICROBICIDE:

A chemical or mixture of chemicals that kills microorganisms. The term is often used in place of “biocide”, “chemical germicide” or “antimicrobial”.

SPOROCIDE:

A chemical or mixture of chemicals used to kill microorganisms and spores.

STERILIZATION:

A process that kills and/or removes all classes of microorganisms and spores.

CHEMICAL DISINFECTION

The following chemicals or germicides are used to disinfect the materials.

1. Chlorine (Sodium hypochlorite)
2. Chloramines
3. Chlorine dioxide (ClO_2)
4. Formaldehyde
5. Alcohols
6. Iodine and Iodophors
7. Hydrogen peroxide
8. Peracid

HEAT DISINFECTION AND STERILIZATION

Saturated steam under pressure (autoclaving) is the most effective way of sterilizing laboratory materials.

The following cycle will surely sterilize the correctly loaded materials.

1. 3 minutes holding time at 134ºC
2. 10 minutes holding time at 126ºC
3. 15 minutes holding time at 121ºC
4. 25 minutes holding time at 115ºC

QUESTION#1: How scientists can protect themselves against microbiological risks in laboratory?

QUESTION# 2: While working in laboratory areas, which cares should be taken?

QUESTION # 3: What is biosafety?

Reference: *Laboratory biosafety manual 3rd edition, world health organization Geneva, 2004*

EXERCISE # 2

EQUIPMENTS / MISCELLANEOUS LAB ITEMS FREQUENTLY USED IN PLANT PATHOLOGY LABORATORY

SR. NO.	EQUIPMENTS/LAB ITEM	PURPOSE
1	Spirit lamp or gas burner	For sterilization of inoculation needles, forceps, scalpel, etc.
2	Autoclave	For sterilization of heat stable liquids — common culture media, dry bulk materials, heat resistant instruments, glassware.
3	Koch's steam	Used for sterilization of media sterilizer or constituents which are damaged by exposure to temperature above 100°C, namely sugar, gelatin, milk.
4	Oven	For sterilization of glassware and metal instruments resistant to high temperature.
5	Gas sterilants	For sterilizing heat sensitive equipments and substrates
6	Liquid filters	For sterilization of heat sensitive solutions
7	Air filters	For sterilization of air for cultures and transfer chambers
8	Liquid disinfectants	Use on working surfaces, instruments, tools, etc.,
9	Laminar Flow Cabinet	It's a hood to avoid contamination
10	Centrifuge Machine	Used to run a solution at high revolution to settle the small particles in solution
11	Hot Plate	Used to warm the liquids and media
12	Liquid Shaker	Used to culture the microbes in liquid media
13	Digital Balance	Used to weigh the small quantities of materials
14	Incubator	Used to optimize the conditions for the growth of cultures
15	Refrigerators	Used to store the specimens and materials at low temperatures

QUESTION # 1: Which equipment is used for sterilization?

QUESTION # 2: What is the difference between sterilization and pasteurization?

QUESTION # 3: Why we use inoculating chamber for inoculation?

EXERCISE # 3

INTRODUCTION TO MICROSCOPE:

A microscope (from the Greek: *mikrós*, "small" and *skopeîn*, "to look" or "see") is an instrument used to see objects that are too small for the naked eye.

MICROSCOPY:

The science of investigating small objects using such an instrument is called microscopy. Microscopic means invisible to the eye unless aided by a microscope.

MAGNIFICATION:

For calculation of the total magnification when viewing an image with a light microscope, first take the power of the objective lens which is at 4x, 10x or 40x and then multiply it by the power of the eyepiece which is generally 10x. It means, a 10x eyepiece used with a 40x objective lens, will produce a magnification of 400x. Magnification is a measure of increase in diameter of an object.

RESOLUTION:

Resolution means the degree of detail. Resolving Power may be described as the ability to measure the separation of images that are close together. The shorter wavelength ensures increased resolution.

PRINCIPLE OF WORKING:

MAGNIFICATION SYSTEM:

Stereo microscopes have two major types of magnification systems. One fixed magnification and other zoom magnification. Primary magnification is a part of fixed magnification which is achieved by set of objective lenses with a set degree of magnification. While with Zoom magnification, range of magnification is already set and magnification can be continuously changed within that range. If one wants more magnification of the object, it can be achieved by using auxiliary objectives that increase total magnification by a set factor. By changing the eyepiece, one can increase or decrease total magnification of both fixed and zoom system. In between these two magnifications, there are fixed-focus convex lenses that are arranged so to achieve the fixed magnification. This system is called "Galilean optical system". But the difference to fixed magnification is that if same arrangement of these convex lenses is physically inverted one will get the different magnification. So to get two different magnifications, same set of lenses can be used; two sets of lenses will provide four magnifications and so on. Galilean optical system is a good replacement of expensive zoom magnification system.

ILLUMINATION:

Fiber optic light source with halogen lamps is used to illuminate specimen which gives high light output for a given power input. Many other light stalks are also used for the same specimen, so increasing the illumination yet further.

VIEWING HEADS:

1. **MONOCULAR**
2. **BINOCULAR**
3. **TRINOCULAR**

1. MONOCULAR

The use of only one eyepiece when viewing the specimen. The monocular microscopes are light weight and inexpensive.

2. BINOCULAR

A microscope having two eyepieces. It is most comfortable to use as a common choice.

3.TRINOCULAR

It has a third eyepiece tube that can be used by another person simultaneously or by a CCD camera. The trinocular option is more expensive than monocular or binocular.

CARE AND WISE USE OF MICROSCOPES

1. Ever hold a microscope firmly by the stand, only. Don't catch it by the eyepiece holder.
2. When to unplug the illuminator; hold the plug (not the cable).
3. After completion of lab work, turn the illuminator off.
4. The stage and lenses should be cleaned before putting away the microscope.
5. Always use a good quality lens tissue or a cotton swab (100% natural cotton) to clean an optical surface. Appropriate lens cleaner or distilled water may be used to help remove dried material. Organic solvents will damage the lens coatings.
6. Cover the microscope with a dust cover when it is not in use.
7. Try to focus smoothly and lightly. Increased resistance during focusing shows that you have reached a limit.

QUESTION # 1 Which is the most preferable microscope for daily use?

QUESTION # 2 What is Microscope?

QUESTION # 3 Differentiate between uni, bi and trinocular?

EXERCISE # 4

DESCRIPTION OF DIFFERENT TYPES OF MICROSCOPES

There are many types of microscopes. Optical microscope was first invented which was followed by many other advance type of microscopes including Electron Microscope and Scanning Probe Microscope. Following are different types of microscopes:

1. Light microscope
2. Compound light microscope
3. Digital microscope
4. Stereomicroscope
5. Fluorescence Microscope
6. Portable microscopes
7. Inverted microscope
8. Electron microscope
    i. Scanning Electron microscope
    ii. Transmission Electron microscope
    iii. Scanning transmission electron microscope
9. Scanning Probe microscope
10. Confocal microscope

1) LIGHT MICROSCOPE

It employs visible light for detection of small objects and it is the most well-used research tool in the field of biology.

2) COMPOUND LIGHT MICROSCOPE

Compound light microscope is a microscope with more than one lens and its own light source. It has ocular lenses in the binocular eyepieces and objective lenses in a rotating nosepiece closer to the specimen. The strongest compound microscopes have magnifying powers of 1,000 to 2,000 X. As it contains its own light source in its base, a compound light microscope is also considered a bright field microscope. Bright field microscopy simply means that the specimen is lightened from below and viewed from above.

3) DIGITAL MICROSCOPE

The digital microscope was invented in Japan in 1986. It makes use of the computer to visualize the objects not visible to the naked eye. A digital microscope has a digital CCD camera attached to it and connected to a LCD or a computer monitor.

4) STEREOMICROSCOPE

A stereo microscope also known as "dissecting microscope", uses two objectives and two eyepieces which makes it possible to view a specimen under angles to the human eyes forming a stereo 3D optical vision. It has two optical paths at slightly different angles allowing to view the three dimensional image under the lenses.

5) FLUORESCENCE MICROSCOPE

A fluorescence microscope or "epifluorescent microscope" is a special type of a light microscope which instead of light reflection and absorption uses fluorescence and phosphorescence to view the samples and their properties. Fluorescence is a physical phenomenon in which a compound absorbs light and re-emits this as light of a usually higher wavelength. Since the wavelengths of the excitation light source and the emitted fluorescence can be separated very well, the fluorescence can be detected with very high sensitivity, making it possible to visualize even single molecules.

6) PORTABLE MICROSCOPES

These are small, durable and portable microscopes sometimes as small as an ink pen. They provide detailed close images of objects and larger single celled organisms. These hand-held microscopes do not need batteries and may operate using natural light while producing high definition of images without blurred edges.

7) INVERTED MICROSCOPE

An inverted microscope has its light source and condenser on the top, above the stage pointing down, while the objectives and turret are below the stage pointing up. Inverted microscope is useful for observing living cells or organisms at the bottom of a large container (e.g. a tissue culture flask) under more natural conditions than on a glass slide. The stage on an inverted microscope is fixed and focus is adjusted by rotating the objective lens along a vertical axis to bring it closer to or further from the specimen.

8) ELECTRON MICROSCOPE

An electron microscope is an advanced microscope with the highest magnification and resolution capacity. In electron microscope, electron beam is used to illuminate the tiniest particles. Electron microscope is a much more powerful tool in comparison to commonly used light microscopes.

8.1) TRANSMISSION ELECTRON MICROSCOPE

The transmission electron microscope (TEM) is operated on the same basic principles as the light microscope but here electrons are used instead of light. This microscope helps to study the ultra structure.

8.2) SCANNING ELECTRON MICROSCOPE

A scanning electron microscope (SEM) is a type of electron microscope that images a sample by scanning it with a beam. The electrons interact with the atoms that make up the sample producing signals which contain information about the sample's surface topography, composition, and matrix.

8.3) SCANNING TRANSMISSION ELECTRON MICROSCOPE

The scanning transmission electron microscope (STEM) is an important tool for the characterization of nanostructures, ensuring a range of different imaging modes with the ability to provide information on elemental composition and electronic structure. The STEM works on the same principle as the normal scanning electron microscope (SEM). The difference with SEM is that thin specimens are used so that transmission modes of imaging are also available.

9. SCANNING PROBE MICROSCOPE (SPM)

These microscopes are used in research and development as standard analysis tools. Images are highly magnified and are visualized as three-dimensional-shaped-specimens in real time. SPMs employ a delicate probe to scan the surface of the specimen eliminating the limitations generally found in electron and light microscopy.

10. CONFOCAL MICROSCOPE

It ensures an optical imaging technique to increase optical resolution and contrast of a micrograph by using point illumination and a spatial pinhole to eliminate out-of-focus light in specimens that are thicker than the focal plane. It provides the reconstruction of three-dimensional structures from the obtained images. This technique has its useful application in life sciences.


JEOL JEM-1010

QUESTION # 1. What is difference between light and compound light microscope?

QUESTION # 2. What are the uses of electron microscope?

QUESTION # 3. What is electron microscopy?

QUESTION # 4. What is difference between scanning and transmission electron microscope?

QUESTION # 5. Which type of electron microscope is used to examine the ultra structure of the specimens?

EXERCISE # 5:

GENERAL REPLICATION CYCLE OF PLANT VIRUS

VIRUS:

Literally the word "virus" means "poison" and the same had been used for a slimy infectious like material which may be the poison or venom. Simply virus is an infectious submicroscopic entity having nucleic acid (either RNA or DNA) and protein coat. It can only be replicated inside the host cell using the host cell machinery. Virus is an obligate unique infectious parasite far different from other microorganisms because of the following characteristics:

1. They are not visible under light microscope.
2. They replicate only in living host cell.
3. They consist of nucleic acid and protein coat.

Nucleic acid contains all the genetic information while protein coat protects the delicate nucleic acid. Nucleic acid may be the single stranded or double stranded. Replication of virus is dependent on the host cell enzyme synthesis machinery. Stages for replication of plant viruses have been described by Verma (2003) as follows.

1. Adsorption
2. Entry
3. Uncoating or disassembly
4. Translation and transcription
5. Assembly or maturation
6. Transportation

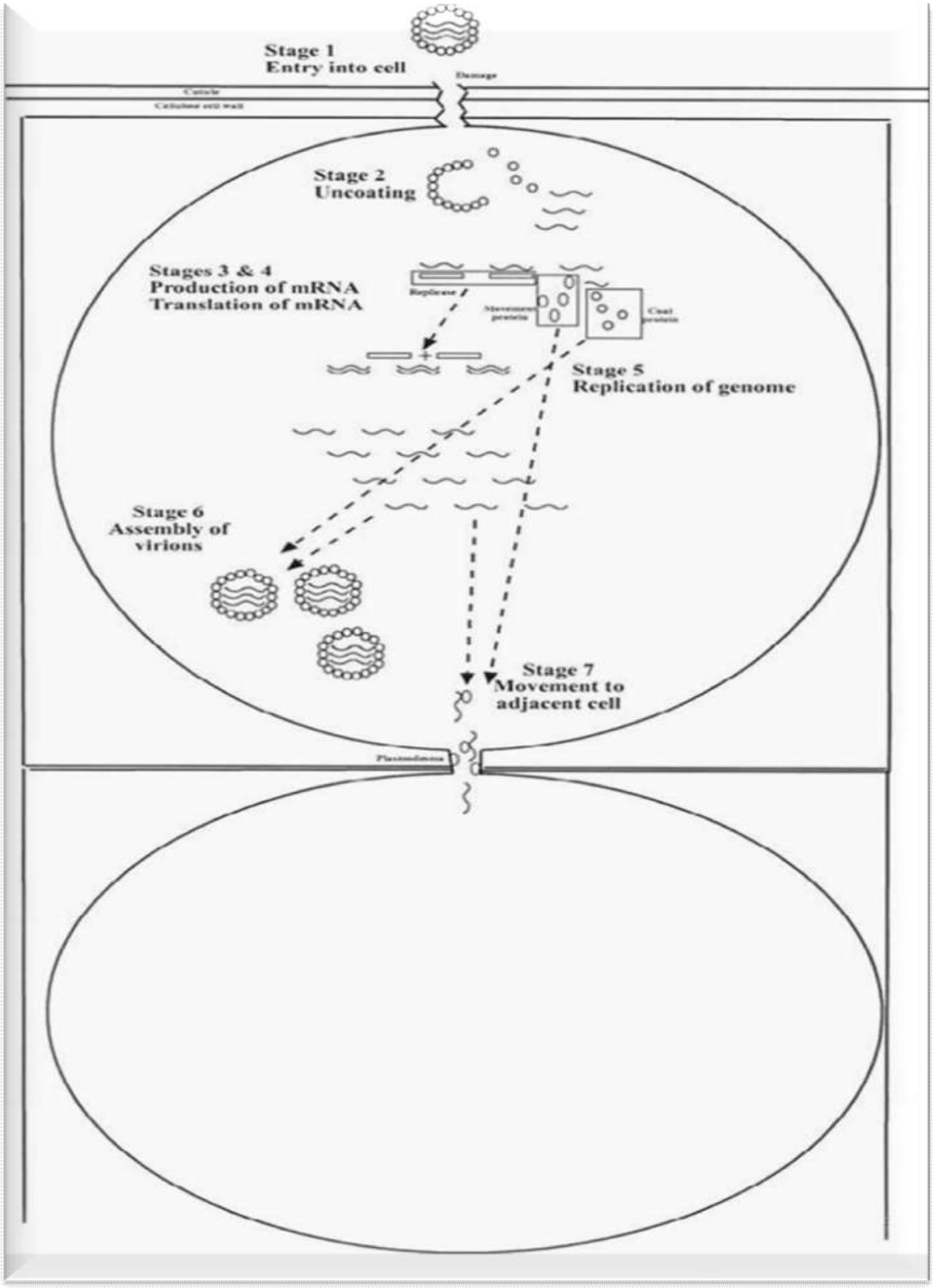
SHAPES

Viruses are of different shapes such as rigid rods, flexuous/filamentous, isometric, polyhedral, twinned or geminate and bacilliform particles.

QUESTION # 1. Define a virus?

QUESTION # 2. Differentiate between animal and plant virus?

QUESTION # 3. What is the biological status of plant virus?


Stage 1
Entry into cell
Stage 2
Uncoating
Stages 3 & 4
Production of mRNA
Translation of mRNA
Stage 5
Replication of genome
Stage 6
Assembly of
virions
Stage 7
Movement to
adjacent cell

EXERCISE # 6

GENERALIZED LIFE CYCLE OF PLANT PATHOGENIC BACTERIA

Bacteria are the prokaryotes, unicellular microorganisms having DNA as genetic material not bounded by membrane. Plant pathogenic bacteria are rod like; diameter ranging from 0.6-3.5 µm. Different forms of flagella may be present on the body of bacteria.

Bacteria reproduce asexually through binary fission or fission at rapid rate. Identification of plant pathogenic bacteria is based on visual observation including symptoms expression, colony pattern on media, microscopic observation, gram staining, serological and molecular techniques.

Plant pathogenic bacteria though cause limited number of diseases in plants but all the diseases are of much economic importance. Soft rot, blight and bacterial canker of vegetable and stone fruits are important bacterial diseases.

QUESTION # 1: What is the difference between Gram +ve and Gram –ve bacteria?

QUESTION # 2: Differentiate between bacterial and fungal cell.

QUESTION # 3: What is bacterium?

EXERCISE # 7

GENERALIZED LIFE CYCLE OF PLANT PARASITIC NEMATODES

The nematodes are invertebrate roundworms that inhabit marine fresh water and terrestrial environments. The majority of nematodes are microscopic, averaging less than a millimeter in length, but some of the animal parasites are quite large and readily visible to the naked eye. The animal and plant parasites are of direct importance in agriculture, environment, human health; however, most nematodes in the environment are not parasites. Nematodes that feed on other organisms are important participants in the cycling of minerals and nutrients in the ecosystem that is fundamental to other biological activity. Some of these nematodes may have major roles in decomposition, including biodegradation of toxic compounds. Insect-parasitic nematodes can be of importance in regulating insect populations, and are being used in the biological control of insect pests e.g. *Steinernema* spp.

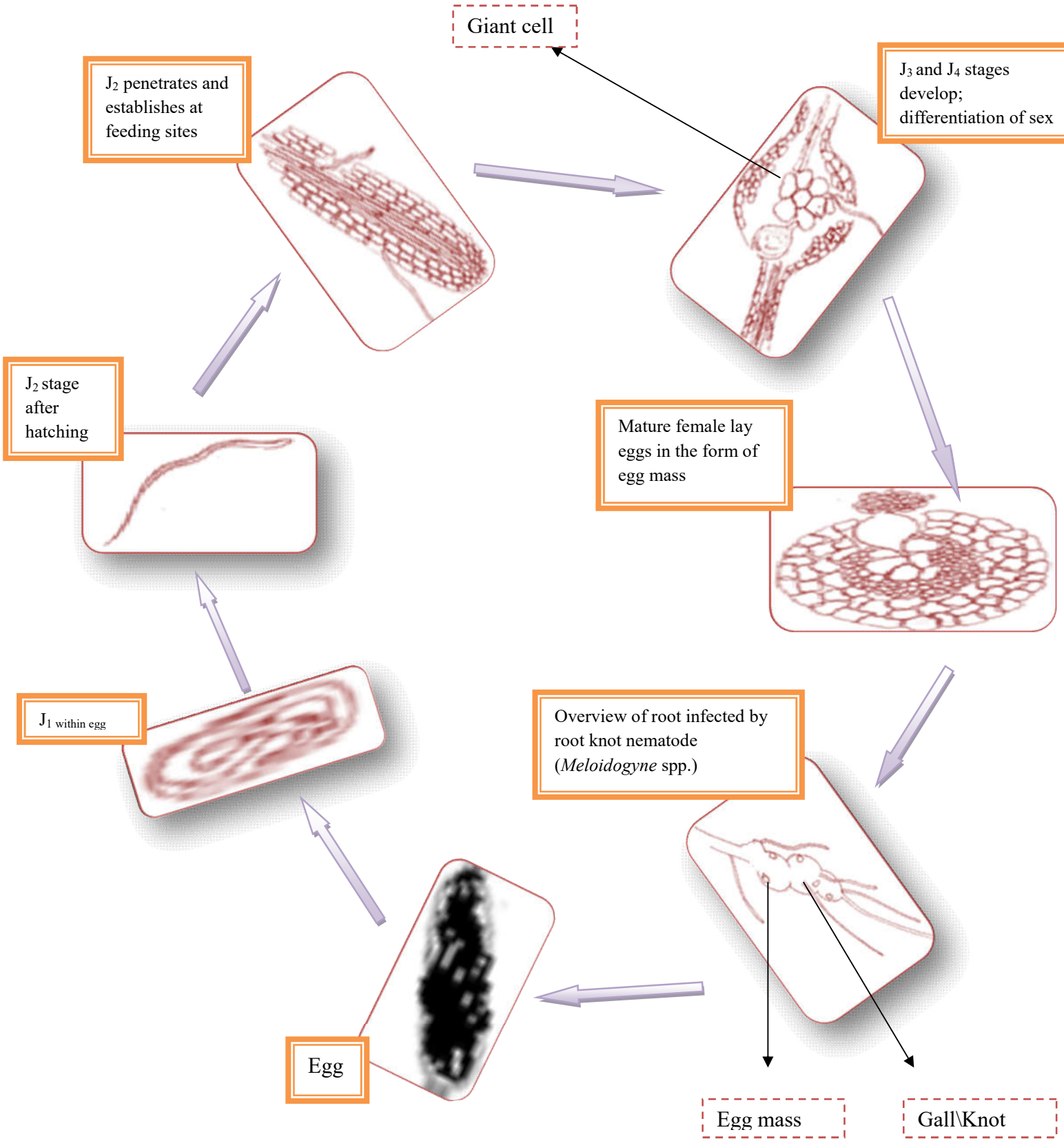
Disease Cycle

The J1 stage resides entirely inside the translucent egg case, where it molts into a J2 nematode. The motile J2 stage is the only stage that can initiate infections. J2s attack growing root tips and enter roots intercellularly. Nematode secretions cause dramatic physiological changes in the parasitized cells, transforming them into giant-cells. If the nematode dies, so will the giant-cells upon which it feeds. J2s do not possess reproductive organs. Root-knot nematodes undergo four juvenile stages, each progressing through a "molting" process similar to that of insects. As a result of this process, juvenile root-knot nematodes have little resemblance to adult males and females. In the J4 stage, the progression from juvenile to globose adult females or to vermiform adult males becomes clearly visible. They emerge as adults from the J4 cuticle.

QUESTION # 1: At what stage nematodes initiate infection?

QUESTION # 2: What is the difference between juvenile and larva of insect?

QUESTION # 3: How much time nematodes require for completing their life cycle?


Giant cell
J2 penetrates and establishes at feeding sites
J3 and J4 stages develop; differentiation of sex
J2 stage after hatching
Mature female lay eggs in the form of egg mass
J1 within egg
Overview of root infected by root knot nematode (Meloidogyne spp.)
Egg
Egg mass
Gall\Knot

EXERCISE # 8

PHANEROGAMIC PARASITES: CUSCUTA, STRIGA, OROBANCHE AND MISTLETOE

Phanerogamic parasites are leaveless higher plants with no chlorophyll and dependent on the host plant for their nutrition. They parasitize the root or stem of the host plant through sending their haustoria into the host tissues and fulfill their nutritional requirement leaving the host plant production less.

Following are the major phanerogamic parasites:

1. Dodder (Stem Parasite)
2. Mistletoe (Stem Parasite)
3. Broom rapes (Root Parasite)
4. Witch weed (Root Parasite)

Question # 1 What is meant by Phanerogamic parasite?

Question # 2 How Phanerogamic parasites obtain their nutrition?

Question # 3 What are different strategies for management of phanerogamic parasites?

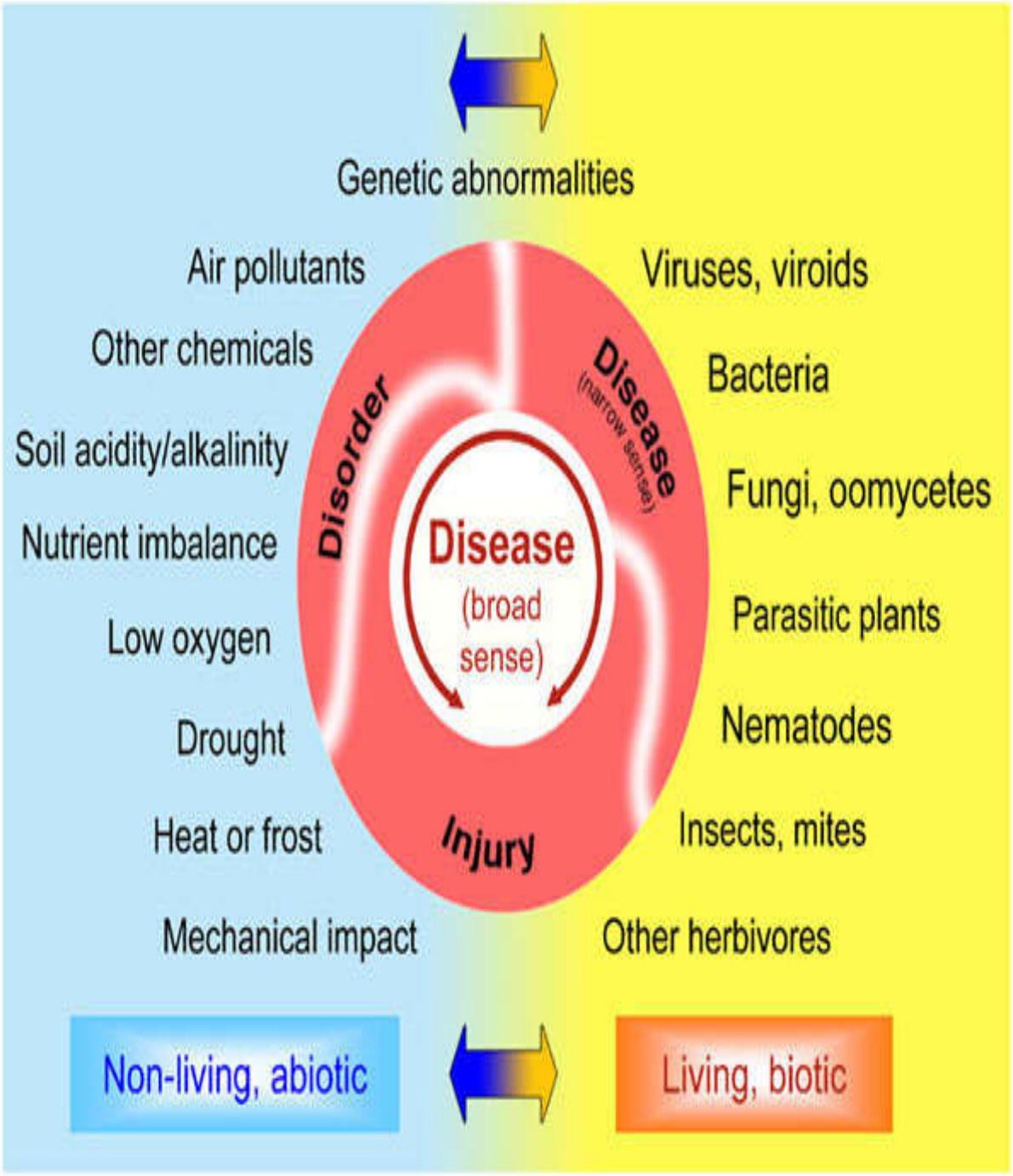
TAXONOMY OF PHANEROGAMIC PLANTS				
Family	**Genus (Common name)**	**Parasitize**	**Characteristics**	**Image**
Cuscutaceae	Cuscuta (dodders)	Stem parasite Alfalfa, onion, potato,	Achlorophyllus, scale like leaves, white flowering and small seeds	
Orobanchaceae	Orobanche (broom rapes)	Root parasite tobacco	Woody stem with achlorophyllus scale like leaves, purple or reddish flowering with brown seeds.	
Lauraceae /Viscaceae	Mistletoes (Dwarf, American, European)	Conifers: stem parasite	Chlorophyllus, true leaves, pink flowering and berry like fruit with small seeds	
Scrophulariaceae	Striga (witch weeds) Mono-cot plant	Root Parasite	Active chlorophyll present, true stem with broad leaves, whitish pink flowering and fruit formation with small seeds	

EXERCISE # 9

ABIOTIC STRESSES IN PLANTS

"Abiotic" literally means without life. Abiotic plant disorders are non-biological factors, usually associated with the plant's environment that affect plants adversely.

1. The environmental factors include temperature, moisture, soil pH, air quality, light regime, and nutrition.
2. If one or more of these factors go above or below the optimum range for a given plant species, plant growth might be abnormal or adversely affected.
3. Abiotic disorders may also be caused by human activities, such as pesticide and fertilizer applications.
4. One important indicator of an abiotic cause for a plant health problem is the distribution of the damage within the environmental unit. Plants generally grow in distinct environmental units such as vegetable gardens. Environmental problems are much more likely to affect most plants in the environmental unit uniformly.
5. Disease and insect problems, on the other hand, tend to occur in clumps or hot spots within the unit, especially early in outbreaks. For example, if frost injury occurs in a vegetable garden, all vegetable plants are likely to have blackened leaves.
6. A fungal pathogen, in contrast, may produce similar dark discoloration, but only on one or two plants in the early stages of the epidemic.


Genetic abnormalities
Air pollutants
Other chemicals
Soil acidity/alkalinity
Nutrient imbalance
Low oxygen
Drought
Heat or frost
Mechanical impact
Viruses, viroids
Bacteria
Fungi, oomycetes
Parasitic plants
Nematodes
Insects, mites
Other herbivores
Disorder
Disease
(narrow sense)
Disease
(broad
sense)
Injury
Non-living, abiotic
Living, biotic

EXERCISE # 10

PREPARATION OF MEDIA FOR CULTURE

Media play an important role not only for the nutrition and growth of the pathogen but also in identification through growth pattern on media. Fungi and Bacteria need synthetic media enriched with essential element for the growth. In general, different types of media are prepared to culture fungi and bacteria but Potato dextrose agar (PDA) for fungi and Nutrient agar for bacteria are being used commonly.

RECIPES OF DIFFERENT MEDIA

POTATO DEXTROSE AGAR

- Potato starch 250 ml
- Dextrose 15g
- Agar-Agar 15g
- Distilled water 750 ml

PROCEDURE TO PREPARE POTATO DEXTROSE AGAR

MATERIAL

Petri dish, Flask, test tubes, Agar-agar, Dextrose, Potato starch, Water

PROCEDURE:

First of all, take potatoes to extract potato starch. Peel the potatoes and cut into small pieces. Preferably 200 g of potato pieces are weighed, washed quickly in running water, placed in 11iter of water and boiled for nearly 1 hour till a mash is formed. The mash is then squeezed through double layer of muslin cloth to obtain as much of the pulp as possible. Discard the potato pieces. Alternatively, potato starch commercially available in the markets can also be used. Take the potato starch 250 ml, add dextrose (15g) into it and slightly shake it so that dextrose completely mixes into starch. Now slowly add agar-agar (15g) into this and continue shaking until the whole contents get mixed thoroughly. Now add the distilled water (750 ml) to make up the volume as per requirement. Plug in the flask and autoclave. After autoclaving, medium is ready to pour into Petri dishes.

POTATO SUCROSE AGAR (for *Fusarium*) (PSA)

It is useful as all-purpose medium.

RECIPE:

- Potatoes 1,800 g
- Water 4,500 ml
- Potato extract 500 ml
- Sucrose 20 g
- Agar 20 g
- Water 500 ml

PROCEDURE:

Potatoes are to be peeled and made into small discs. Potato discs are to be suspended in muslin in water and boiled for 10 minutes. Potatoes are discarded and sterile liquid is stored in large glass containers in refrigerator. When required, potato extract is mixed with molten agar and sucrose. pH is adjusted to 6.5. Volume is made up to 1 Liter. Sterilization has to be done at 15 p.s.i. for 20 minutes.

QUESTION # 1: Name the general purpose media.

QUESTION # 2: How can we extract starch from potatoes?

QUESTION # 3: What is agar-agar?

QUESTION # 4: Why the fresh starch extracted from potatoes is more beneficial than commercial grade starch?

EXERCISE # 11

PREPARATION OF TEMPORARY MOUNT

Temporary mount of diseased specimen is frequently required for microscopic examination. Such mounts can be made as follows:

- Take a clean slide and cover slip.
- Place a drop of water on the slide.
- Now add the specimen which needs to be examined. Care must be taken that the specimen should not be of large size. Size of specimen should be in few millimeters so that it could easily settle down in drop of water.
- In many cases it may be necessary to tease the specimen with dissecting needle so that it spreads uniformly in a thin layer and does not form a clamp which will hamper the observation.
- Then the cover slip is placed on the slide. To avoid the air bubble in mount it is necessary to place the cover slip carefully. First place the one edge of cover slip in contact with water then with the help of dissecting needle gently and slowly lower the cover slip.
- Instead of water, lectophenol can also be used for mount preparation but in this case specimen should be stained with cotton blue.

QUESTION # 1 How will you prepare a temporary slide from culture of a fungus contained in a Petri dish?

QUESTION # 2 What precaution is needed to place the cover slip on the slide?

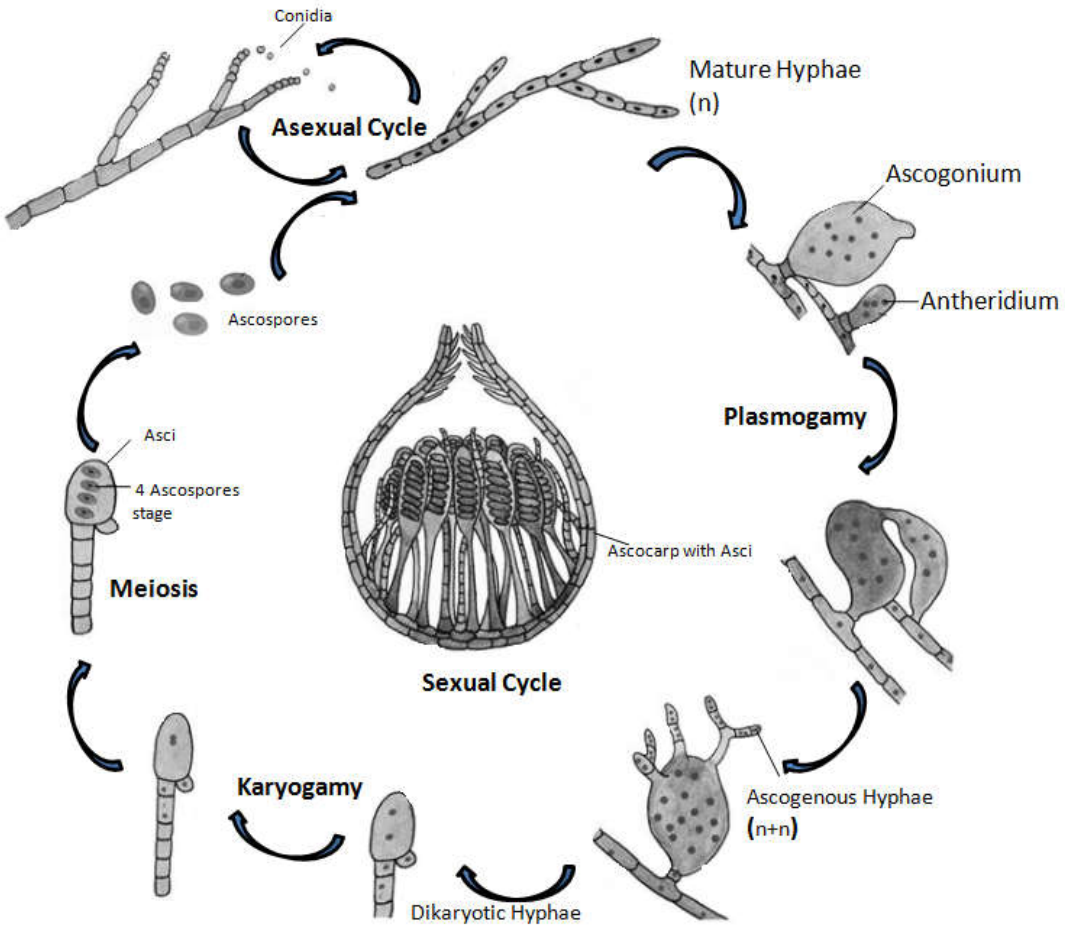
QUESTION # 3 Which magnification is required for initial observations?

EXERCISE # 12-A

INTRODUCTION TO FUNGI

GENERALIZED LIFE CYCLE OF ASCOMYCOTA

The Ascomycota are a Division/Phylum of the kingdom Fungi, and subkingdom Dikarya. Its members are commonly known as the sac fungi. They are the largest phylum of Fungi, with over 64,000 species. The defining feature of this fungal group is the "ascus", a microscopic sexual structure in which nonmotile spores, called ascospores, are formed. However, some species of the Ascomycota are asexual, meaning that they do not have a sexual cycle and thus do not form asci or ascospores (Wikipedia).



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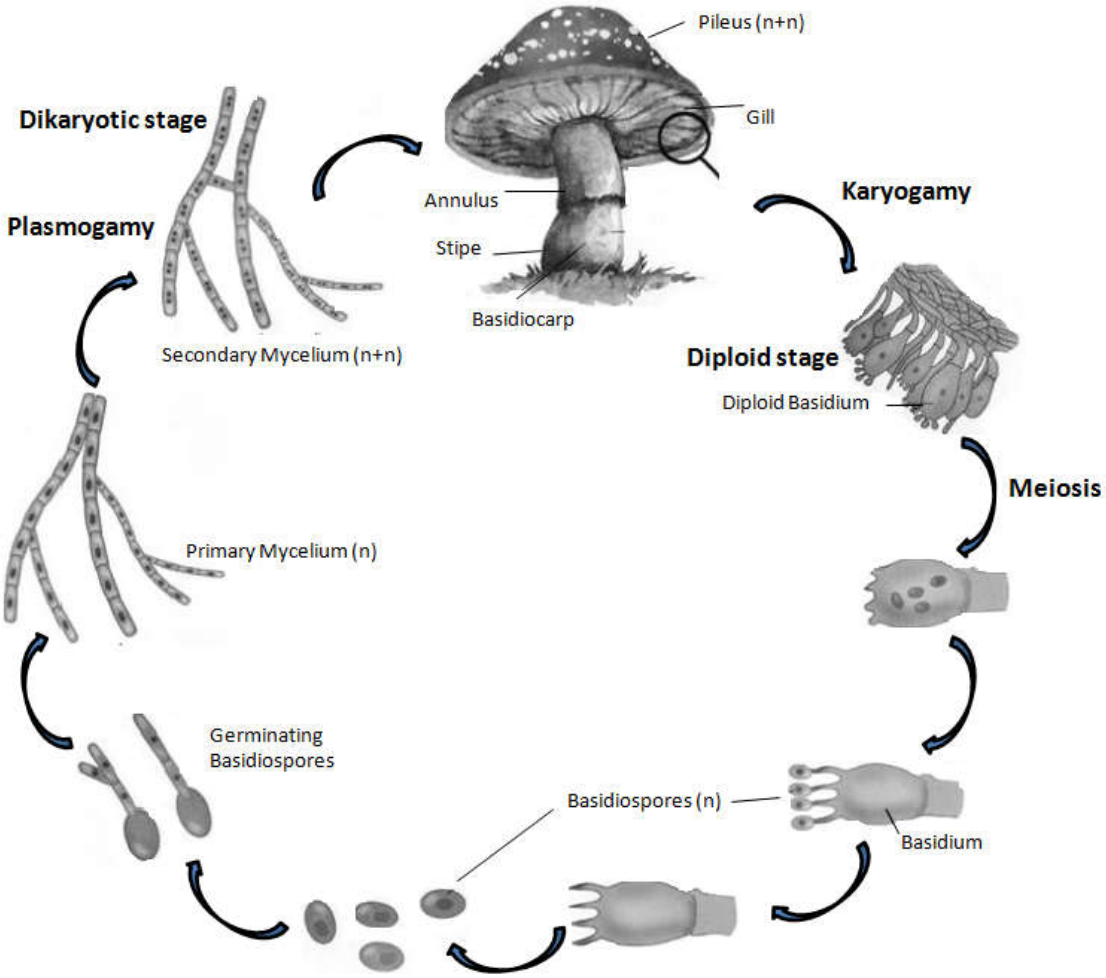
EXERCISE # 12-B

GENERALIZED LIFE CYCLE OF BASIDIOMYCOTA

Basidiomycota is one of two large phyla that, together with the Ascomycota, comprise the subkingdom Dikarya (often referred to as the "higher fungi") within the kingdom Fungi. Basically, Basidiomycota are filamentous fungi composed of hyphae (except for yeasts), and reproducing sexually via the formation of specialized club-shaped end cells called basidia that normally bear external meiospores (usually four). These specialized spores are called basidiospores (Wikipedia).



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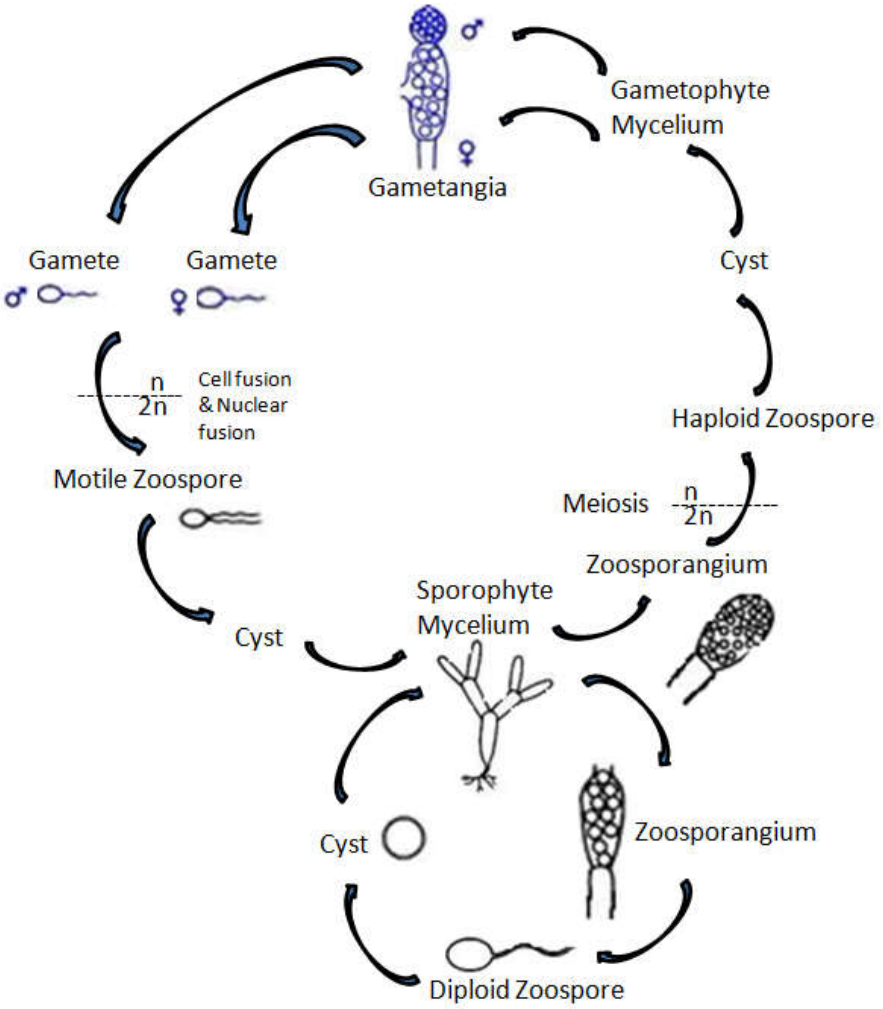
EXERCISE # 12-C

GENERALIZED LIFE CYCLE OF CHYTRIDIOMYCOTA

Chytridiomycota is a division of the Fungi kingdom. The name is derived from the Greek *chytridion*, meaning "little pot", describing the structure containing unreleased spores. Many chytrids are aquatic (mostly found in fresh water). There are approximately 1,000 chytrid species, in 127 genera, distributed among 5 orders. The chytrids are the most primitive of the fungi and are mostly saprobic (degrading chitin and keratin). The species has an interesting life cycle. The thallus (body) is attached by rhizoids, and has an erect trunk on which reproductive organs are formed at the end of branches. The life cycle has the ability to change from haploid and diploid generations (Wikipedia).



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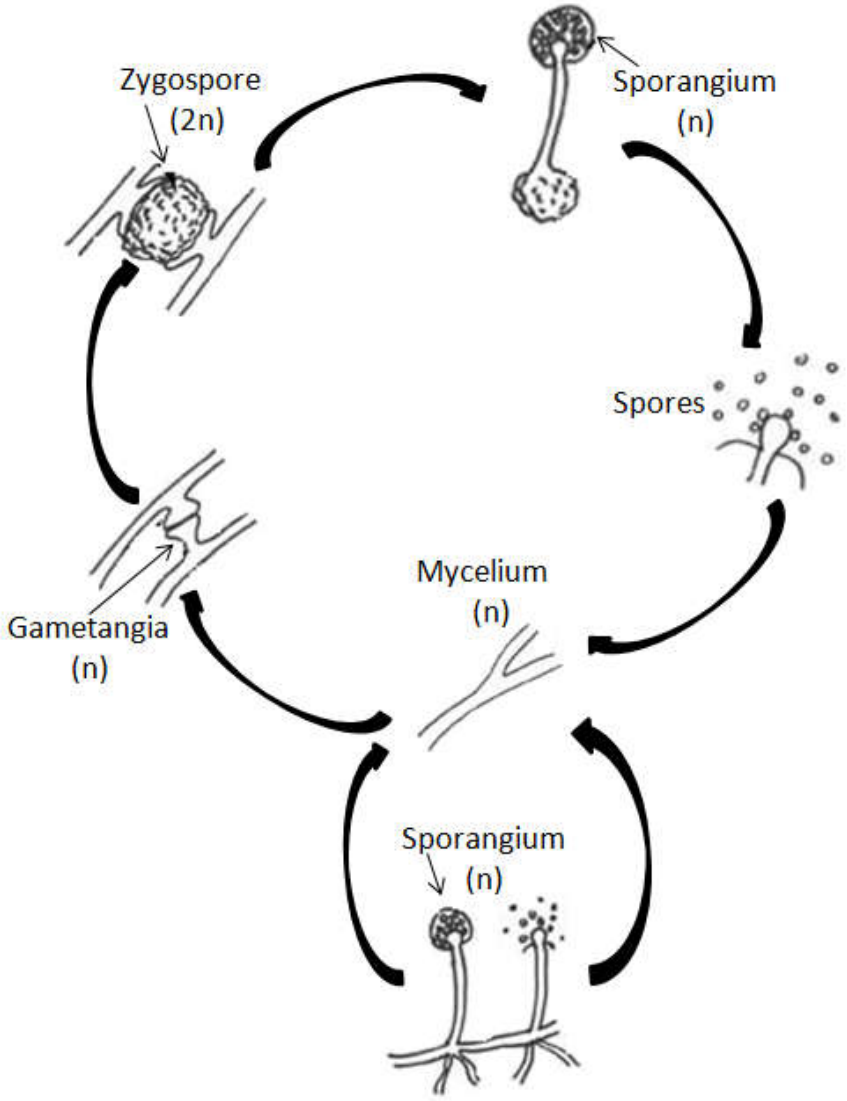
EXERCISE # 12-D

GENERALIZED LIFE CYCLE OF ZYGOMYCOTA

Zygomycota classification makes up only about 1% of true Fungi. There are only about 900 species. The most familiar is the mold that affects strawberries and other fruits. Zygomycota are commonly thought of as bread molds, but there are many species of fungi within this classification that form symbiotic relationships with plants or infect animal hosts. It is believed that zygomycota have zygotic or haplontic life cycles. Zygomycota are able to reproduce both sexually and asexually (Wikipedia).



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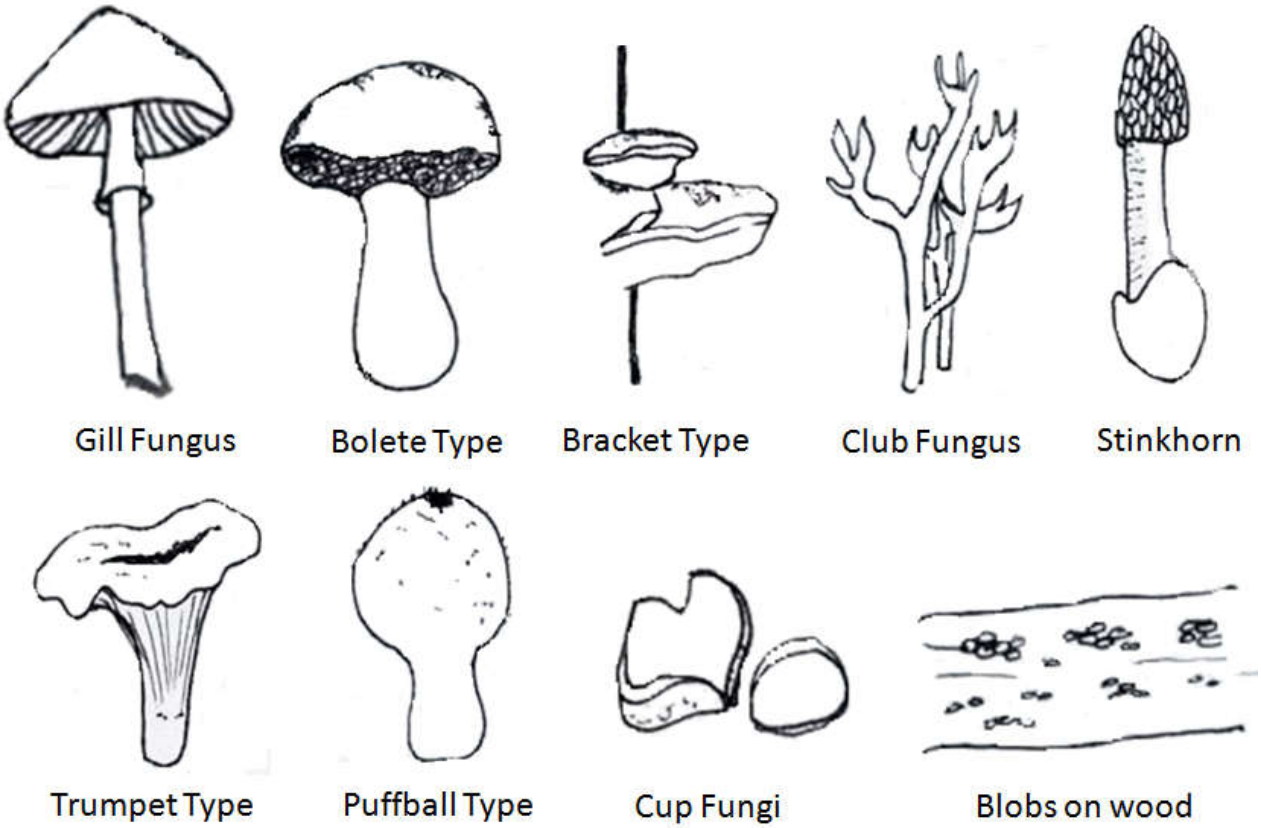
EXERCISE # 12-E

TYPES OF FILAMENTOUS FUNGI



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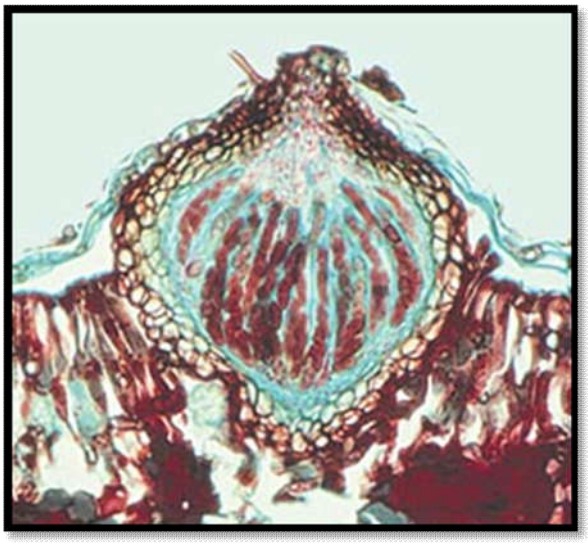
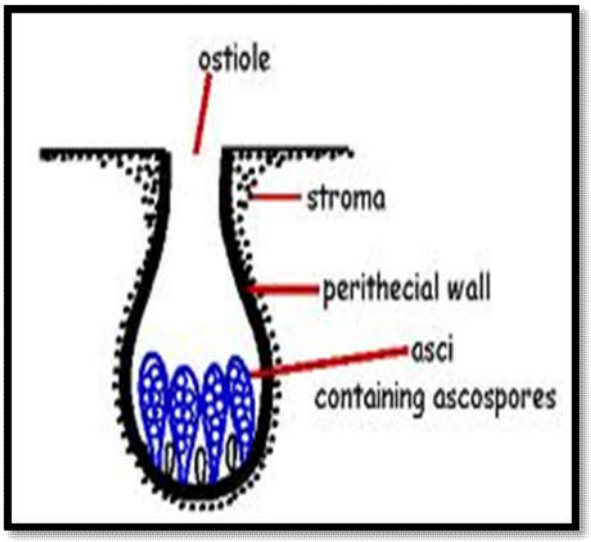
EXERCISE # 12-F

SEXUAL AND ASEXUAL FRUITING BODIES OF ASCOMYCOTA

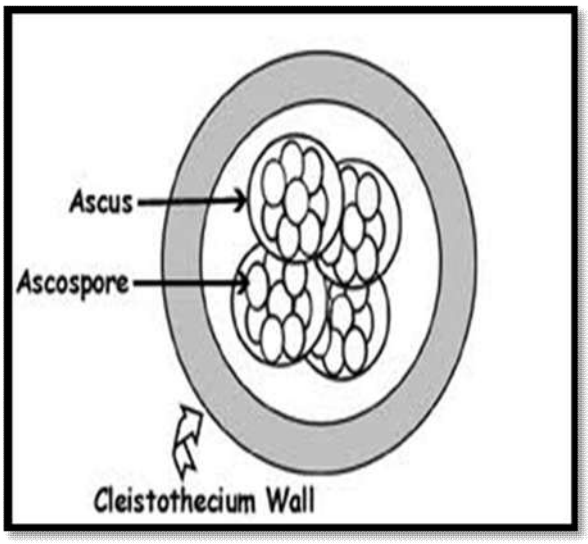
SEXUAL FRUITING BODIES



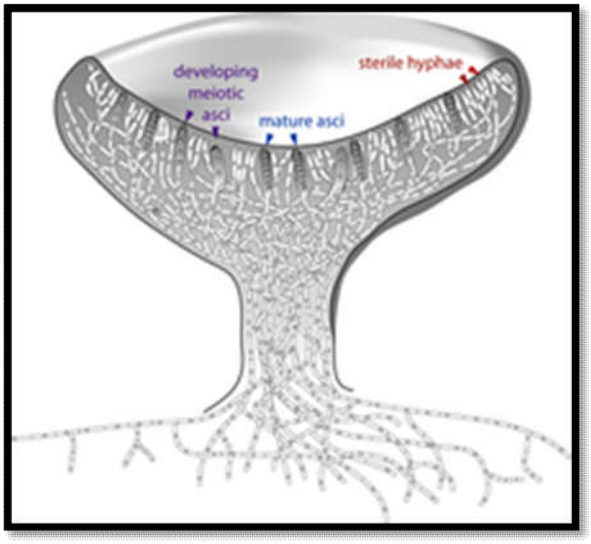
PSEUDOTHECIUM



PERITHECIUM

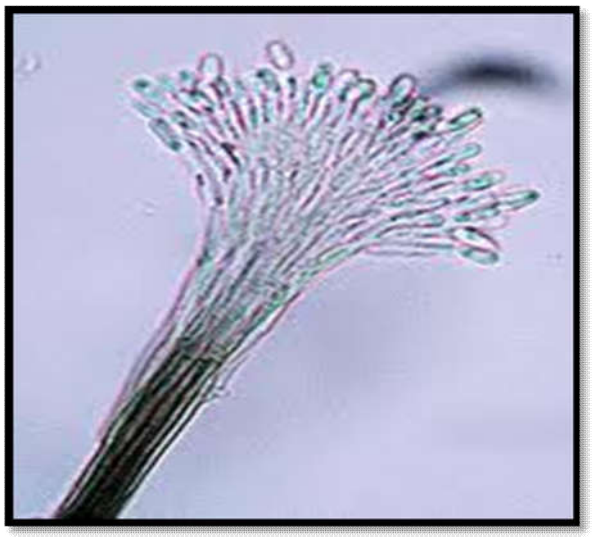


CLEISTOTHECIUM

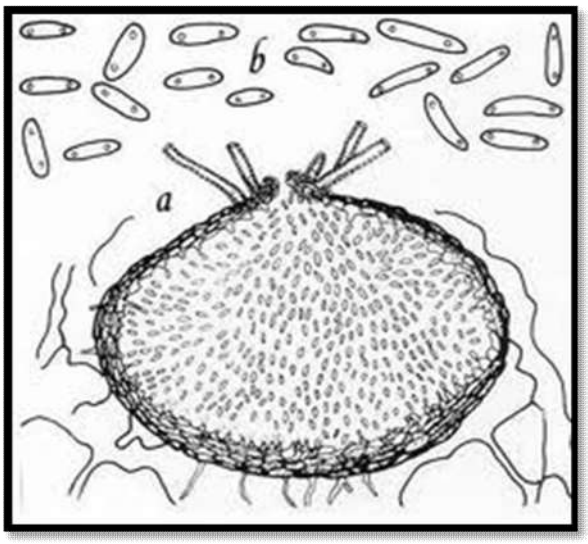


APOTHECIUM

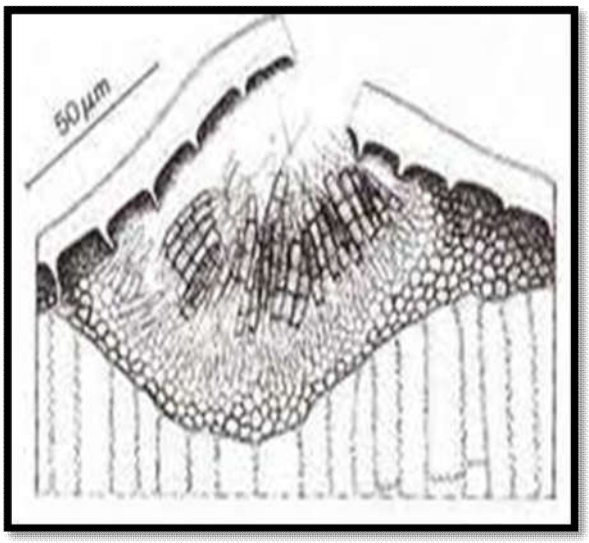
ASEXUAL FRUITING BODIES



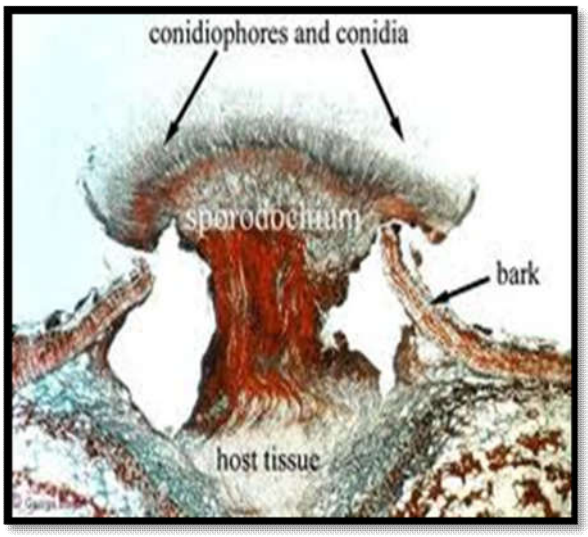
SYNNEMATA



PYCNIDIUM



ACERVULUS



SPORODOCIUM