Practical Manual

# MYCOLOGY

## APP-501 3(2+1)


*For*

**M.Sc. (Ag.) Plant Pathology**

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**Department of Plant Pathology**
**College of Agriculture**

**Rani Lakshmi Bai Central Agricultural University**
**Jhansi, Uttar Pradesh-284003**

Practical manual

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## M.Sc. (Ag.) Plant Pathology

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## CONTENT

# PRACTICAL NO. 1

## Detailed comparative study of different groups of fungi

**Objective:** To study the comparative differences in different groups of fungi

**Outline classification of fungi**

The following classification is adapted from the 9th and 10th editions of The Dictionary of the Fungi (Kirk et al., 2001, 2008), but it has been amended to adopt the phylogenetic arrangement emerging from the AFTOL (Assembling the Fungal Tree of Life) project funded by the US National Science Foundation (visit: http://www.aftol.org/; Blackwell et al., 2006), as set out by Hibbett et al. (2007).

Presently, then, the true fungi which make up this monophyletic clade called kingdom Fungi comprises the seven phyla:

| **Kingdom Fungi (8 Phyla)** | **Kingdom Chromista (3 phyla)** | **Kingdom Protozoa (1 Phylum, 3 classes)** |
|---|---|---|
| Chytridiomycota (2 classes) | Hyphochitriomycota (1 class) | Protostelea |
| Neocallimastigomycota (1 class) | Labrynthulomycota (1 class) | Myxogastrea |
| Blastocladiomycota (1 class) | Oomycota (1 class) | Dictyostelia |
| Zygomycota (4 subphyla) | | |
| Ascomycota (3 subphyla: 15 classes) | | |
| Glomeromycota (1 class) | | |
| Microsporidia | | |
| Basidiomycota (3 subphyla; 16 classes) | | |

**Summarised classification of Kingdom Fungi**

The most recently-published phylogenetic classification of Fungi (Hibbett et al., 2007)

**Kingdom: Fungi**

**Phylum: Chytridiomycota**

Water moulds that live as aquatic saprotrophs or parasites in fresh water and soils; a few are marine. Chytrids produce motile asexual zoospores (with a single posterior flagellum, both a kinetosome and non-functional centriole, nine flagellar props, and a microbody-lipid globule complex) in zoosporangia. Golgi apparatus with stacked cisternae; nuclear envelope fenestrated at poles during mitosis. Thallus may be unicellular or filamentous, and holocarpic (where all of the thallus is involved in formation of the sporangium) or eucarpic (where only part of the thallus is converted into the fruiting body, monocentric, polycentric or filamentous. Sexual

reproduction with zygotic meiosis where known; sometimes produce motile sexual zoogametes. Considered to be the most ancestral group of fungi.

**Class : Chytridiomycetes**

**Order:** Chytridiales

**Class: Monoblepharidomycetes**

**Order**: Monoblepharidales; example genus: *Monoblepharis*

**Phylum: Neocallimastigomycota**

Thallus monocentric or polycentric; anaerobic, found in digestive system of larger herbivorous mammals and possibly in other terrestrial and aquatic anaerobic environments; lacks mitochondria but contains hydrogenosomes of mitochondrial origin; zoospores posteriorly unflagellate or polyflagellate, kinetosome present but non-functional centriole absent, kinetosome-associated complex composed of a skirt, strut, spur and circumflagellar ring, microtubules extend from spur and radiate around nucleus, forming a posterior fan, flagellar props absent; nuclear envelope remains intact throughout mitosis.

**Class: Neocallimastigomycetes**

**Order:** Neocallimastigales; example genus: *Neocallimastix*

**Phylum: Blastocladiomycota**

Very like the chytrids, characteristically, the Blastocladiomycota have life cycles with what is described as a sporic meiosis; that is, meiosis results in the production of haploid spores that can develop directly into a new, but now haploid, individual.

**Class: Blastocladiomycetes**

**Order:** Blastocladiales

**Phylum: Microsporidia**

No subdivision of the group is proposed yet because of the lack of well-sampled multigene phylogenies within the group. Microsporidia are unicellular parasites of animals and protists with highly reduced mitochondria. Microsporidia may be a sister group of the rest of the Fungi, but this suggestion may have arisen from incomplete sampling.

**Phylum: Glomeromycota**

Until recently, arbuscular mycorrhizal (AM) fungi have generally been classified in the Zygomycota (being placed in the Order Glomales), but they do not form the zygospores characteristic of zygomycota, and all 'glomalean' fungi form mutualistic symbioses.

**Class: Glomeromycetes**

**Order:** Archaeosporales; example genera: *Archaeospora, Geosiphon*.

**Order:** Diversisporales; example genera: *Acaulospora, Diversispora, Pacispora*.

**Order:** Glomerales; example genus: *Glomus.*

**Order:** Paraglomerales; example genus: *Paraglomus.*

**Phylum: Ascomycota**

This is the largest group of fungi, and the lifestyles adopted cover the complete range from saprotrophs, to symbionts (notably lichens), and to parasites and pathogens (plant pathogens are particularly numerous, but there are many important human pathogens in this group also). The Ascomycota are characterised by having sexual spores (ascospores) formed endogenously within an ascus A layered hyphal wall with a thin relatively electron-dense outer layer and a thicker electrontransparent inner layer also appears to be diagnostic.

**Subphylum: Taphrinomycotina**

**Class: Taphrinomycetes**

**Order:** Taphrinales

**Class: Neolectomycetes**

**Order:** Neolectales; example genus: *Neolecta*

**Class: Pneumocystidomycetes**

**Order:** Pneumocystidales; example genus: *Pneumocystis*.

**Class: Schizosaccharomycetes**

**Order:** Schizosaccharomycetales; example genus: *Schizosaccharomyces.*

**Subphylum: Saccharomycotina**

**Class: Saccharomycetes**

**Order:** Saccharomycetales;

**Subphylum: Pezizomycotina**

**Class: Arthoniomycetes**

**Order**: Arthoniales

**Class: Dothideomycetes**

**Class: Eurotiomycetes**

**Class: Pezizomycetes**

**Class: Lichinomycetes**

**Class: Leotiomycetes**

**Class: Lecanoromycetes**

**Class: Laboulbeniomycetes**

**Class: Sordariomycetes**

**Phylum: Basidiomycota**

Saprotrophic or parasitic on plants or insects; filamentous; hyphae septate, the septa typically inflated (dolipore) and centrally perforated; mycelium of two types, primary (homokaryotic) of uninucleate cells, succeeded by secondary (heterokaryotic), consisting of dikaryotic cells, this often bearing bridge-like clamp connections over the septa; asexual reproduction by fragmentation, oidia (thin-walled, free, hyphal cells behaving as spores) or conidia; sexual reproduction by fusion of hyphae with each other or with hyphal fragments or with germinating spores (somatogamy), resulting in dikaryotic hyphae that eventually give rise to basidia, either singly on the hyphae or in variously shaped basidiomata.

**Class: Pucciniomycetes**

**Order:** Septobasidiales; example genera: *Septobasidium, Auriculoscypha*.

**Order**: Pachnocybales; example genus: *Pachnocybe.*

**Order:** Helicobasidiales; example genera: *Helicobasidium, Tuberculina*.

**Order:** Platygloeales; example genera: *Platygloea, Eocronartium*.

**Order:** Pucciniales; example genera: *Puccinia, Uromyces*.

**Class: Cystobasidiomycetes**

**Order:** Cystobasidiales; example genera: *Cystobasidium, Occultifur, Rhodotorula*.

**Order:** Erythrobasidiales; example genera: *Erythrobasidium, Rhodotorula, Sporobolomyces, Bannoa*.

**Order:** Naohideales; example genus: *Naohidea.*

**Class: Agaricostilbomycetes**

**Order**: Agaricostilbales; example genera: *Agaricostilbum, Chionosphaera*.

**Order:** Spiculogloeales; example genera: *Mycogloea, Spiculogloea*.

**Class: Microbotryomycetes**

**Order**: Heterogastridiales; example genus: *Heterogastridium*.

**Order**: Microbotryales; example genera: *Microbotryum, Ustilentyloma*.

**Order:** Leucosporidiales; example genera: *Leucosporidiella, Leucosporidium, Mastigobasidium*.

**Order**: Sporidiobolales; example genera: *Sporidiobolus, Rhodosporidium, Rhodotorula*.

**Class: Atractiellomycetes**

**Order:** Atractiellales; example genera: *Atractiella, Saccoblastia, Helicogloea, Phleogena*.

**Class: Classiculomycetes**

**Order:** Classiculales; example genera*: Classicula, Jaculispora*.

**Class: Mixiomycetes**

**Order**: Mixiales; example genus: *Mixia.*

**Class: Cryptomycocolacomycetes**

**Order**: Cryptomycocolacales; example genera: *Cryptomycocolax, Colacosiphon*

**Subphylum: Ustilaginomycotina (equivalent to the traditional Ustilaginomycetes)**

**Class: Ustilaginomycetes**

**Order:** Urocystales; example genera: *Urocystis, Ustacystis, Doassansiopsis*.

**Order:** Ustilaginales; example genera: *Ustilago, Cintractia*.

**Class: Exobasidiomycetes**

**Subphylum: Agaricomycotina (equivalent to the traditional Hymenomycetes or Basidiomycetes)**

**Class: Tremellomycetes**

**Class: Dacrymycetes**

**Class: Agaricomycetes Subclass: Agaricomycetidae**

**Subclass: Phallomycetidae Order: Geastrales**

**Class: Wallemiomycetes**

**Class: Entorrhizomycetes**

**Kingdom Chromista**

**Phylum Hyphochytriomycota**

**Order:** Hyphochytriales; example genera: *Hyphochytrium*, *Rhizidiomyces.*

Phylum Oomycota

**Order Leptomitales**; example genera: *Apodachlyella, Ducellieria, Leptolegniella, Leptomitus*. Order Myzocytiopsidales; example genus: *Crypticola*.

**Order Olpidiopsidales**; example genus: *Olpidiopsis*.

**Order Peronosporales**; example genera: *Albugo, Peronospora, Bremia, Plasmopara*.

**Order Pythiales**; example genera: *Pythium, Phytophthora, Pythiogeton.*

**Order Rhipidiales**; example genus: *Rhipidium.*

**Order Salilagenidiales**; example genus: *Haliphthoros.*

**Order Saprolegniales**; example genera: *Leptolegnia, Achlya, Saprolegnia*.

**Order Sclerosporales;** example genera: *Sclerospora, Verrucalvus*.

**Order Anisolpidiales;** example genus: *Anisolpidium.*

**Order Lagenismatales;** example genus: *Lagenisma*.

**Order Rozellopsidales;** example genera: *Pseudosphaerita, Rozellopsis*.

**Order Haptoglossales;** example genera: *Haptoglossa, Lagena, Electrogella, Eurychasma, Pontisma*, *Sirolpidium.*

**Kingdom Protozoa**

**Phylum Plasmodiophoromycota** Obligate intracellular symbionts or parasites of plant, algal or fungal cells living in freshwater or soil habitats

**Class Plasmodiophoromycetes**

**Order**: Plasmodiophorales; example genera: *Plasmodiophora, Polymyxa*.

**Phylum Myxomycota** Free-living unicellular or plasmodial amoeboid slime moulds

**Class Dictyosteliomycetes**

**Class Myxomycetes**

**Class Protosteliomycetes**

**Phylum Acrasiomycota** Amoeboid slime moulds; generally saprotrophic, found on a very wide range of decaying plant material. A total of 14 species assigned to 6 genera

**Class Acrasiomycetes**

**Order Acrasiales**; example genera: *Acrasis, Copromyxa, Guttulinopsis, Fonticula*

**Phylum Choanozoa Amoebidiales and Eccrinales**

**Class Mesomycetozoea**

**Order** Amoebidiales

**Order** Eccrinales

**Activity:**

1. Collect isolates of fungi belongs to each phylum and show distinguishing features.
2. Get the culture belongs to different phylum and record the cultural characteristics

# PRACTICAL NO. 2

## Saccardoan classification and classification based on conidiogenesis

**Objective:** To study the different forms of conidia based on conidiogenesis based on Saccardoan classification

Fungi in the Ascomycetes and Basidiomycetes groups produce a type of asexual reproductive structure known as conidia. Some fungi only produce these asexual propagules and have not been observed to have a sexual state. These fungi were classified as Fungi Imperfecti or Deuteromycetes. With advances in molecular sequencing, some of them is being integrated into the classification system based on their sexual reproductive structures.

Despite not having a sexual state, mitosporic fungi play significant roles ecologically, medically, and industrially. Therefore, it is important to identify and name them accurately. In the past, two classification approaches have been used for these fungi, one based on the overall morphology of the conidia and conidiomata, and the other based on conidial development. Recently, identification manuals have combined these two approaches.

The Saccardoan System, developed in 1886, classified Fungi Imperfecti based on the spore characteristics of pigmentation, septation, and form. The primary combinations of these characters were used to classify the species of Fungi Imperfecti.

**Activity:** Examine the species of fungi provided (cultures and slides). Draw the conidia of each species under the terms that best describe them (be sure to write the name of the species under your drawing.

A. Hyaloamerosporae – hyaline or brightly colored single-celled conidia.

B. Phaeoamerosporae – dark pigmented single-celled conidia.

C. Hyalodidymosporae – hyaline or brightly colored, two celled condia.

D. Phaeodidymosporae – dark pigmented, two celled conidia.

E. Hyalophragmosporae – hyaline or brightly colored, two to many septate conidia.

F. Phaeophragmosporae – dark pigmented, two to many septate conidia.

G. Hyalodictyosporae – hyaline or brightly colored, transversely and longitudinally septate conidia

H. Phaeodictyosporae – dark pigmented, transversely and longitudinally septate conidia.

I. Hyalo or Phaeoscolecosporae – hyaline, brightly colored, or dark pigmented, long, curved, often sigmoidal conidia.

J. Hyalo or Phaeohelicosporae – hyaline, brightly colored, or dark pigmented, coiled conidia.

K. Hyalo or Phaeostaurosporae – hyaline, brightly colored, or dark pigmented, star shaped (arms radiating out from a central point) conidia.

## PRACTICAL NO. 3

## Collection of cultures and live specimens, preservation of plant parasitic fungi

**Objectives:** To collect and preserve plant disease samples

Preservation means killing or restricting the growth of an organism in or on the substrate on which it grows. Preservation of disease materials (herbaria) on their natural substrates as dry specimens or wet specimens is essential for conducting systematic mycological work and important taxonomic research on various micro-organisms.

**Materials required:** Polythene bags, Newsprint paper • Hand saw, Trowel, Pruning shear, knife, Scissors • Hand lens • Pencil, Ink markers • Vasculum, Plant press, Paper bags, Envelopes • Ice box • Manual

**Specimens**

A herbarium specimen may be a single sporocarp or a portion of it, dried culture, slide or the material on its host or substrate (e.g. leaf, stem, bark, rock, soil, paper, cloth). The following two types of preservation methods are used for diseased plant specimen:

**1) Dry Preservation**: It involves following steps:

a) **Collection and drying:** The sample should have distinctively visible symptoms. Dry the specimen in layer of blotting sheets under sunlight or in hot air oven for few days.

b) **Labelling and packaging:** The material should be kept in good herbarium packets. This is attached to a chart paper sheets. The two sides of packet are folded first, then bottom flap and finally top flap. The name of pathogen, host, locality, date, name of scientist who identified the specimen, should be mentioned on the label.

c) **Disinfection and storage**: The specimen folders are fumigated with methyl bromide vapours in fumigation chamber for 24-48 hrs before storage.

**2) Wet Preservation**

Washed fresh diseased specimens are put in a boiling mixture of 1 part of glacial acetic acid saturated with normal copper acetate crystals and 4 parts of water till the green colour reappears and then kept preserved in 5 per cent formalin in the glass jars.

All mounted or preserved specimens must be labeled with as much of the following information as far as possible:

1. Host (name of the diseased plant)
2. Name of the disease Parasite (the name of the organism causing the disease)
3. Place where collected (nearest town and state is usually sufficient)
4. Date collected
5. Name of the collector

**Size of the specimen**: A specimen should ideally be 25–40 cm long and up to 26 cm wide, allowing it to fit on a standard herbarium mounting sheet which measures 42 x 27 cm. This is also the approximate size of tabloid newspapers. Plant parts that are too large for a single sheet may be cut into sections pressed on a series of

sheets, for example a palm or cycad frond. Long and narrow specimens such as grasses and sedges can be folded once, twice or even three times at the time of pressing. In this way a plant of up to 1.6 metres high may be pressed onto a single sheet. For very small plants, a number of individuals may be placed on each sheet.

**Activity 1**: Prepare herbarium of Plant disease with all following details in it:

1. Host (name of the diseased plant)
2. Name of the disease Parasite (the name of the organism causing the disease)
3. Place where collected (nearest town and state is usually sufficient)
4. Date collected
5. Name of the collector

**Activity 2:** Collect disease sample and preserve in the glass bottle following wet preservation protocol.

## PRACTICAL NO. 4

## Preparation of Potato dextrose Agar media

**Objective:** To prepare Potato dextrose Agar media

**Materials required:** For the preparation of potato dextrose agar medium the following ingredients in different quantities are used

| | | | |
|---|---|---|---|
| (i) | Peeled potato slices | - | 200g |
| (ii) | Dextrose | - | 20g |
| (iii) | Agar- agar | - | 20g |
| (iv) | Distilled water | - | 1000 ml |

**Method:**

(1) Potato slices are cooked in 500 ml of water.

(2) Then filtered with the help of muslin cloth.

(3) Agar-agar is melted in 500 ml of water.

(4) Potato juice is added to the melted agar.

(5) Volume is made 1000 ml by adding required water.

(6) Again lit is filtered through muslin cloth.

(7) Dextrose is added in this mixture and shaken well.

(8) Medium is sterilized in an autoclave at 1.1kg/$cm^2$ pressure for 20 minutes at temperature of 121.6°C. Thus the medium is ready for use.

**Activity 1**: Prepare one litre of Potato dextrose Agar medium. Note the materials required and quantity of the components.

## PRACTICAL NO. 5

## Isolation of plant pathogens from Diseased Plant Tissues

**Objective**: To isolate plant pathogens from Diseased Plant Tissues

Tissues sampled during the active stage of an infection are likely to have within them only the pathogen responsible for the infection; the surfaces of such tissues, however, are usually contaminated with saprophytic organisms. The steps of isolation of the pathogen have been given in the flowchart below:

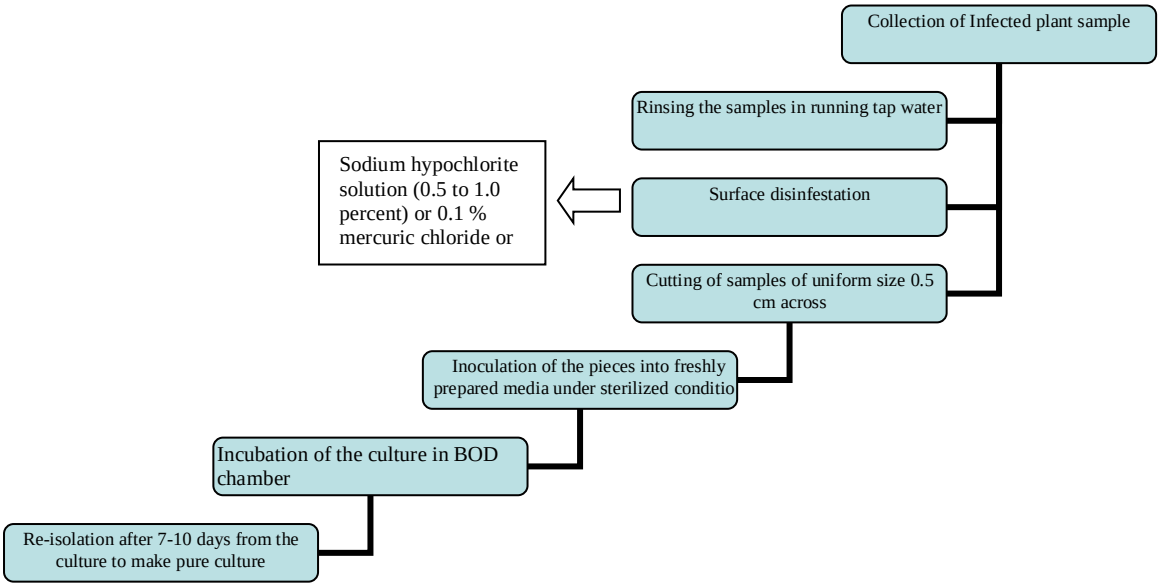



**Fig. 1. Flowchart showing steps of isolation of pathogen from plant tissues**

**Activity 1.** Isolate the pathogen from the given sample and note the different morphological features observed under the microscope and identify it based on morphology and cultural characteristics.

## PRACTICAL NO. 6

## Study of different types of mycelium, asexual spores and fruiting bodies

**Objective:** To study different types of mycelium, asexual spores and asexual fruiting bodies

**Mycelium:** Network of hyphae is called as mycelium. It may be aseptate or septate.

i. **Aseptate Mycelium-** When the hyphae are undivided by cross-walls (septa) it is known as septate mycelium. This type of mycelium is found in lower fungi.

ii. **Septate Mycelium-** When the mycelium is divided by cross walls (septa) at certain intervals, it is known as septate mycelium. In the septa (singular septum), there is a minute hole, which is known as "septal pore." This type of mycelium is found in higher fungi.

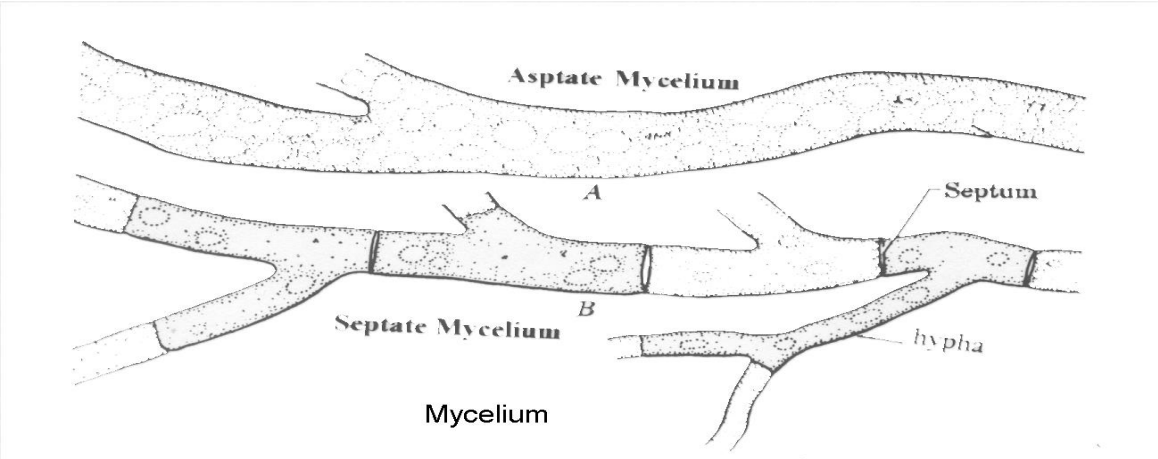



**Fig. 2. Structure of fungal mycelium**

**Different Types of Asexual Spores:** Asexual spores are those in which sex is not involved. Generally five types of asexual spores are produced in fungi. They are as follows:-

1. **Arthrospores** (Oidia): Formed in chains (basipetal) on short conidiophores, single celled, barrel or drum shaped.
2. **Chlamydospores**: Formed singly or in chains, which may be terminal or intercalary, provided with an envelop (covering).
3. **Blastospores:** Spores formed by process of budding, which are single celled, first formed in chains but later separated from each other.

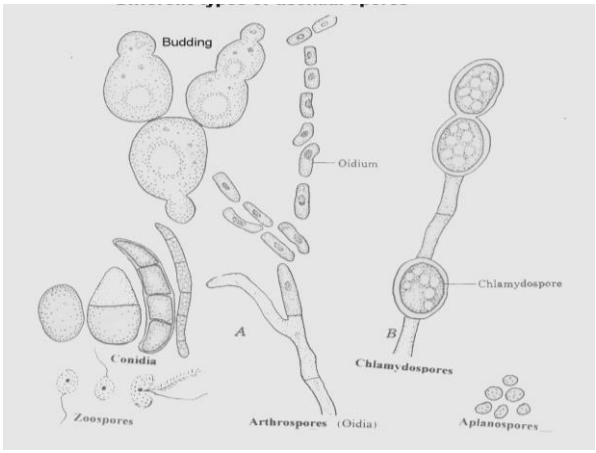



**Fig. 3. Different types of fungal asexual spores**

4. **Conidia**: Formed at the tip or side of the hypha (Conidiophore), may be formed singly or in chains, quite variable in shape, size, septation, colour and also in ornamentation.

**5. Zoospores:** Pear or kidney shaped, single shaped, naked, motile (flagellate), produced in sporangium (zoosporangium).

**6. Aplanospores:** Oval or spherical in shape, single celled, non-motile (aflagellate) and produced mostly in elliptical spora ngium.

**Asexual fruiting bodies:**

**1. Pycnidia:** These are spherical or flask shaped structures in which the conidia or produced. They have the natural opening known as ostiole through which the conidia are liberated. This type of structure is produced in order Spaeropsidales of sub division Deuteromycotina.

**2. Acervuli**: These are mat or cushion shaped structure formed below the cuticle or epidermis of the host. They may be provided with sterile hair like structures known as setae.

**3. Sporodochia**: These are the cushion-shaped structure on which the conidiophores are produced.

**4. Synnemata**: In these structures the conidiophores are grouped together at the base and free towards apex.

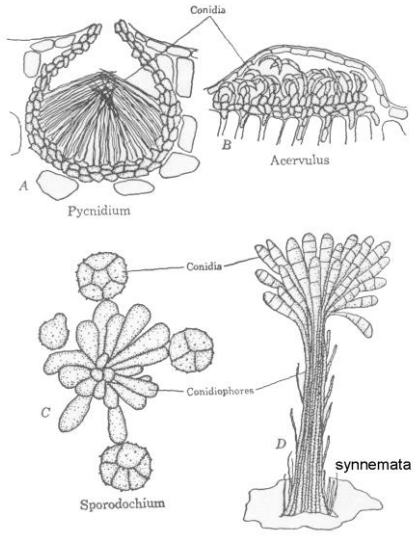



**Fig. 4 Different types of asexual fruiting bodies**

**Activity:** 1. Examine the representative slides of asexual fruiting bodies and asexual spores and note down the characteristic features of the structures observed.

**Activity:** 2 Enlist the organisms against the particular asexual fruiting bodies produced by them.

# PRACTICAL NO. 7

## Study of different types sexual spores and sexual fruiting bodies

**Objective:** To study different types sexual spores and sexual fruiting bodies

**Different types of sexual spores**

Following four types of sexual spores are formed in fungi, which are produced by various methods and they form the bases for the classification of fungi in different sub-divisions.

**1. Oospores:** Mostly spherical in shape, formed in the oogonium, usually smooth walled. They are formed by gametangial contact (oogamy), characteristics of phylum Iliptic.

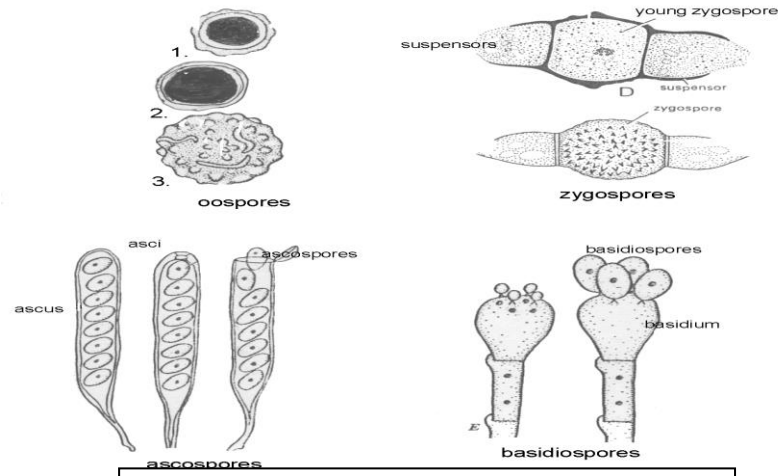



**Fig. 5 Different types of sexual spores**

**2. Zygospores:** Black in colour, rough-walled, warty in appearance and provided with suspensors. They are formed by gametangial copulation (zygogamy), characteristics of sub-division Zygomycotina.

**3. Ascospores:** Produced in asci, definite in number (usually 8). They are formed by spermatization/somatogamy, characteristics of sub-division Ascomycotina.

**4. Basidiospores:** Borne on the basidium, definite in number (usually 4). They are formed by spermatization/somatogamy, characteristics of sub-division Basidiomycotina.

### Different Types of Ascocarps:

1. **Cleistothecia (-um):** Spherical in shape, black in colour, hard in structure and without any natural opening. Asci come out by tearing or breaking of the cleistothecium. Cleistothecia are also provided with appendages.

2. **Perithecia (-um):** Flask shaped with natural opening known as "**ostiole**", some time having long neck. Asci are produced in the perithecium at basal region. Paraphyses may also be present in between the asci.

3. **Apothecia (-um):** The ascocarp, which produces its asci in an open disc or cup shaped structure, is called as apothecium. It is exposed and form the layer of asci in a "hymenium" among them paraphyses may also be present.

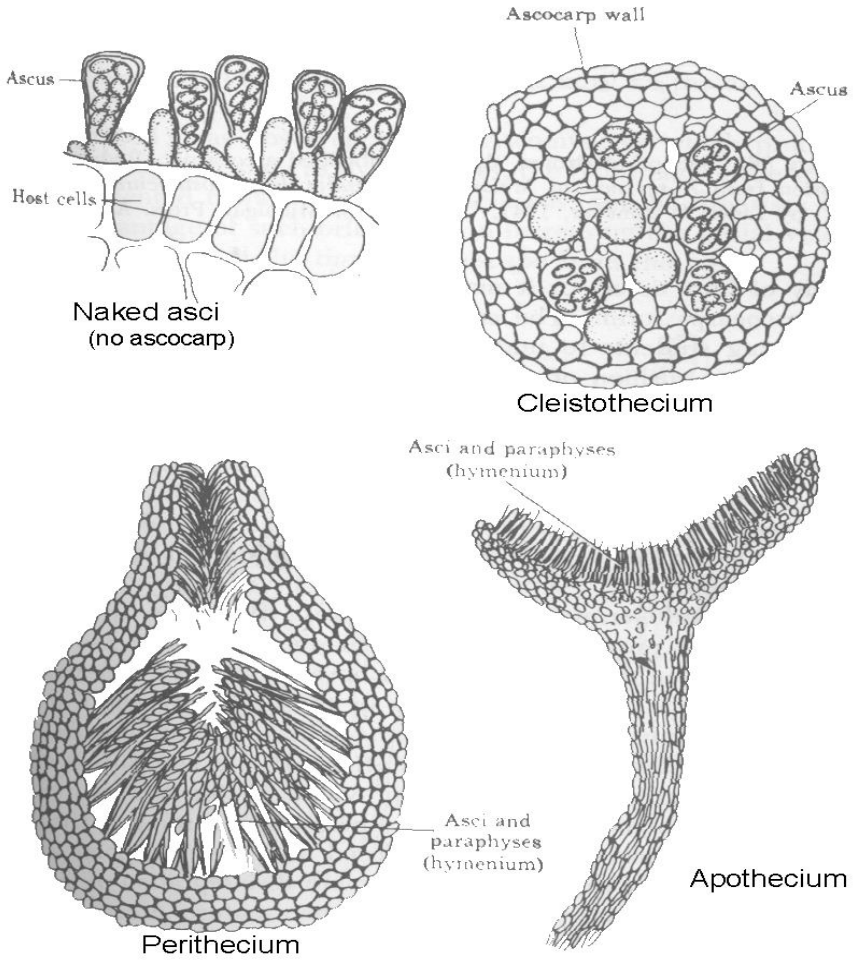



**Fig.6 Different types of Ascocarps**

**Activity:** 1. Examine the representative slides of asexual fruiting bodies and asexual spores and note down the characteristic features of the structures observed.

**Activity:** 2 Enlist the organisms against the particular asexual fruiting bodies produced by them.

## PRACTICAL NO. 8

## Somatic and reproductory structures of *Pythium* and *Phytophthora*

**Objective:** To study the somatic and reproductive structures of *Pythium* and *Phytophthora* and their differences

**1. Genus – *Pythium*** (Damping off)

| Systematic Position | |
|---|---|
| **Phylum** | - Oomycota |
| **Class** | - Oomycetes |
| **Order** | - Peronosporales |
| **Family** | - Pythiaceae |

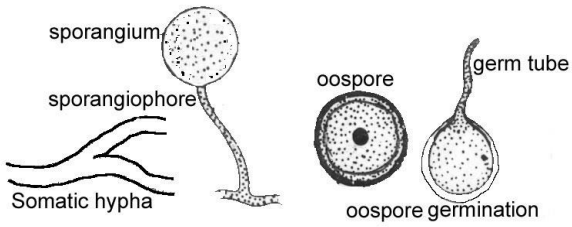



**Fig. 7 Sporangium and oospore of *Pythium* spp**

**Mycelium –** Aseptate, branched, cottony white.

**Sporangiophores –** Different from vegetative hyphae, erect, simple and bearing sporangia singly.

**Sporangia-** Spherical or globose, sometimes filamentous or toruloid.

**Ooospores –** Thick walled, spherical, usually smooth and three layered and plerotic.

**Important species-** *P. aphanidermatum, P. ultimum, P. graminicolum* (damping off disease)

**2. Genus – *Phytophthora***

**Mycelium –** Aseptate, coenocytic, branched.

**Sporangiophores –** of indeterminate growth, zig-zag, sympodially branched, noduate (with nodular swellings)

**Important species-** *P. infestans* (Late blight of potato).

**Sporangia-** Single celled, lemon shaped and papillate.

**Ooospores –** Spherical in shape, smooth walled and aplerotic.

| Systematic Position | | |
|---|---|---|
| Phylum | – | Oomycota |
| Class | – | Oomycetes |
| Order | - | Peronosporales |
| Family | - | Pythiaceae |

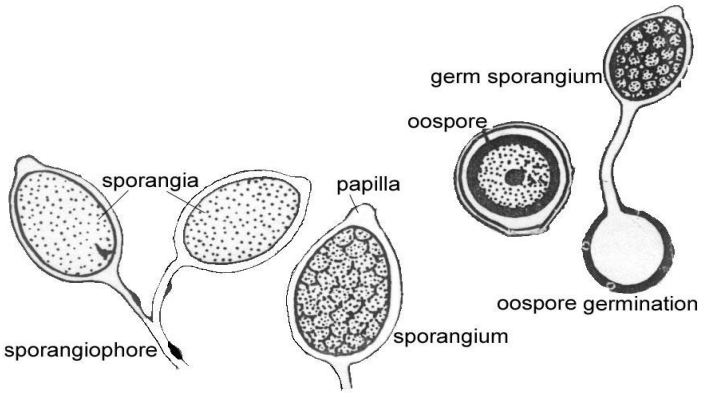



**Fig. 8 Sporangium and oospore of *Phytophthora* spp**

**Activity:**

1. Examine the representative slides of *Pythium* and *Phytophthora* and note down the characteristic features of the mycelium, sporangia and the spores. Draw a proper neat and clean pencil sketch diagram. Note the location of the antheridium in relation to the oogonium. How do oospores differ from oogonia?
2. Isolation of Phytophthora from plant tissue and soil

**Materials required:**

Root, stem, or leaves from diseased plant

Soil sampling tools

Plastic bags

Scalpel with #10 blade

Petri Plates

Sieves

Magnetic strirrer

Balance, weigh boats

BOD incubator

V8 Media

Compound microscope

**3. Observation of zoospores and their characteristics.**

**i) Zoospore motility**: Sporangia in an aqueous preparation have been placed in water, chilled, and then warmed to induce the formation of motile zoospores. Observe the release of zoospores with a dissecting microscope

ii) **Attraction of zoospores to plant roots**: Place the roots of a pea seedling into an aqueous suspension of *Pythium* zoospores in a Petri dish, and let stand for 5-10 minutes. Observe the suspension with a dissecting microscope for the accumulation of zoospores around the root zone of elongation.

iii) **Diagram your observations from the prepared cultures and slides:**

a) Sporangia and zoospores (include vesicles if observed)

b) Oogonia and antheridia

c) Oospores

**4. List the differences in the characteristic features of *Pythium* and *Phytophthora*.**

**Conclusive Questions:**

1. What fungal structure differentiates species of Pythium from species of Phytophthora? What is the location of this structure and its role in the Pythium life cycle?

2. How can wet, poorly drained soil contribute to root rots caused by Pythium and Phytophthora?

3. There will be no sexual reproduction in Phytophthora infestans, if only one mating type present in the pathogen population. However, both mating types have been found. If you suspected that both mating types were present in your potato field:

a. Where would you look to determine if sexual reproduction was occurring?

b. What fungal structure would be evidence of sexual reproduction?

## PRACTICAL 9
## Somatic and reproductory structures of downy mildews and *Albugo* spp.

**Objective:** To study Somatic and reproductory structures of downy mildews and *Albugo* spp.

**1. Genus – *Peronospora*** (Downy mildew)

| | |
|---|---|
| **Mycelium** | Aseptate, coencocytic, branched, hyaline, endophytic and intercellular. |
| **Conidia** | Single celled, spherical or oval in shape and borne singly. |
| **Branching** | Dichotomous at acute angles. Last (ulti |
| **Sterigmata** | Long and pointed and bearing conidia singly. |
| **Ooospores** | Spherical and reticulate in *Peronospora parastitica* (downy mildew of Crucifers). |
| **Conidiophores** | Arise from the stomatal openings. They are slender, long, 2/3 portion unbranched and only 1/3 portion is branched. |

| **Systematic Position** | |
|---|---|
| Phylum | – Oomycota |
| Class | – Oomycetes |
| Order | - Peronosporales |
| Family | - Peronosporaceae |

**Important species-** *Peronospora parastitica* (downy mildew of Crucifers), *P. tabacinia* (downy mildew of tobacco). *P. pisi* (downy mildew of pea).

**2. Genus – *Sclerospora***

**Mycelium –** Aseptate, coencocytic, branched, hyaline, endophytic and intercellular.

**Sporangiophores–** Arise from the stomatal openings. They are short and broader towards apex.

| **Systematic Position** | |
|---|---|
| Phylum | - Oomycota |
| Class | - Oomycetes |
| Order | - Peronosporales |
| Family | - Peronosporaceae |

**Branching –** Dichotomous or even trichotomous. Last branch is changed into the sterigmata.

**Sterigmata –** Short and swollen and bearing sporangia singly.

**Sporangia-** Borne singly, single celled and sometimes papillate also.

**Ooospores –** Irregular in appearance because the sporangial wall shrinks and touches the oosporic wall at several places.

**Important species** is *Sclerospora graminicola*, which causes green ear disease of Bajra.

**3. Genus: *Plasmopara***

**Mycelium –** Aseptate, coencocytic, branched, hyaline, endophytic and intercellular.

**Sporangia:** The sporangia were hyaline, oval shaped formed on right angle sporangiophore. **Sporangiophore:** The sporangiophore were hyaline, straight or slightly curved

**Branching –** Right angle branched.

**Sterigmata:** Sterigmata are mostly trichotomous

**Ooospores –** large, 25–50 µm in diameter, thick-walled spherical

**Important species*: Plasmopara viticola***

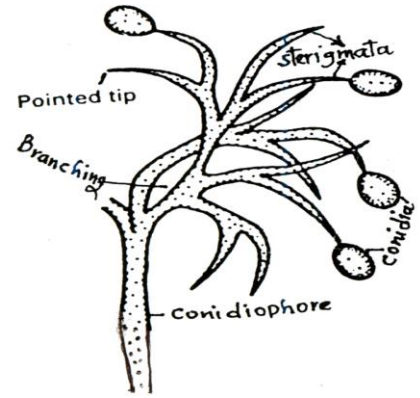
**4. Genus: *Bremia***

**Mycelium –** Aseptate, coencocytic, branched, hyaline, endophytic and intercellular. **Sporangiophores branched more or less like Peronospora but ends with a** disc-like or saucer shaped, sterigmata bearing structure

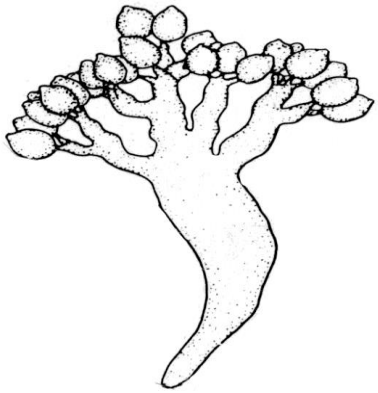
**Important species** is *Bremia lectuca*

**5. Genus: *Basidiophora***

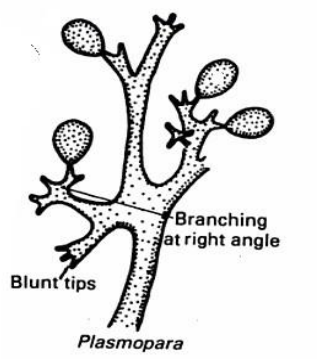
Sporophores unbranched, apex swollen and with short sterigmata bearing papillate sporangia germinating by zoospores; oospores aplerotic



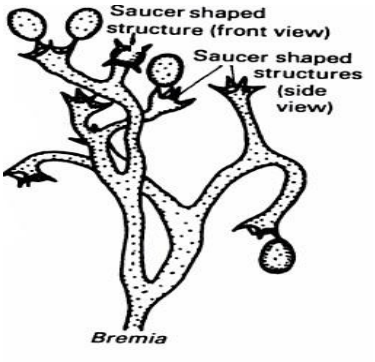

***Peronospora* spp**



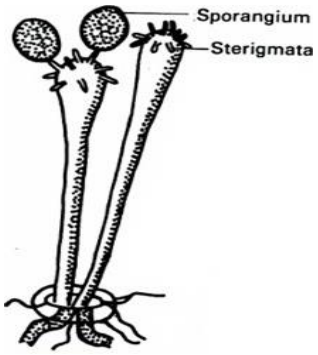
***Sclerospora* spp**




***Plasmopara* sppp**




***Bremia* spp**




***Basidiophora* spp**

**Fig.9 Sporangiophore of different Genus causing downy mildews.**

**6. Identification of Genus –** *Albugo* spp (White blister/rust)

**Mycelium –** Aseptate, coencocytic, branched, hyaline, intercellular with knob shaped haustoria.

**Sporangiophores–** Club shaped (clavate), simple, forming palisade layer below the epidermis, lateranl wall thickened and laterally free, bearing sporangia in basipetal chains.

| **Systematic Position** | |
|---|---|
| Phylum | –Oomycota |
| Class | – Oomycetes |
| Order | - Albuginales |
| Family | - Albuginaceae |

**Sporangia-** Single celled, globose and produced in chains in basipetal succession and attached with each other with a gelatinous pad known as “disjuctor”.

**Ooospores –** Rough and warty in appearance and yellow in colour.

**Important species** – *Albugo candida* (white blister / white rust of crucifers).

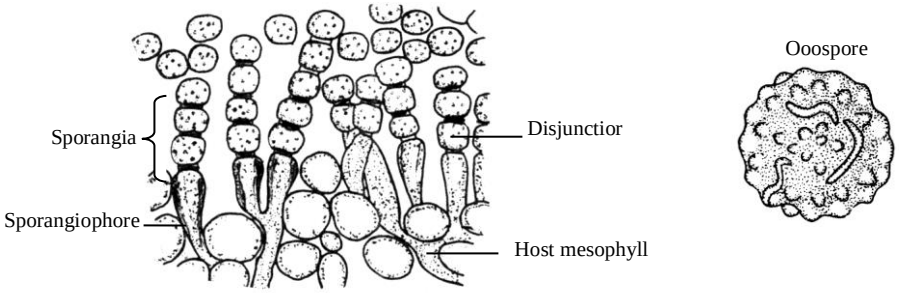



Fig. 10 Sporangiophore of *Albugo candida* producing conidia in chains

## Activity:

1. Prepare slides by taking any downy mildew infected plant sample (Mustard, cucurbits,
2. Note the observations and draw a neat and clean diagram of the characteristic features observed.

| Host | Genus | Branching of the sporangiophore | Sterigmata |
|---|---|---|---|
| | | | |
| | | | |
| | | | |
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| | | | |
| | | | |

## PRACTICAL NO. 10

## Zygomycetes: Sexual and asexual structures of *Mucor*, *Rhizopus* spp. under Phylum Zygomycota

**Objective:** Sexual and asexual structures of *Mucor, Rhizopus* spp

**1. Genus – *Mucor*** (Bread mould)

**Mycelium** : Aseptate, branched, cottony white without stolons and rhizoids.

**Sporangiophores** : Arise singly, simple, aseptate, bearing sporangia singly.

**Sporangia** : Spherical or globose, smooth walled, fragile, columellate and multi-spored

**Columella** : Central portion in the sporangium which is sterile and "Dome shaped"

**Aplanospores** : Oval or spherical in shape and single celled.

**Zygospores-** : Rough walled, black, warty in appearance and provided with "suspensors".

| **Systematic Position** | |
|---|---|
| Phylum | – Zygomycota |
| Class | – Zygomycetes |
| Order | - Mucorales |
| Family | - Mucoraceae |

**Important species:** *M. mucedo*, *M. basiliformis.*

**2. Genus – *Rhizopus*** (Bread mould)

Characters of this genus are *Mucor*- like except the formation of stolons and rhizoids, sporangiophores arise in-groups from rhizoids.

**Important species:** *R. stolonifer*

| **Systematic Position** | |
|---|---|
| Phylum | – Zygomycota |
| Class | – Zygomycetes |
| Order | - Mucorales |
| Family | - Mucoraceae |

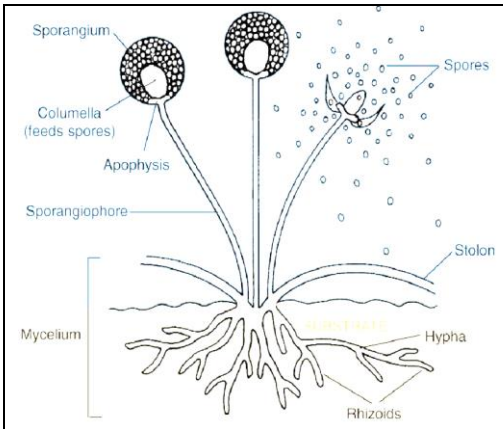


**Fig. 11.Spore bearing structure of *Rhizopus* spp**

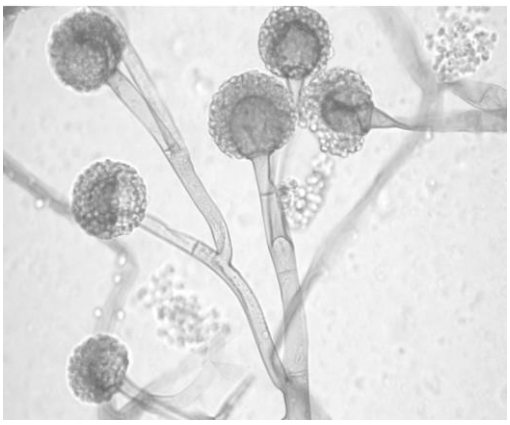

**Fig. 12.Spore bearing structure of *Mucor* spp**

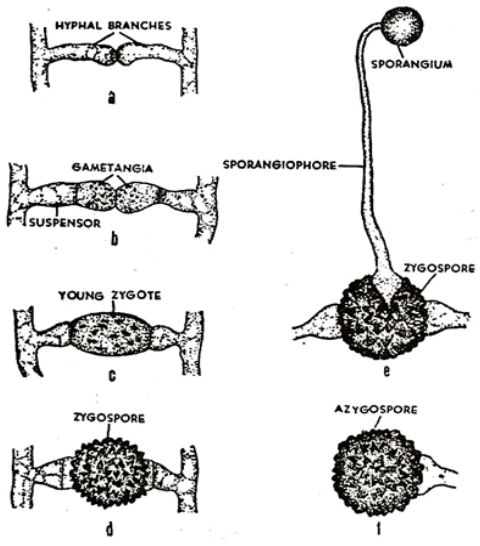

Fig. 194. *Mucor* : a-d—stages in the formation of Zygospore; e—zygospore germinating; f—an Azygospore.

**Fig.13 Gametangial copulation in Zygomycota**

**Activity:**

1. Observed *Rhizopus* and *Mucor* culture available in the Laboratory. Note down its characteristic features.

| | |
|---|---|
| | |
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| | |
| | |
| | |

2. If 2 different strains (called + and – strains) are placed together on a culture medium (or in nature), the hypha will grow towards each other and conjugation will occur. This produces a sexual spore called a zygospore—a diploid sexual spore.

A. On 10X and 40X, identify hyphae, sporangia, and sporangiospores.

B. Differentiate between the sexual zygospores and the sporangiospores on the slides.

**Conclusive questions:**

1. What are the vegetative structures present in *Rhizopus* and *Mucor*?

2. What is the form of sexual reproduction in *Rhizopus* and *Mucor*?

## PRACTICAL NO. 11

## Identification of the plant pathogens under the Phylum Basidiomycota

**Objective:** To study the structures of teliospores and sorus of different genus under the Phylum Basidiomycota

**1. Genus – *Sphacelotheca***

**Phylum-**Basidiomycota
**Class –** Teliomycetes
**Order-** Ustilaginales
**Family –** Ustilaginaceae

**Sorus** : Conical or cylindrical covered with the peridium and filled with black spore powder.

**Columella** : In the central portion of sorus, slender on curved, made up of host tissues in *S. sorghi*

**Teliospores:** Round to shortly oval, dark brown in mass but olive brown singly, smooth walled. Mass but olive brown singly, smooth walled.

Important spp. *S. sorghi* (Grain smut of Jowar), *S. cruenta* (Loose smut of jowar), *S. reiliana* (Head smut of Jowar)

**2. Genus – *Tolyposporium***

**Phylum–** Basidiomycota
**Class –** Teliomycetes
**Order-** Ustilaginales
**Family –** Tilletiaceae

**Sorus** : Though formed in various parts of the host, is more common in the ovary.

**Teliospores** : They are formed in the form of "spore balls" which are covered by member of host origin.

**Important species**: *T. penicillariae* (smut of bajra), *T. ehrenbergii* (long smut of jowar)

**3. Genus- *Tilletia***

**Phylum–** Basidiomycota
**Class –** Teliomycetes
**Order-** Ustilaginales
**Family –**Tilletiaceae

The disease caused by *Tilletia* are called as "Bunt"

**Teliospores** :Teliospores are large, 16-54 smooth, verrucose

Important species :*T. caries & T. foetida* (stinking smut or hill bunt)

**4. Genus – *Neovossia***

Grains partially or wholly converted into black powdery mass enclosed by membrane (*N.indica*).

**Teliospores:** Dark brown, spherical to oval with reticulations on the epispore which appear as curved spines.

**Important species**: *N. indica* (Karnal bunt of wheat), *N. horrida* (Bunt of rice)

**5. Genus – *Ustilago***

**Sorus:** The teliosorus without a peridium; the black dusty teliospores are covered by a membrane of host origin.

**Teliospores:** Small globose to oval or lliptical less than 20 μm in diameter in most of the species the outer wall (episopore) is minutely echinulate but sometimes smooth also (*U. hordei*).

Important species: *U. segetum tritici* (*U. tritici*)

*U. nuda* – (Loose smut of barley)

*U. maydis* (corn smut)

*U. scitaminea* (whip smut of sugar came)

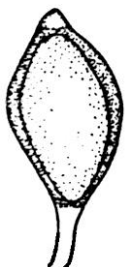
**Phylum**– Basidiomycota
**Class** – Teliomycetes
**Order**-Ustilaginales
**Family** –Ustilaginaceae

**Activity:**

1. Examine various parts of plant infected with smut and note the smut balls on the above ground plant parts. Illustrate.
2. Mount some smut spores (teliospores) in lactophenol and study under the oil immersion of objective. The teliospores of the Ustilaginaceae produce a 3-septate (four-celled) promycelium from which the basidiospores are formed laterally. The teliospores have minute spines on their surfaces. They are thick-walled, and dark in color. Note the size of the spores. Ilustrate.

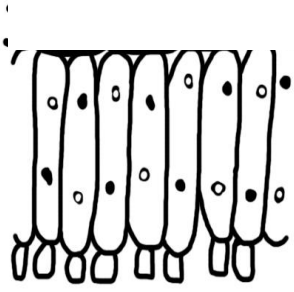
**Teliospores of Rust Fungi**

| *Uromyces* | *Puccinia* | *Melampsora* |
|---|---|---|
| **1.** Teliospores are stalked | 1. Teliospores are bicelled | **1.** Teliospores single celled, |
| **2.** They are single celled | 2. They are stalked | **2.** They are sessile and cylindrical in shape |
| **3.** Apex of teliospores is thickened | | **3.** Form layer below the epidermis |



*Uromyces*

*Puccinia*



*Melampsora*

## PRACTICAL 12

## Difference between Uredial and telial stage of rust fungi

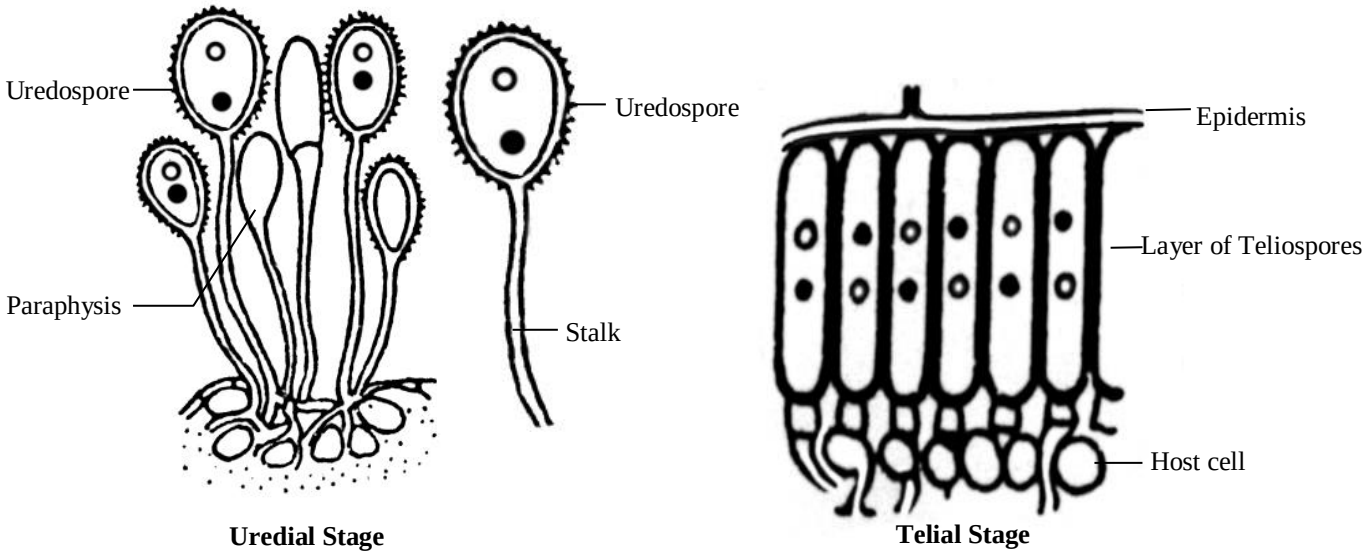
**Objective:** To observe the structural difference in Uredial and telial stage of rust fungi

**(A) Uredial Stage**

1. Epidermis ruptured Uredospore
2. Uredospores stalked
3. Uredospores finely echinulate
4. Capitate paraphyses also present

**(B) Telial Stage**

1. Epidermis intact (unbroken)
2. Teliospores sessile
3. They are single celled, cylindrical in shape
4. Teliospores form layer below epidermis




**Uredial and Telial Stages of *Melampsora***

## Activity

**Materials needed:**

**Infected plant parts:** Aboveground parts of corn plant infected with smut.

Infected wheat stems with the black stem rust.

**Fresh basidiocarp of *Agaricus* sp., *Pleurotus ostreatus***.

Media and chemicals: MEA; Calcium sulfate (gypsum); Pasteurized medium clay loam or peat / limestone mixture (calcium carbonate).

**Plant and animal materials**: Fresh Horse or chicken manure; Wheat or barley straw; Wheat bran; Grain (e.g., rye, wheat, or sorghum).

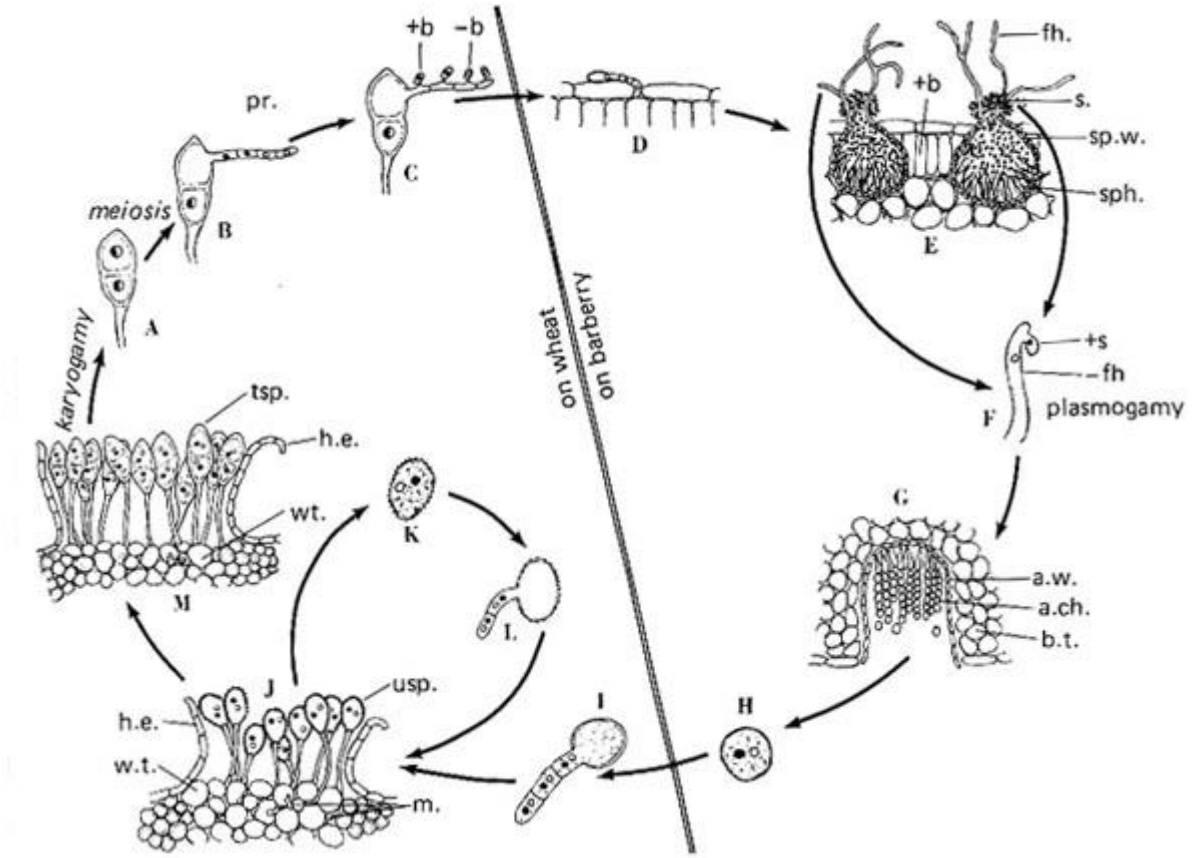
**Procedure:**

1- Under the microscope examine prepared slides of wheat leaves infected with the rust. The

Uredinia (Stage 2) can be seen on the upper epidermis.

Scrape a uredinium with a scalpel and mount some urediniospores onto a slide in lactophenol cotton blue. Study the spores under the oil immersion objective. Study a prepared slide showing cross section of a wheat stem bearing uredinia. Observe the origin of the uredinium and its effect on the host epidermis. Note the long stalks of the urediniospores. Illustrate and record your observations.

1. If wheat straw bearing the teletial stage (Telia, Stage III) of the fungus is available you could observe the black pustules from which the disease "black stem rust of wheat" takes its name. Teliospores can then be studied in a lactophenol mount under the oil immersion objective; their color, number of cells in each, and thickness of the wall could also be noted. Illustrate and record your observations.
2. Study prepared slides showing cross sections of a wheat stem bearing telia. Note how the teliospores are borne in the telia and note their position in relation to the host cells and to each other. Illustrate and record your observations.
3. Label the following diagram showing life cycle of *Puccinia graminis*




**Conclusive Questions:**

1. Explain how the rusts obtain nourishment from the host?
2. Define basidium; clamp connection; dolipore septum; teliobasidium; dikaryotic;

spermatiophore; spermogonium; autoecious rusts; heteroecious rusts.

3. How do long-cycle rusts differ from short-cycled rusts?

4. How do the rusts reproduce sexually?

5. The uredospores are considered to be the conidia of the rusts. Why?

6. What structures represent the basidia of the rusts? Explain.

## PRACTICAL NO. 13

## General characters of fungi under the class Class: Eurotiomycetes under the Phylum Ascomycota

**Objectives:** To identify the general characters of fungi under the class Class: Eurotiomycetes

**1. Genus – *Aspergillus*** (Black mould)

**Mycelium –** Well developed, branched, septate, hyaline and submerged in the substratum.

**Conidiophores–** Arise from the "**foot cell,"** aseptate, simple, terminating into vesicle.

**Sterigmata–** Two rows of the sterigmata are formed on the vesicle. sterigmata are bottle shaped.

| Systematic Position | |
|---|---|
| Phylum | –Ascomycota |
| Class | –Eurotiomycetes |
| Order | - Eurotiales |
| Family | - Trichocomaceae |

**Conidia-** Borne on secondary sterigmata in long basipetal chains. They are globose, single celled, and echinulate.

**Important species:** *A. niger*, *A. flavus*, *A. fumigatus*

**Perfect Stage:** *Eurotium*

| Systematic Position | |
|---|---|
| Phylum | –Ascomycota |
| Class | –Eurotiomycetes |
| Order | - Eurotiales |
| Family | - Trichocomaceae |

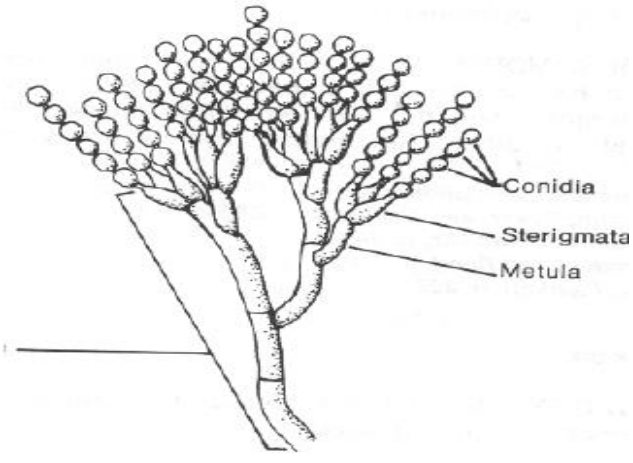



**Fig.16 Conidiophore bearing conidia of *Penicillium* spp**

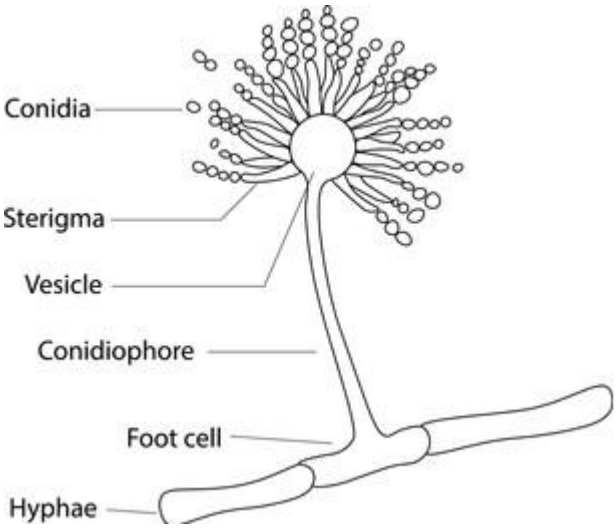



**Fig.17 Conidiophore bearing conidia of *Aspergillus* spp**

**2 Genus – *Penicillium*** (Blue / green mould)

**Mycelium –** Well developed, branched, septate, hyaline and submerged in the substratum.

**Conidiophores–** Septate and branched without forming vesicle. Foot cells absent.

**Sterigmata–** Single row of sterigmata is formed. They are peg like.

**Conidia-** Borne on sterigmata in long basipetal chains. They are, single celled, globose to ovoid, smooth walled and resemble as "glass beads".

**Important species:** *P. notatum, P. chrysogenum*

**Perfect Stage:** *Talaromyces*

**Activity 1.** Observed *Penicillium* and *Aspergillus* culture available in the Laboratory. On 10X and 40X, identify hyphae, conidia fruiting structures, and the asexual conidiospores. Draw neat and labeled diagram of the spores bearing conidiophores.

| **Point of differences** | ***Aspergillus*** | ***Penicillium*** |
|---|---|---|
| Root cell | | |
| Conidiophores | | |
| Vesicle | | |
| Sterigmata | | |
| Conidia | | |
| 6. Perfect stage | | |

**Activity 2:** Examine under the low and high power objectives prepared slides showing different types of ascocarps: cleistothecia (c.s. in ascocarp of *Erysiphe graminis*; cleistothecia, whole mount, *Uncinula sallics*), perithecia (median sec. in apple leaf infected with *Venturia inequalis*; *Xylaria* sp.), apothecia (l.s., apothecium, *Morchella* sp.), ascostroma (c.s. stroma, *Claviceps purpurea*, causal agent of ergot of Gramineae). Illustrate it.

## PRACTICAL NO. 14

## General characters of fungi under the Class: Sodariomycetes under the Phylum Ascomycota

**Objective:** To study the different features of different genera under Class: Sodariomycetes

**1. Genus – *Fusarium***

| **Systematic Position** |
|---|
| Phylum–Ascomycota |
| Class –Sordariomycetes |
| Order- Hypocreales |
| Family- Nectriaceae |

**Mycelium**: Septate, branched, pinkish brown in colour.

**Sporodochia:** Spherical, oval or ovate.

**Conidiophores:** Short, aseptate or septate, usually simple may be branched also bearing conidia singly.

**Conidia:** Micrococonidia – usually single celled or bicelled

Macroconidia – many celled (2-7), sickle shaped and knotched at the base.

Chlamydospores- formed in mycelium and macroconidia.

**Important species:** *F. oxysporum* (wilt diseases), *F. udum* (wilt of pigeonpea).

**Perfect Stage:** *Gibberella* and *Nectria.*

**2. Genus *Claviceps* (Ergot)**

| **Systematic Position** |
|---|
| **Phylum** – Ascomycota |
| **Class** –Sordariomycetes |
| **Order-** Hypocreales |
| **Family** – Clavicipitaceae |

The genus *Claviceps*, causes the important disease "Ergot"particularly of the cereals and millets.

Common species is *C. purpurea* (Ergot of rye).

**Mycelium-** Septate and branched, destroying ovary tissues and replacing it by cottony white mycelial mat forming conidiophores bearing conidia at their tips.

**Conidia** –Minute, oval and single celled forming "Honey dew" stage (Nector like secretion).

**Sclerotia** –Black, hard and variable in shape and actually the ovaries being destroyed and replaced by sclerotia.

**Perithecia-**Flask-like, ostiolate.

**Asci** – Several in a perithecium, and are elongated, cylindrical in shape.

**Ascospores**- Formed 8 in number in each ascus, which are long and thread like.

Important sp. *C. purpurae* (Ergot of rye), *C. microcephate* (Ergot of bajra)

**3. Genus – *Pyricularia***

| **Systematic Position** |
|---|
| Phylum-Ascomycota |
| Class –Sordariomycetes |
| Order- Magnaporthales |
| Family-Magnaporthaceae |

**Conidiophores:** Straight, septate (with 2-4 septa), slender and thickened at the base.

**Conidia:** Pyriform (pear shaped) to obclavate base rounded tapering at the apex, 2- septate (three celled), slightly darkened. One to many conidia may found on a single conidiophore.

**Important spp.** *P. oryzae* (blast of paddy)

**Perfect stage** – *Magnaporthe oryzae*

**4. Genus – *Colletotrichum***

**Mycelium**: Septate, light brown, branched

**Acervuli:** Cushion shaped and provided with sterile, hair like black structure setae on acervuli.

**Conidiophores:** Short, aseptate and unbranched

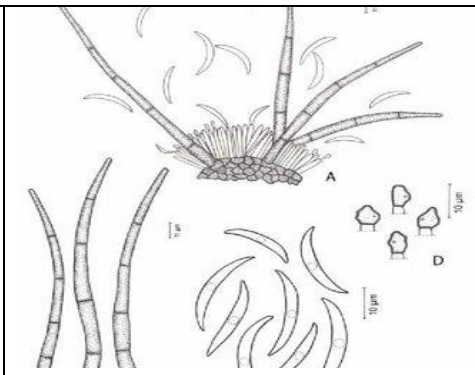
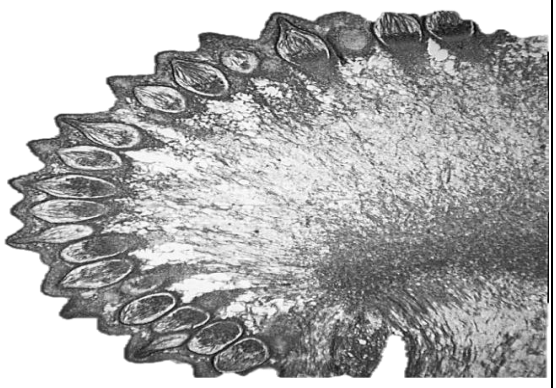
**Conidia:** Single celled, falcate, often with oil globule

**Important species-** *F. calcatum* (red rot of sugarcane), *C. truncatum* (Anthracnose of pulses)

**Perfect stage-** *Glomerella, Physalospora*

| **Systematic Position** | |
|---|---|
| Phylum | –Ascomycota |
| Class | –Sodariomycetes |
| Order | - Glomerellales |
| Family | - Glomerellaceae |

**Activity: Identify the organism based on the features provided below and write their characteristics:**

| | |
|---|---|
|  |  |
| **A** | **B** |
|  |  |
| **C** | **D** |

## PRACTICAL NO. 15

## General identification characters of fungi under the Phylum Ascomycota Class: Dothideomycetes

**Objective:** To study the different features of different genera under Class: Dothideomycetes

**1. Genus – *Helminthosporium***

**Systematic Position**
Phylum–Ascomycota
Class – Dothideomycetes
Order- Pleosporales
Family-Pleosporaceae

**Conidiophores:** Straight or zig-zag having knee joints (geniculate).

**Conidia:** Conidia are produced singly at the apex and at knee-joints of the conidiophores. They are cylindrical, multi-septate, mostly with rounded ends.

**Important spp.** *H. gramineum* (stripe disease of barley) and *H. oryzae* (brown spot of paddy).

**Perfect stage** – *Cochliobolus* and *Pyrenophora.*

**2. Genus – *Alternaria***

**Systematic Position**
Phylum–Ascomycota
Class- Dothideomycetes
Order - Pleosporales
Family- Pleosporaceae

**Conidiophores:** Septate, simple or some times branched.

**Conidia:** Conidia borne usually in chains (acropetal).

Sometimes solitary also. Conidia are provided with cross as well as longitudinal or oblique septa (muriform).

Conidia are also provided with beak, which may vary from very short to very long according to species.

**Important spp.** *A. solani* (early blight of potato), *A. brassicae* (Alternaria blight of crucifers), *A. triticina* (Leaf blight of wheat).

**Perfect stage** – *Pleospora*

**3. Genus – *Cercospora***

**Systematic Position**
Phylum–Ascomycota
Class –Dothideomycetes
Order- Capnodiales
Family- Mycosphaerellaceae

**Conidiophores:** Straight or zig-zag having knee joint (geniculate).

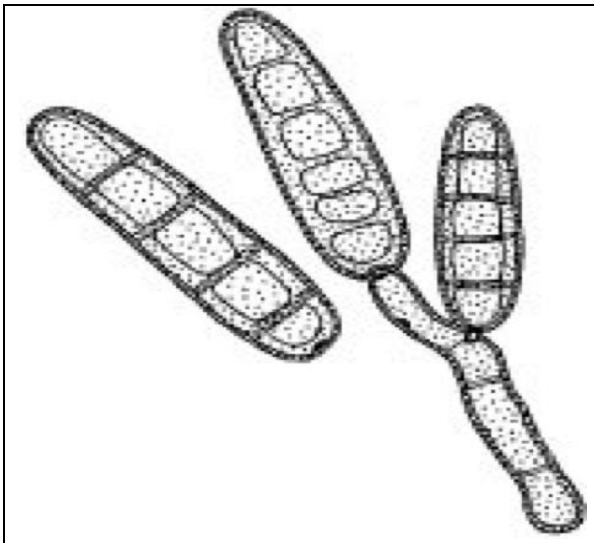
**Conidia:** Conidia are produced singly at the apex and at knee joints of the conidiophores.

They are acicular, multi-septate, tip acute and base broad.

**Important spp**. *C. personata* and *C. arachidicola*, which cause tikka disease of groundnut.

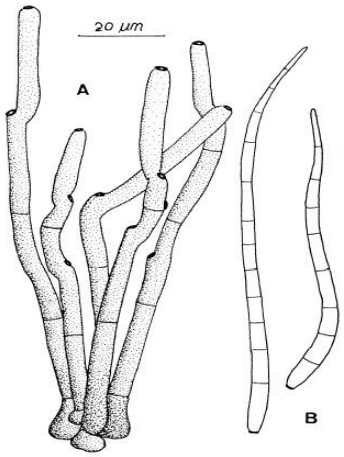
**Perfect stage** - *Mycosphaerella*

**Activity: Identify the organism based on the features provided below and write their characteristics**



**A**


**B**




**C**

## PRACTICAL NO. 16

## General identification characters of fungi under the Phylum Ascomycota Class: Leotiomycetes

**Objective:** 1. To understand the different forms of Cleistothecia in different powdery mildew genera

2.To study the different identification features of *Sclerotinia*

**I. Identification of powdery mildew genera, Class: Leotiomycetes based on Cleistothecia**

**1. Mycelium internal and Cleistothecium with several asci**

i. Appendages with bulbous base: *Phyllactinia*, Anamorph: *Ovulariopsis*

ii. Appendages hypha-like: *Leveillula*, Anamorph: *Oidiopsis*

**2. Mycelium superficial and Cleistothecium with several asci**

i. Appendages hypha-like*: Erysiphe*

ii. Appendages dichotomously-branched: *Microsphaera*

iii. Appendages with coiled tips: *Uncinula*

iv. Anamorph: *Oidium*

v. Appendages branched, hypha-like *Golvinomyces*

**3. Cleistothecium with single ascus**

i. Appendages dichotomously branched tip: *Podosphaera*

ii. Hypha-like: *Sphaerotheca*

The diagram is indicated in fig. no.18

**II. Genus – *Sclerotinia***

**Systematic Position**

**Phylum –** Ascomycota

**Class –** Leotiomycetes

**Order-** Helotiales

**Family –** Sclerotiniaceae

**Mycelium** Septate and branched mostly white in colour.

**Conidiophores** long septate land branched.

**Conidia-** oval or lemon shaped, single celled and formed in chains.

**Sclerotia** Black, hard, variable in shape

**Apothecia** Long and stalked cup or disc shaped.

**Asci** Clavate, slightly thickened at apex, with paraphyses.

**Ascospores** 8 in number in each ascus, single celled round, or elliptical or elongated.

**Important sp.** *S. sclerotiorum* causing root rot and white rot disease.

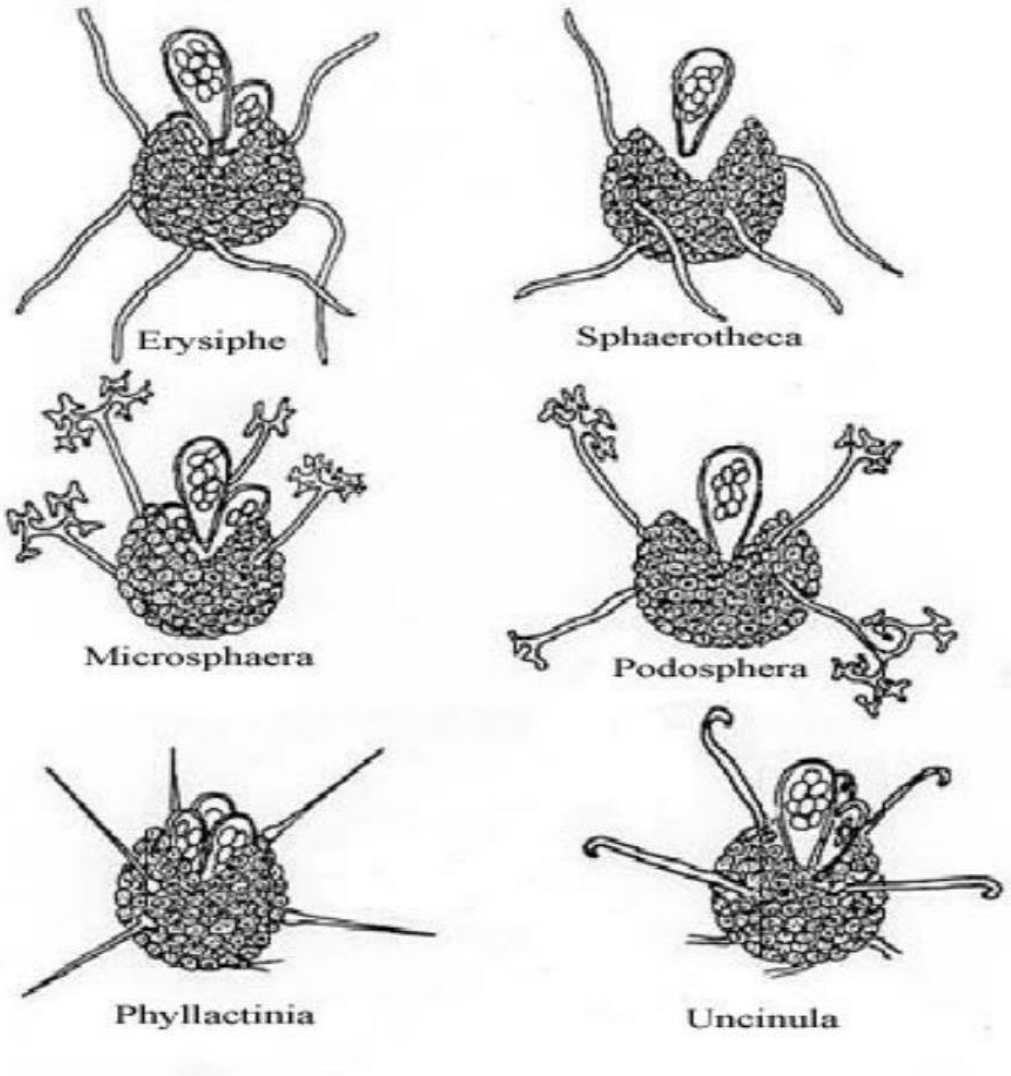



**Fig. 18 Different features of Cleistothecia produced by powdery mildew fungi**

**Activity: 1.** Collect the powdery mildew disease sample from the crop cafeteria and prepare slides and observe under the microscope. Note down the following characteristics and identify the genera based on morphological observation. Draw a neat and label diagram of the features observed.

| | A | B | C |
|---|---|---|---|
| **Mycelium** | | | |
| **Asexual stage** | | | |
| **Conidiophores** | | | |
| **Conidia** | | | |
| **Sexual stage** | | | |
| **Cleistothecia** | | | |
| **Appendages** | | | |
| **Asci** | | | |
| **Ascospores** | | | |
| **Host** | | | |
| **Genus** | | | |

**2.** Examine permanent preparations of l.s. through the head of a sclerotium under low magnification and draw a general view, and then under higher magnification draw a section showing a part of a perithecium. The surface of the stroma head is covered with sunken perithecia (ascostroma). The inoperculate asci have a typical cap at their apex that is penetrated by a pore 2- Study the preserved specimen of the sclerotia available. Illustrate and record your observations.

## PRACTICAL NO. 17

## General identification characters of fungi under the Phylum Ascomycota Class Taphrinomycetes

**Objective:** To understand the identifying characters of the genus *Taphrina* spp. and its life cycle

| Systematic Position |
|---|
| **Phylum –** Ascomycota |
| **Class –**Taphrinomycetes |
| **Order-** Taphrinales |
| **Family –** Taphrinaceae |

**1. Genus *Taphrina* (Leaf curl fungus)**

Mainly the spp. of this genus cause the disease symptoms as leaf curl, puckering, pockets and witches broom. The most important species is *T. deformans*, the cause of "Peach leaf curl".

**Mycelium –**Composed of septate hyphae, consisting of typically binucleate cells. There hyphae may be intercellular on sub-cuticular or may grow within the walls for the epidermal cells.

**Asci-** Naked, (without forming any fruiting body (ascocarp), forming the layer of naked (Hymenium on the epidermis of the host, and each ascus harving 8 ascospores).

**Ascospores-** Eight in number, mostly located at upper portion of asci, single celled, round or ovoid.

Important and species. *T. deformans* (Peach leaf curl)

*T. pruni* (plum pocket)

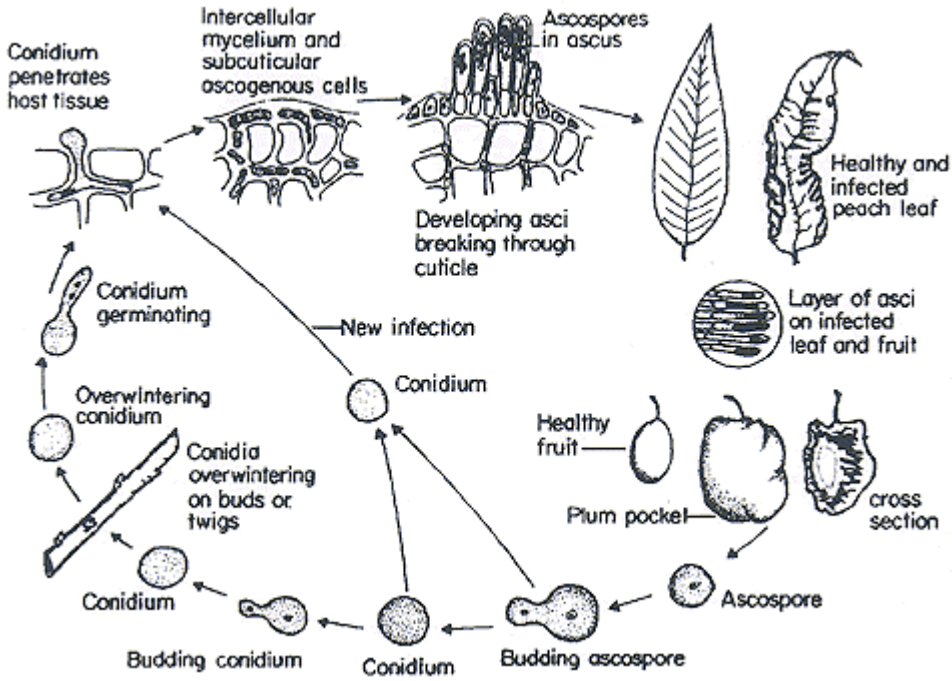



**Fig. 19 Life cycle of *Taphrina pruni***

**Conclusive Questions:**

1. How does the binucleate condition arise in *Taphrina deformans*?

2. How does *Taphrina deformans* reproduce asexually?

3. How does *Taphrina deformans* overwinter?

4. Collect the sample of plant infected with Sclerotinia rot from the crop cafeteria and prepare slides and observe under the microscope. Note down the important observations

## PRACTICAL 18

## Application of molecular approaches and techniques for identification of fungal pathogens.

**Objective: To identify fungal isolates through molecular characterization**

**Procedure**

1. Set water bath at 60° C.
2. Preheat the extraction buffer to 60°C followed by addition of mycelial mate into be pre-heated buffer, mixProperly at 10 min interval by moving the tubes.
3. Cool the samples to room temperature.
4. Add equal vol. of PCI(Phenol: chloroform: Isoamylalcohal) (25:24:1) to the sample.
5. Centrifuge at 12,500rpm for 20min at 25° C.
6. Spell out the supper aqueous phase and repeat the step no-4.
7. Add Isoamyl alcohol (ice cooled) in to the sample and incubate for Ihr at -20°C.
8. Precipitate the DNA by centrifuge at 5°C at 10000 rpm for 10 min
9. Add wash buffer followed by ethanol to the sample.
10. Allow ethanol to evaporate.
11. Dissolve DNA pellet in Nucleus free water & TE buffet.
12. Store at 4°C until use.

**Basic PCR Protocol**

1. Place a 96 well plate into the ice bucket as a holder for the 0.2 ml thin walled PCR tubes. Allowing PCR reagents to be added into cold 0.2 ml thin walled PCR tubes will help prevent nuclease activity and nonspecific priming.
2. Pipette the following PCR reagents in the following order into a 0.2 ml thin walled PCR tube: Sterile Water, 10X PCR buffer, dNTPs, $MgCl_2$, primers, and template DNA . Since experiments should have at least a negative control, and possibly a positive control, it is beneficial to set up a Master Mix in a 1.8 ml microcentrifuge tube (See explanation in Notes).
3. In a separate 0.2 ml thin walled PCR tubes add all the reagents with the exception of template DNA for a negative control (increase the water to compensate for the missing volume). In addition, another reaction (if reagents are available) should contain a positive control using template DNA and or primers previously known to amplify under the same conditions as the experimental PCR tubes.

4. *Taq* DNA polymerase is typically stored in a 50% glycerol solution and for complete dispersal in the reaction mix requires gentle mixing of the PCR reagents by pipetting up and down at least 20 times. The micropipettor should be set to about half the reaction volume of the master mix when mixing, and care should be taken to avoid introducing bubbles.
5. Put caps on the 0.2 ml thin walled PCR tubes and place them into the thermal cycler . Once the lid to the thermal cycler is firmly closed start the program.
6. When the program has finished, the 0.2 ml thin walled PCR tubes may be removed and stored at 4 °C. PCR products can be detected by loading aliquots of each reaction into wells of an agarose gel then staining DNA that has migrated into the gel following electrophoresis with ethidium bromide. If a PCR product is present, the ethidium bromide will intercalate between the bases of the DNA strands, allowing bands to be visualized with a UV illuminator.

**Activity:** Extract DNA and quantity it. Check its quality on nanodrop. Send the sample for sequencing. Get the sequence and prove the identity of the pathogen