PRACTICAL MANUAL

on

MYCOLOGY

Course No. PPA 501 Credit Hrs. 3(2+1)

M.Sc. (Ag.) Plant Pathology

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Syllabus: Mycology

Practical: The study covers fungal taxonomy, morphology, and identification techniques, with a focus on the classification of fungi, including Saccardoan and conidiogenesis-based systems. It also examines the vegetative structures and fruiting bodies of slime molds, stramenopiles, and true fungi.

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Practical No. 1

Objective: Detailed comparative study of different groups of fungi

Activity: Elaborate comparative differences in different groups of plant pathogenic fungi

Phylum: Chytridiomycota	……
Phylum: Ascomycota	……
Phylum: Basidiomycota	……
Phylum: Oomycota	……
Phylum: Plasmodiophoromycota	……

Practical No. 2

Objective: Saccardoan classification and classification based on conidiogenesis

Activity: Draw the conidia of each species, using the terms that best describe them. Be sure to label each drawing with the name of the corresponding species.

Hyaloamerosporae: ……..… ……..… ……..… ……..… ……..… ……..… ……..…	
Phaeoamerosporae: ……..… ……..… ……..… ……..… ……..… ……..…	
Hyalodidymosporae: ……..… ……..… ……..… ……..… ……..… ……..…	
Phaeodidymosporae: ……..… ……..… ……..… ……..… ……..… ……..…	
Hyalophragmosporae: ……..… ……..… ……..… ……..… ……..… ……..…	

Phaeophragmosporae: ……..... …… …… …… …… ……	
Hyalodictyosporae: ……..… …… …… …… …… ……	
Phaeodictyosporae: ……..… …… …… …… …… ……	
Hyalo or Phaeoscolecosporae: …… …… …… …… …… ……	
Hyalo or Phaeohelicosporae: ……..… …… …… …… …… ……	
Hyalo or Phaeostaurosporae: ……..… …… …… …… …… ……	

Practical No. 3

Objective: Vegetative structures and different types of fruiting bodies produced by slime molds, stramenopiles and true fungi

Activity: Study the different types of mycelium, spores, and fruiting bodies.

1. Write about and draw the types of mycelium

…… …… …… …… ……	
…… …… …… …… ……	
…… …… …… …… ……	
…… …… …… …… ……	

2. Write about and draw different types of asexual spores:

…… …… …… …… …… ……	
…… …… …… ……	

…… ……	
…… …… …… …… …… ……	
…… …… …… …… …… ……	

3. Write about and draw different types of sexual spores:

…… …… …… …… …… ……	
…… …… …… …… …… ……	
…… …… …… …… …… ……	
…… …… …… ……	

Practical No. 4

Objective: Myxomycotina: Fructification, plasmodiocarp, sporangia, plasmodium and aethalia

Activity: Write about and draw structures of plasmodiocarp, sporangia, plasmodium and aethalia

…… …… …… …… …… ……	
…… …… …… …… …… ……	
…… …… …… …… …… …… ……	
…… …… …… …… …… …… ……	
…… …… …… …… …… …… ……	

Practical No. 5

Objective: Oomycota; somatic and reproductory structures of Pythium, Phytophthora, downy mildews and *Albugo*

Activity: Observe the slides, state the systematic position of the fungal genera, and draw and record their features while describing the given genera.

Genus: *Pythium*

Systematic position: …… …… …… …… …… …… …… …… …… …… ……	**Diagram**

Characteristics: ……
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Genus: *Phytophthora*

Systematic position: …… …… …… …… …… …… …… …… …… …… ……	**Diagram**

Characteristics: ……

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Record the characteristic differences in morphology of *Pythium* and *Phytophthora*

Characteristics	***Pythium* spp**	***Phytophthora* spp**
Mycelium		
Sporangiophores		
Sporangia		
Oospores		
Haustoria		
Vesicle		
Zoospore formation		

State the systematic position of the Genera given in the space below. Record the characteristic morphology of Genus – *Peronospora* (Downy mildew), *Sclerospora* and draw a neat and labeled

diagram of the spores along with conidiophores.

Systematic position: …….. …… …….. …… …… …….. …… …….. …… ……	**Systematic position:** …….. …… …….. …… …… …….. …… …….. …… ……

Characteristics	***Sclerospora***	***Peronospora***
Mycelium		
Conidia		
Branching		
Sterigmata		
Oospores		
Conidiophores		

Record the characteristic morphology of *Albugo candida* (White blister/rust) and draw a neat and labeled diagram of spores

Systematic position: …….. …… …… …… …… …… …… …… …… …… ……	**Diagram**

Characteristics	***Sclerospora***	***Peronospora***
Mycelium		
Sporangiophores		
Sporangia		
Oospores		

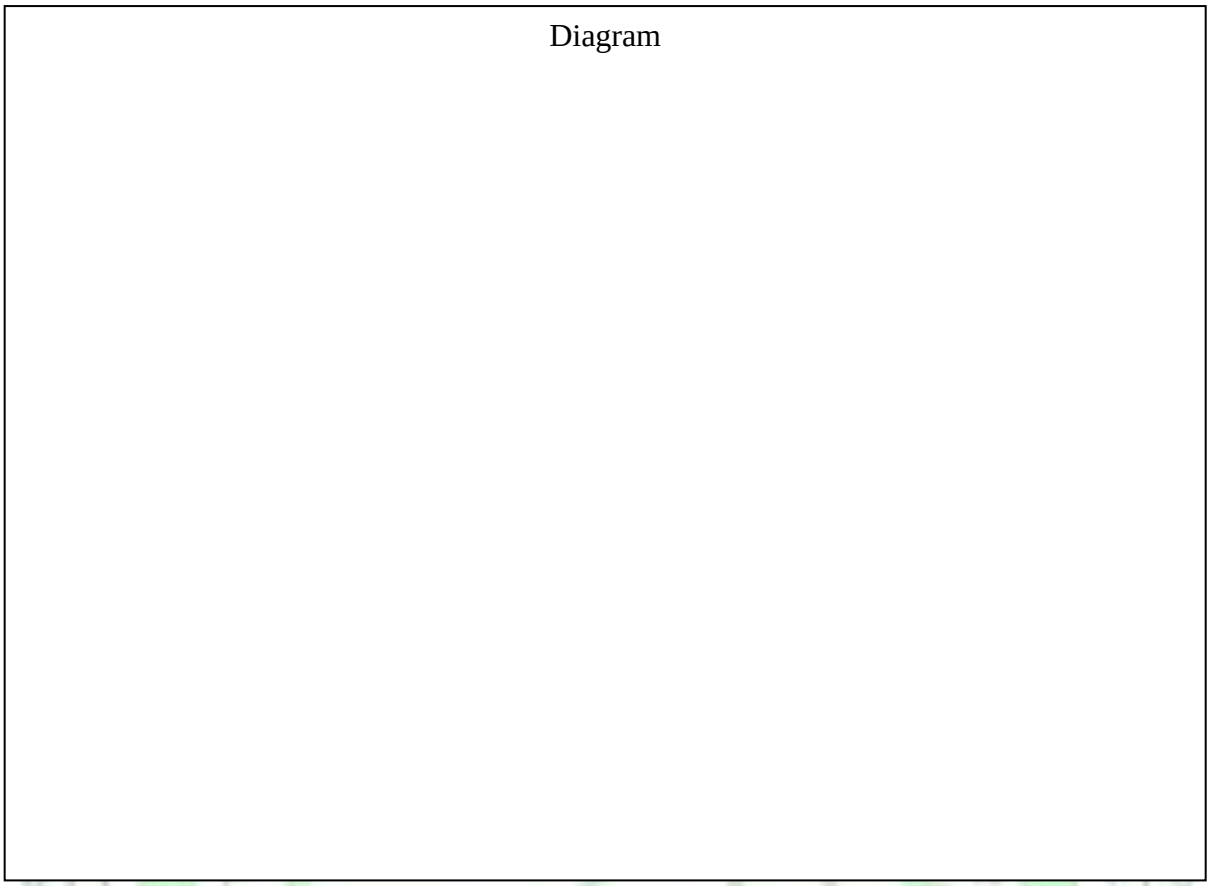
Practical No. 6

Objective: Zygomycota: Sexual and asexual structures of Mucor and Rhizopus

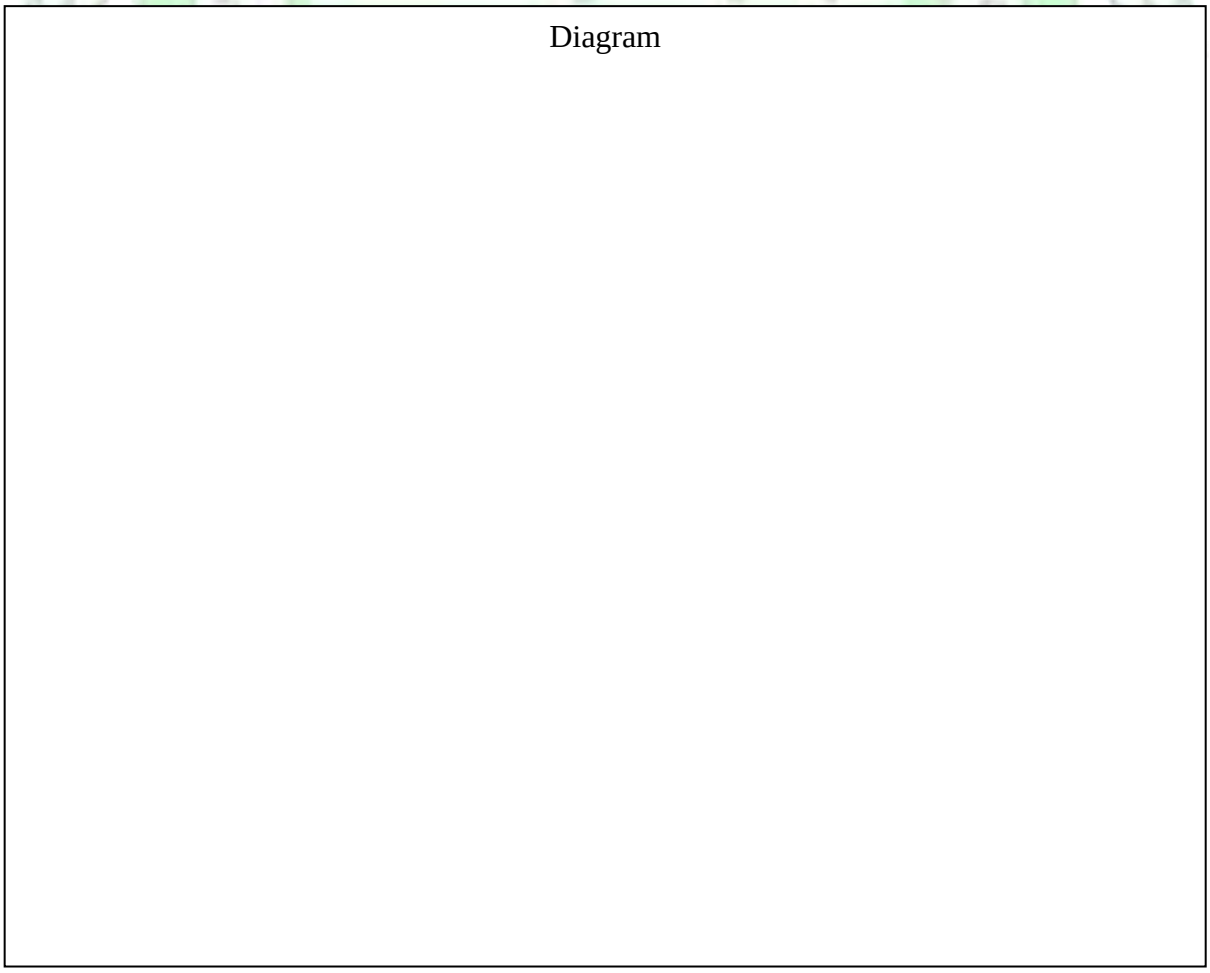
Activity: Record the characteristic morphology of Genus – *Mucor* (Bread mould) and *Rhizopus* and draw a neat and labeled diagram of their spores.

Systematic position: …….. …… …….. …… …… …….. …… …….. …… ……	**Systematic position:** …….. …… …….. …… …… …….. …… …….. …… ……

Characteristics	***Mucor***	***Rhizopus***
Mycelium		
Sporangiophores		
Sporangia		
Columella		
Aplanospores		
Zygospores		



Diagram



Diagram

Practical No. 7

Objective: Ascomycetes: fruiting structures, Erysiphales, and Eurotiales

Order: Eurotiales

Systematic position: …….. …… …….. …… …… …….. …… …….. …… ……	**Systematic position:** …….. …… …….. …… …… …….. …… …….. …… ……

Characteristics	*Aspergillus*	*Penicillium*
Mycelium		
Foot Cell		
Conidiophore		
Vesicle		
Sterigmata		
Conidia		
Perfect Stage		

Diagram	Diagram

Order: Erysiphales

Draw different forms of Cleistothecia in different powdery mildew genera

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			

Practical No. 8

Objective: General identification characters of Pyrenomycetes, Discomycetes, Loculoascomycetes and Laboulbeniomycetes

Activity: Record the identification characters of Pyrenomycetes, Discomycetes, Loculoascomycetes and Laboulbeniomycetes and draw a neat and labeled diagram of their sexual fruiting bodies

1. Pyrenomycetes

General characters: …… …… …… …… …… …… …… …… …… …… ……	**Diagram**
Enlist plant pathogenic fungi belonging to the class Pyrenomycetes: …… …… ……	

2. Discomycetes

General characters: …… …… …… …… …… …… …… …… …… …… ……	**Diagram**
Enlist plant pathogenic fungi belonging to the class Discomycetes: …… …… ……	

3. Loculoascomycetes

General characters: ……	**Diagram**
…… …… …… …… …… …… …… …… …… ……	
Enlist plant pathogenic fungi belonging to the class Loculoascomycetes: …… …… ……	

4. Laboulbeniomycetes

General characters: ……	**Diagram**
…… …… …… …… …… …… …… …… …… ……	
Enlist plant pathogenic fungi belonging to the class Loculoascomycetes: …… …… ……	

Practical No. 9

Objective: **Basidiomycetes; characters, ultrastructures and life cycle patterns in Ustilaginomycetes and Teliomycetes**

Activity: Record characteristic morphology of the following Genera and draw a neat and labeled diagram of spores.

1. Genus: *Uromyces*

General characters: ……	**Diagram**
…… …… …… …… …… …… …… …… …… …… ……	

2. Genus: *Melampsora*

General characters: ……	**Diagram**
…… …… …… …… …… …… …… …… …… …… ……	

3. Genus: *Ustilago*

General characters: ……	**Diagram**
…… …… ……	

…… …… …… …… …… …… …… ……	

4. Genus: *Tilletia*

General characters: ……	**Diagram**
…… …… …… …… …… …… …… …… …… …… ……	

5. Genus: *Puccinia*

General characters: ……	**Diagram**
…… …… …… …… …… …… …… …… …… …… …… …… ……	

Draw life cycle of *Puccinia*

Draw life cycle of *Ustilago*

Practical No. 10

Objective: Deuteromycetes: characters of Hyphomycetes

Activity: Record characteristic morphology of Hyphomycetes and draw the disgram of asexual fruiting structures and spores formed by important fungi

Hyphomycetes

General characters: ……	**Diagram**
…… ……	

Enlist plant pathogenic fungi belonging to the class Hyphomycetes: ……

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Practical No. 11

Objective: Deuteromycetes: characters of Coelomycetes

Activity: Record characteristic morphology of Coelomycetes and draw the disgram of asexual fruiting structures and spores formed by important fungi

Coelomycetes:

General characters: ……	**Diagram**
…… ……	

Enlist plant pathogenic fungi belonging to the class Coelomycetes: ……

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Practical No. 12

Objective: Collection of diseased live samples

Activity: Collect diseased samples, study symptoms and observe infected parts under microscope

Materials required: ……

……

Details of collected diseased samples

Disease name	Host	Causal organism	Fungal structures observed under microscope

Practical No. 13

Objectives: Preservation of diseased plant samples

Activity: Collect disease samples from the University research fields and prepare a herbarium with all necessary details provided below.

a. Host (name of the diseased plant): .....

b. Name of the pathogen (organism causing the disease): .....

c. Place where collected: .....

d. Date of collection: .....

e. Name of the collector: .....

Materials required: .....

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Procedure for dry preservation: .....

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

Materials required: .....

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Procedure for dry preservation: .....

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Practical No. 14

Objective: Staining and slide preparation

Materials required: ……

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Procedure: ……

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Preparation of Fungal Stain: ……

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Use of Stain: ……

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Precautionary Measures: ……

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Practical No. 15

Objective: Preparation of Potato Dextrose Agar medium

Activity: Prepare one litres of Potato dextrose Agar medium. Describe procedure and quantity of the components.

Materials required: .....

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Procedure: .....

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Practical No. 16

Objective: Isolation and purification of pathogen

Activity: Isolate and purify plant pathogens from diseased plant tissues

Materials required: ……

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Procedure for isolation – Flowchart

Practical No. 17

Objective: Demonstration of Koch's Postulates

Activity: Inoculate the host plant with the given plant pathogen sample and re-isolate it.

Materials required: ……

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Procedure for inoculation: ……

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Procedure for re-isolation: ……

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Practical No. 18

Objective: Application of molecular approaches and techniques for identification of fungal pathogens.

Activity: To identify fungal isolates through molecular characterization

Write the procedure of fungal DNA isolation: ……

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Write the basic steps of Polymerase Chain Reaction (PCR): ……

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CLASSIFICATION OF FUNGI

This 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 reflect 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 outlined by Hibbett *et al*. (2007). Currently, the true fungi, which form a monophyletic clade known as the kingdom *Fungi*, consist of 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)		

Classification of Kingdom Fungi

Kingdom: Fungi

Phylum: *Chytridiomycota*
- Water molds living as aquatic saprotrophs or parasites.
- Produce motile asexual zoospores; sexual reproduction with zygotic meiosis.
- Classes: *Chytridiomycetes* (Order: *Chytridiales*) and *Monoblepharidomycetes* (Order: *Monoblepharidales*; genus *Monoblepharis*)

Phylum: *Neocallimastigomycota*
- Anaerobic fungi in herbivorous mammals; lack mitochondria, contain hydrogenosomes.
- Class: *Neocallimastigomycetes* (Order: *Neocallimastigales*; genus *Neocallimastix*)

Phylum: *Blastocladiomycota*
- Similar to chytrids, characterized by sporic meiosis.
- Class: *Blastocladiomycetes* (Order: *Blastocladiales*)

Phylum: *Microsporidia*
- Unicellular parasites with highly reduced mitochondria, potentially a sister group to fungi.

Phylum: *Glomeromycota*
- Arbuscular mycorrhizal fungi, forming mutualistic symbioses.
- Class: *Glomeromycetes*
  - Orders: *Archaeosporales* (genus *Archaeospora*), *Diversisporales* (genus *Acaulospora*), *Glomerales* (genus *Glomus*), *Paraglomerales* (genus *Paraglomus*)

Phylum: *Ascomycota*
- Largest group, ranging from saprotrophs to pathogens, characterized by ascospores in an ascus.
- Subphyla:
  - *Taphrinomycotina* (Class: *Taphrinomycetes*, *Neolectomycetes*, *Pneumocystidomycetes*, *Schizosaccharomycetes*)
  - *Saccharomycotina* (Class: *Saccharomycetes*)
  - *Pezizomycotina* (Classes: *Arthoniomycetes*, *Dothideomycetes*, *Eurotiomycetes*, *Pezizomycetes*, *Lichinomycetes*, *Leotiomycetes*, *Lecanoromycetes*, *Laboulbeniomycetes*, *Sordariomycetes*)

Phylum: *Basidiomycota*
- Saprotrophic or parasitic fungi; filamentous with septate hyphae.
- Classes:
  - *Pucciniomycetes* (Orders: *Septobasidiales*, *Pucciniales*)
  - *Cystobasidiomycetes* (Orders: *Cystobasidiales*, *Erythrobasidiales*)
  - *Agaricostilbomycetes* (Orders: *Agaricostilbales*, *Spiculogloeales*)
  - *Microbotryomycetes*, *Atractiellomycetes*, *Classiculomycetes*, *Mixiomycetes*, *Cryptomycocolacomycetes*
  - Subphyla: *Ustilaginomycotina* (*Ustilaginomycetes*), *Agaricomycotina* (*Tremellomycetes*, *Agaricomycetes*), *Phallomycetidae*

Kingdom: *Chromista*
- **Phylum**: *Hyphochytriomycota* (Order: *Hyphochytriales*)
- **Phylum**: *Oomycota* (Orders: *Leptomitales*, *Peronosporales*, *Pythiales*, *Saprolegniales*)

Kingdom: *Protozoa*

- **Phylum**: *Plasmodiophoromycota* (Class: *Plasmodiophoromycetes*)
- **Phylum**: *Myxomycota* (Classes: *Dictyosteliomycetes*, *Myxomycetes*, *Protosteliomycetes*)
- **Phylum**: *Acrasiomycota* (Class: *Acrasiomycetes*)

Phylum: *Choanozoa* (Class: *Mesomycetozoea*)

SACCARDOAN CLASSIFICATION

Some fungi produce only asexual propagules and have not been observed in a sexual state. These fungi were traditionally classified as *Fungi Imperfecti* or *Deuteromycetes*. However, with advancements in molecular sequencing, many of these fungi are now being integrated into classification systems based on their sexual reproductive structures.

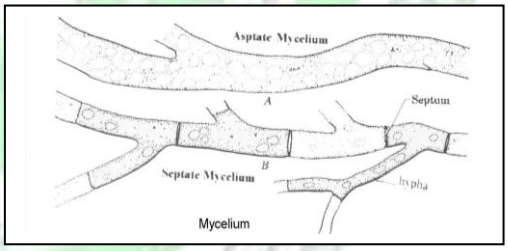
Despite lacking a sexual state, mitosporic fungi play significant ecological, medical, and industrial roles. Accurate identification and naming of these fungi remain essential. Historically, two classification approaches have been used: one based on the overall morphology of the conidia and conidiomata, and the other on conidial development. Modern identification manuals now combine both approaches for more precise categorization.

The Saccardoan System, developed in 1886, classified *Fungi Imperfecti* primarily based on spore characteristics such as pigmentation, septation, and form. These attributes were used in various combinations to classify species within the group.

- Hyaloamerosporae – Hyaline or brightly colored, single-celled conidia.
- Phaeoamerosporae – Dark-pigmented, single-celled conidia.
- Hyalodidymosporae – Hyaline or brightly colored, two-celled conidia.
- Phaeodidymosporae – Dark-pigmented, two-celled conidia.
- Hyalophragmosporae – Hyaline or brightly colored, two or more septate conidia.
- Phaeophragmosporae – Dark-pigmented, two or more septate conidia.
- Hyalodictyosporae – Hyaline or brightly colored, transversely and longitudinally septate conidia.
- Phaeodictyosporae – Dark-pigmented, transversely and longitudinally septate conidia.
- Hyalo/Phaeoscolecosporae – Hyaline, brightly colored, or dark-pigmented, long, curved, often sigmoidal conidia.
- Hyalo/Phaeohelicosporae – Hyaline, brightly colored, or dark-pigmented, coiled conidia.
- Hyalo/Phaeostaurosporae – Hyaline, brightly colored, or dark-pigmented, star-shaped conidia (with arms radiating from a central point)

VEGETATIVE STRUCTURES AND TYPES OF FRUITING BODIES IN FUNGI

Mycelium: The network of hyphae is referred to as the *mycelium*. It can be either *aseptate* or *septate*.

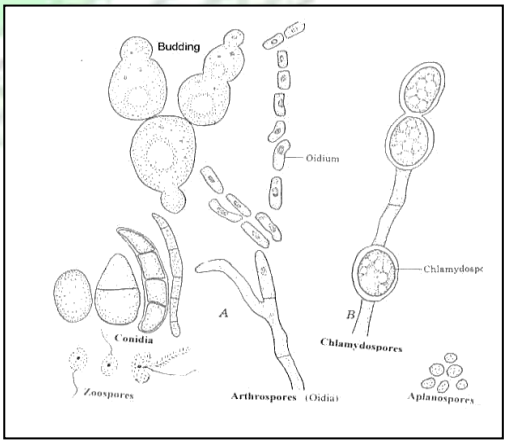


Aseptate Mycelium: When the hyphae lack cross-walls (septa), it is called aseptate mycelium. This type is commonly found in lower fungi.

Septate Mycelium: When the mycelium is divided into compartments by cross walls (septa) at intervals, it is referred to as septate mycelium. Each septum has a small pore, known as a "septal pore," allowing cytoplasmic flow. This type is characteristic of higher fungi.

Different Types of Asexual Spores

1. **Arthrospores (Oidia)**: These spores are barrel- or drum-shaped, single-celled, and form in chains (basipetal) on short conidiophores.
2. **Chlamydospores**: Single or chain-forming spores that can be terminal or intercalary and are encased in a protective envelope.
3. **Blastospores**: Single-celled spores formed by budding. Initially produced in chains, they later separate from each other.
4. **Conidia**: These spores develop at the tips or sides of hyphae (conidiophores). They can be solitary or in chains and vary widely in shape, size, septation, color, and ornamentation.
5. **Zoospores**: Motile (flagellated), pear- or kidney-shaped spores produced in sporangia (zoosporangia). These are naked and flagellated.
6. **Aplanospores**: Non-motile, oval or spherical spores produced in elliptical sporangia. These spores lack flagella.



Asexual Fruiting Bodies

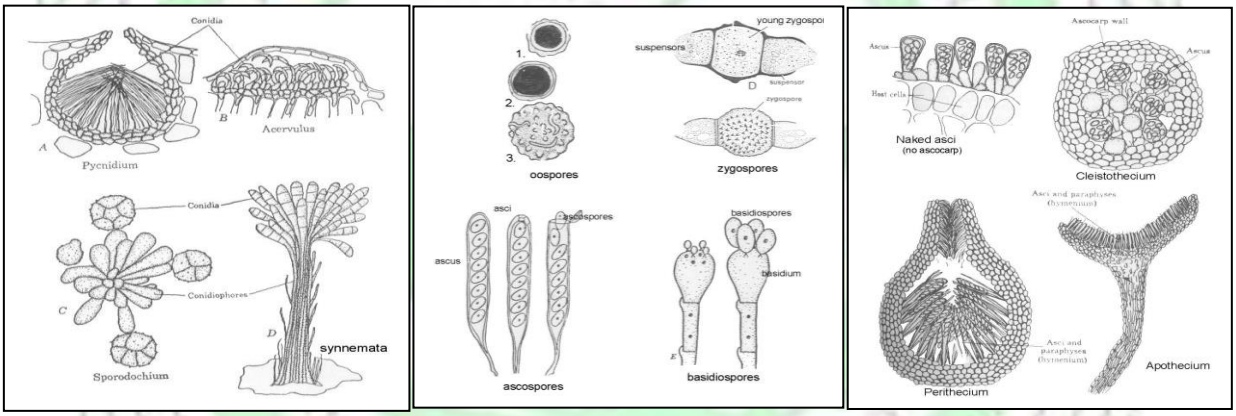
1. **Pycnidia**: Spherical or flask-shaped structures containing conidia, with a natural opening called an *ostiole* through which the spores are released. Commonly found in the order Sphaeropsidales (subdivision Deuteromycotina).
2. **Acervuli**: Cushion-like structures formed beneath the host's cuticle or epidermis, sometimes featuring hair-like sterile structures called *setae*.
3. **Sporodochia**: Cushion-shaped fruiting bodies that bear conidiophores on their surface.
4. **Synnemata**: Conidiophores grouped at the base but free towards the apex, forming a structure resembling a stalk.

Different Types of Sexual Spores

1. **Oospores**: Typically spherical and smooth-walled, these spores are formed in the *oogonium* through gametangial contact (*oogamy*).
2. **Zygospores**: Rough-walled and black, zygospores are formed by gametangial fusion (*zygotamy*). They are characteristic of the subdivision Zygomycotina.
3. **Ascospores**: Produced in groups of eight within *asci*, ascospores result from spermatization or somatogamy, typical of the subdivision Ascomycotina.
4. **Basidiospores**: Formed in groups of four on a *basidium*, these spores are produced through spermatization or somatogamy and are characteristic of the subdivision Basidiomycotina.

Different Types of Ascocarps: There are three main types of ascocarps (fruiting bodies) in Ascomycota fungi:

1. **Cleistothecia**: These spherical, black, and hard fruiting bodies lack natural openings. Asci are released when the cleistothecium ruptures. They often have appendages.
2. **Perithecia**: Flask-shaped fruiting bodies with a natural opening called an *ostiole* and sometimes a long neck. Asci are formed at the base of the perithecium, often accompanied by sterile structures called *paraphyses*.
3. **Apothecia**: These open, cup- or disc-shaped ascocarps have exposed asci arranged in a *hymenium* layer. *Paraphyses* may also be present among the asci.



Myxomycota: General Characteristics and Classification

General Characteristics:

1. **Lack of Cell Wall**: Myxomycota do not possess a cell wall.
2. **Swarm Cells**: These cells feature two unequal anterior whiplash flagella.

Classes of Myxomycota:

1. **Class Myxomycetes**: Characterized by a free-living plasmodium.
2. **Class Plasmodiophoromycetes**: Defined by endoparasitic plasmodium.

Historically, slime molds were classified as animals and termed *Mycotozoa* due to their vegetative phase resembling a plasmodium. They exhibit a free-living, acellular, multinucleate somatic plasmodium and produce flagellated swarm cells within a sporophore, which typically develops a peridium that encloses the spores.

Plasmodium: The plasmodium is a mass of protoplasm enclosed by a thin plasma membrane and a gelatinous sheath. It lacks a fixed size or shape and exhibits fluid and gelatinous regions. The fluid portion of the protoplast forms a branched network that streams through the gelatinous portions.

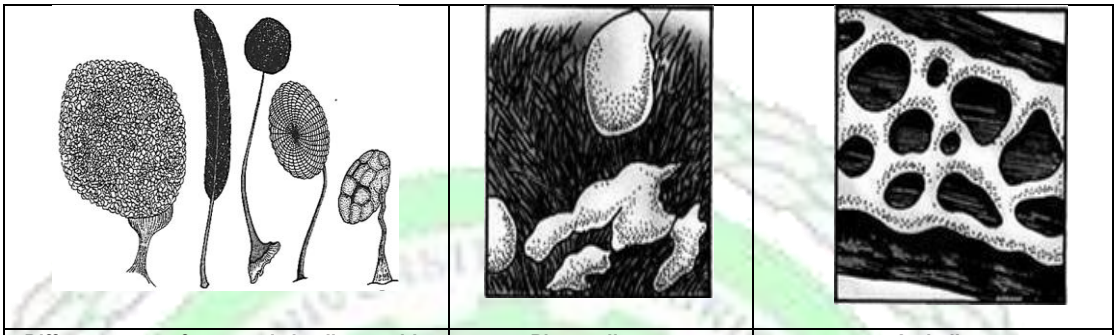
Types of Plasmodia:

1. **Sporangiogenous Plasmodium**: Formed asexually, it contains thin-walled zoosporangia that produce one or more secondary or sporangial zoospores.
2. **Cystogenous Plasmodium**: Formed sexually, it consists of thick-walled cysts, each producing a single primary zoospore

(cyst zoospore). Cysts may be free or fused and function as resting spores.

Types of Sporophores (Reproductive Organs) in Class Myxomycetes:

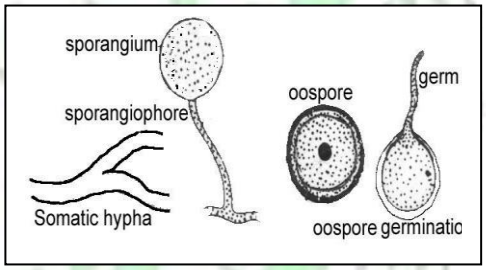
1. **Sporangium**: Can be stalked or sessile (stalkless), with its own peridium. It may also have a thin, cellophane-like base called the hypothallus. Inside the sporangium, there are spores and capillitium. Example: *Physarum*.
2. **Plasmodiocarp**: Similar to a stalkless sporangium, it forms when protoplasm concentrates around some of the main veins of the plasmodium and secretes a membrane around itself. Example: *Trichia*.
3. **Aethalia**: A collection of sporangia that have not separated into individual units. In some aethalia, the walls of individual sporangia are visible, while in others, they are less distinct. Example: *Lycogala*



Different types of sporangia in slime molds	Plasmodiocarp	Aethalia

Oomycota: General Characteristics and Classification

1. Members can be aquatic or terrestrial, and may be saprophytes or obligate parasites.
2. Typically eucarpic and coenocytic.
3. Composed of cellulose; lacks chitin.
4. **Asexual Reproduction**: Achieved through zoospores produced in zoosporangia. Zoospores are biflagellate (one whiplash and one tinsel flagellum), and can be anteriorly or laterally positioned, equal in size.
5. **Sexual Reproduction**: Oogamous, involving gametangial contact. The contents of the antheridium (male gametangium) are transferred to the oogonium (female gametangium) containing the oosphere (egg) through a fertilization tube. The zygote resulting from sexual reproduction is called an oospore. This oospore serves as the sexual resting spore, which is a defining feature of oomycetes.



6. The oospore is diploid and gives rise to diploid mycelium or gametangia.
7. Occurs in the gametangia rather than the zygote.

Genus *Pythium* (Damping Off):

- **Mycelium**: Aseptate, branched, and cottony white.
- **Sporangiophores**: Erect, simple, and bearing sporangia singly, differing from vegetative hyphae.
- **Sporangia**: Spherical or globose, occasionally filamentous or toruloid.
- **Oospores**: Thick-walled, spherical, usually smooth, and three-layered.
- **Important Species**: *P. aphanidermatum*, *P. ultimum*, *P. graminicola* (causing damping off disease).

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

Genus *Phytophthora*:

- **Mycelium**: Aseptate, coenocytic, and branched.
- **Sporangiophores**: Indeterminate growth, zig-zag, sympodially branched, and nodulate.
- **Sporangia**: Single-celled, lemon-shaped, and papillate.
- **Oospores**: Spherical, smooth-walled, and aplerotic.
- **Important Species**: *P. infestans* (causing late blight of potato).

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

Sporangiophore of different Genus causing Downy Mildews:

Genus *Peronospora*:

- **Mycelium**: Aseptate, coenocytic, branched, hyaline, endophytic, and intercellular.
- **Conidia**: Single-celled, spherical or oval, and borne singly.
- **Branching**: Dichotomous at acute angles.
- **Sporangiophores**: Long, pointed, and bearing conidia singly.
- **Oospores**: Spherical and reticulate in *Peronospora parasitica*.
- **Important Species**: *P. parasitica* (downy mildew of crucifers), *P. tabacinia* (tobacco), *P. pisi* (pea).

Genus *Sclerospora*:

- **Mycelium**: Aseptate, coenocytic, branched, hyaline, endophytic, and intercellular.
- **Sporangiophores**: Short, broader at the apex, arising from stomatal openings.
- **Branching**: Dichotomous or trichotomous.
- **Sterigmata**: Short, swollen, bearing sporangia singly.
- **Sporangia**: Single-celled, sometimes papillate.
- **Oospores**: Irregular, due to the sporangial wall shrinking and contacting the oosporic wall.
- **Important Species**: *Sclerospora graminicola* (green ear disease of Bajra).

Genus *Plasmopara*:

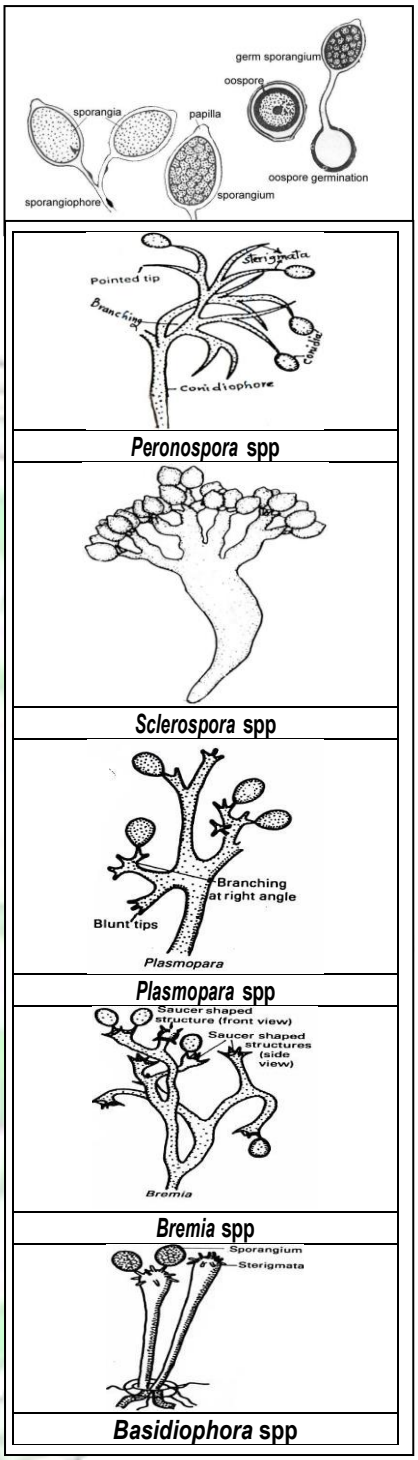
- **Mycelium**: Aseptate, coenocytic, branched, hyaline, endophytic, and intercellular.
- **Sporangia**: Hyaline, oval, formed on right-angle sporangiophores.
- **Sporangiophores**: Hyaline, straight or slightly curved.
- **Branching**: Right-angle branched.
- **Sterigmata**: Mostly trichotomous.
- **Oospores**: Large, spherical, thick-walled (25–50 µm in diameter).
- **Important Species**: *Plasmopara viticola*.

Genus *Bremia*:

- **Mycelium**: Aseptate, coenocytic, branched, hyaline, endophytic, and intercellular.
- **Sporangiophores**: Branch like *Peronospora*, ending with a disc-like or saucer-shaped structure.
- **Important Species**: *Bremia lactucae*.

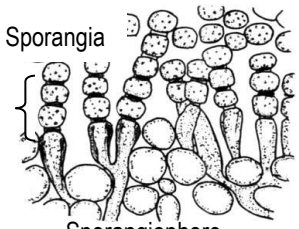
Genus *Basidiophora*:

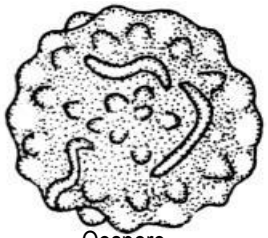
- **Sporophores**: Unbranched, swollen at the apex, with short sterigmata bearing papillate sporangia.
- **Oospores**: Aplerotic.



Sporangiophore of Genus *Albugo* (White Blister/Rust) producing conidia in chains:

- **Mycelium**: Aseptate, coenocytic, branched, hyaline, intercellular with knob-shaped haustoria.
- **Sporangiophores**: Club-shaped (clavate), simple, forming a palisade layer below the epidermis, with lateral walls thickened and laterally free.
- **Sporangia**: Single-celled, globose, produced in chains with a gelatinous pad ("disjuncture").
- **Oospores**: Rough, warty, and yellow.
- **Important Species**: *Albugo candida* (white blister or white rust of crucifers).





Systematic Position

Phylum – Oomycota

Class – Oomycetes

Order - Albuginales

Family - Albuginaceae

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, multispored.
- **Columella**: The sterile, dome-shaped central portion of the sporangium.
- **Aplanospores**: Oval or spherical, single-celled spores.
- **Zygospores**: Rough-walled, black, warty, and provided with suspensors.
- **Important Species**: *Mucor mucedo*, *Mucor basiliformis*.

Systematic Position

Phylum – Zygomycota

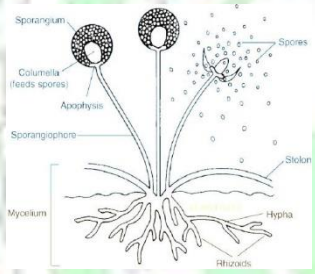
Class – Zygomycetes

Order - Mucorales

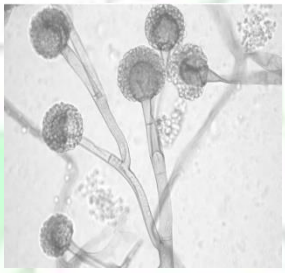
Family - Mucoraceae

Genus: *Rhizopus* (Bread Mould)

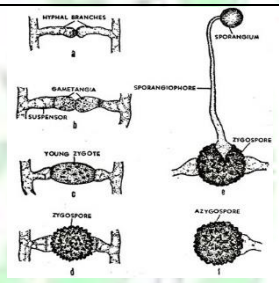
Characteristics: Similar to *Mucor*, but the formation of stolons and rhizoids differentiates *Rhizopus*. Sporangiophores arise in groups from rhizoids. **Important Species**: *Rhizopus stolonifer*.



Spore bearing structure of *Rhizopus* spp



Spore bearing structure of *Mucor* spp



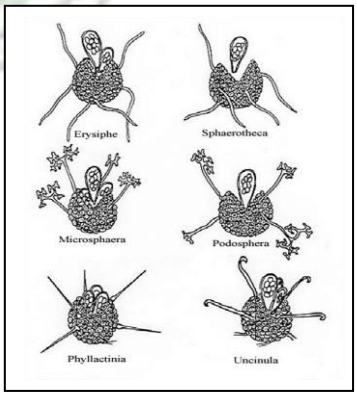
Gametangial copulation in *Zygomycota*

Ascomycota: General Characteristics and Classification

1. Produces a definite number of sexual spores (typically eight) within a sac-like structure called the *ascus*.
2. Mycelium is septate, branched, and organized into tissues known as *plectenchyma*.
3. Produces a sexual fruiting body known as the *ascocarp*, which contains the asci.
4. Lacks motile spores; asexual reproduction occurs through conidia.
5. A brief dikaryotic phase exists in the ascogenous hypha or ascogenous cell.

Identification of Powdery Mildew Genera (Class: Leotiomycetes) based on Cleistothecia

1. **Mycelium Internal and Cleistothecium with Several Asci**:
   - **Phyllactinia**: Appendages with a bulbous base (*Anamorph*: Ovulariopsis).
   - **Leveillula**: Hypha-like appendages (*Anamorph*: Oidiopsis).
2. **Mycelium Superficial and Cleistothecium with Several Asci**:
   - **Erysiphe**: Hypha-like appendages.
   - **Microsphaera**: Appendages with dichotomously branched tips.
   - **Uncinula**: Appendages with coiled tips.
   - Anamorph*:* Oidium.
   - **Golvinomyces**: Branched, hypha-like appendages.
3. **Cleistothecium with a Single Ascus**:
   - **Podosphaera**: Dichotomously branched appendages.
   - **Sphaerotheca**: Hypha-like appendages.



Different features of Cleistothecia produced by powdery mildew fungi

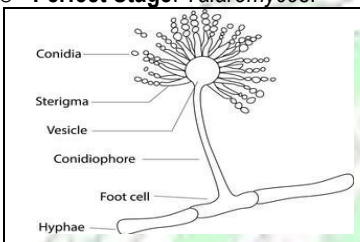
General Characteristics of Fungi in Class: Eurotiomycetes

Genus – *Aspergillus* (Black Mould)

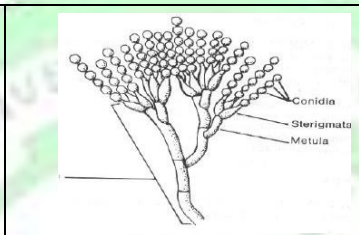
- **Mycelium**: Well-developed, branched, septate, hyaline, and submerged in the substrate.
- **Conidiophores**: Aseptate, simple structures that arise from a "foot cell," terminating in a vesicle.
- **Sterigmata**: Two rows of bottle-shaped sterigmata are formed on the vesicle.
- **Conidia**: Globose, single-celled, echinulate (spiny), and borne in long basipetal chains on secondary sterigmata.
- **Important Species**: *Aspergillus niger*, *A. flavus*, *A. fumigatus*.
- **Perfect Stage**: *Eurotium*.

Genus – *Penicillium* (Blue/Green Mould)

- **Mycelium**: Well-developed, branched, septate, hyaline, and submerged in the substrate.
- **Conidiophores**: Septate and branched, without forming a vesicle. Foot cells are absent.
- **Sterigmata**: Single row of peg-like sterigmata is formed.
- **Conidia**: Globose to ovoid, single-celled, smooth-walled, and borne in long basipetal chains, resembling "glass beads."
- **Important Species**: *Penicillium notatum*, *P. chrysogenum*.
- **Perfect Stage**: *Talaromyces*.



***Aspergillus* spp**.



***Penicillium* spp.**

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

General Identification Characters of Different Ascomycete Classes

1. Pyrenomycetes (Sordariomycetes)

- **Fruiting Body**: Characterized by perithecia, which are flask-shaped fruiting bodies with a narrow opening (ostiole).
- **Asci**: Produced inside the perithecia, typically cylindrical, and arranged in a basal layer.
- **Ascospores**: Usually unicellular to multicellular, often pigmented, and discharged through the ostiole.
- **Mycelium**: Septate, well-developed, and usually forms a plectenchymatous structure around the perithecia.
- **Habitat**: Mostly saprophytic or parasitic on plants.
- **Example Genera**: *Neurospora*, *Claviceps*.

2. Discomycetes (Leotiomycetes/Helotiales)

- **Fruiting Body**: Characterized by apothecia, which are open, disc- or cup-shaped fruiting bodies that expose the asci.
- **Asci**: Produced in a hymenial layer on the surface of the apothecium; usually cylindrical and numerous.
- **Ascospores**: Generally small, unicellular or septate, and colorless (hyaline).
- **Mycelium**: Well-developed and septate, often forming organized tissue structures (plectenchyma).
- **Habitat**: Found on decaying plant matter, as parasites, or symbiotically (e.g., in lichens).
- **Example Genera**: *Helvella*, *Morchella* (morels).

3. Loculoascomycetes (Dothideomycetes)

- **Fruiting Body**: Characterized by pseudothecia, which are flask-shaped or loculate fruiting bodies without a true perithecial wall.
- **Asci**: Bitunicate (having a double-layered wall), formed within cavities (locules) in the stroma.
- **Ascospores**: Multicellular, often darkly pigmented, and may have transverse and longitudinal septa.
- **Mycelium**: Septate, often forming stromatic tissues that enclose the locules.
- **Habitat**: Mostly parasitic on plants, causing leaf spots, cankers, and other diseases.
- **Example Genera**: *Venturia*, *Pleospora*.

4. Laboulbeniomycetes

- **Fruiting Body**: Produce small, flask-shaped perithecia, often attached to the external body surface of arthropods (insects).
- **Asci**: Produced inside perithecia and typically few in number (usually two or four).
- **Ascospores**: Often elongate and spindle-shaped, discharged through an ostiole in the perithecium.
- **Mycelium**: Minimal or absent; the fungi are largely reduced in form and directly attached to the host.
- **Habitat**: Obligate parasites on arthropods, forming tiny thallus-like structures.
- **Example Genera**: *Laboulbenia*, *Hesperomyces*.

Basidomycota: General Characteristics and Classification

1. Produce sexual spores (basidiospores) on the outside of a specialized spore producing structure called basidium.
2. A typical basidium is a club shaped structure, bearing specially 4 basidiospores on pointed projections called sterigmata.
3. Basidiospores are haploid, uninucleate and are the result of plasmogamy, karyogamy and meiosis.
4. Dikaryotic phase dominates the life cycle.
5. Presence of clamp connections on the mycelium.
6. Presence of dolipore septum, except in rusts and smuts.
7. Absence of motile spores.

Genus – *Sphacelotheca*

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)

Genus – *Tolyposporium*

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

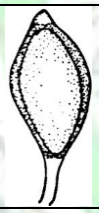
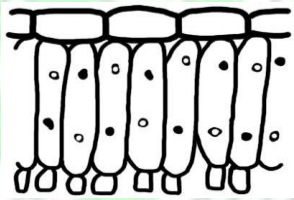
Genus- *Tilletia*: The disease caused by *Tilletia* are called as "Bunt"

Teliospores	Teliospores are large, 16-54 smooth, verrucose
Important spp.	*T. caries & T. foetida* (stinking smut or hill bunt)

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 spp:	*U. segetum tritici* (*U. tritici*), *U. nuda* – (Loose smut of barley), *U. maydis* (corn smut), *U. scitaminea* (whip smut of sugar came)

Teliospores of Rust Fungi

Uromyces	***Puccinia***	***Melampsora***
Teliospores stalked, single celled	Teliospores stalked, bicelled	Teliospores single celled, sessile, cylindrical in shape and form layer below the epidermis
		

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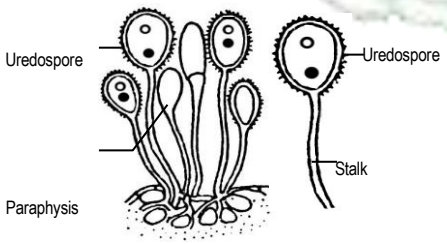
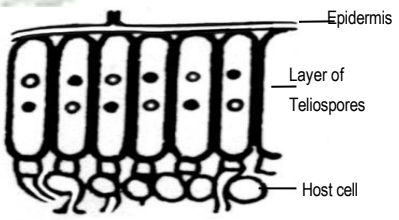
Difference between Uredial and telial stage of rust fungi

Uredial Stage	Telial Stage
Epidermis ruptured Uredospore	Epidermis intact (unbroken)
Uredospores stalked	Teliospores sessile
Uredospores finely echinulate	They are single celled, cylindrical in shape
Capitate paraphyses also present	Teliospores form layer below epidermis
 **Uredial Stage**	 **Telial Stage**

DEUTEROMYCOTA: GENERAL CHARACTERISTICS AND CLASSIFICATION

Fungi imperfecti, also known as **Deuteromycetes**, are a group of fungi that reproduce asexually through **conidia** or by **fragmentation** of hyphae or modified mycelium. Conidia are non-motile asexual spores produced at the tip or side of a sporogenous cell. These fungi are termed **"imperfect"** due to the absence of observed sexual reproduction or perfect stages in their life cycle.

Many fungi classified under Deuteromycetes have no known sexual stage or teleomorph. In cases where a perfect stage is discovered, the fungi are reclassified according to their sexual fruiting bodies, often falling under **Basidiomycota** or **Ascomycota**.

Important Characteristics of Class Coelomycetes

- **Conidia**: Borne on conidiogenous cells, with or without conidiophores, and enclosed in specialized asexual fruiting bodies like **pycnidium** (flask-shaped) or **acervulus** (flat or saucer-shaped).
- Coelomycetes are divided into two form-orders:
    1. **Sphaeropsidales**: Characterized by the production of conidia in a **pycnidium**.
    2. **Melanconiales**: Characterized by the production of conidia in an **acervulus**.

Important Characteristics of Class Hyphomycetes

- **Conidia**: Borne directly on hyphae, either singly or in aggregates. Conidiophores bearing conidia may arise separately or in clusters from the mycelium.
- **Conidiophores**: May be aggregated or solitary, forming various spore-producing structures.
- **Sclerotia**: Some members form **sclerotial bodies**, which are hardened mycelial structures that help in survival during unfavorable conditions.
- **Identification**: Based primarily on the morphology and arrangement of conidia and conidiophores.

Hyphomycetes are divided into four form-orders:

1. **Hyphomycetales (Moniliales/Hyphales)**: Includes fungi that produce conidia in loose arrangements on simple or branched conidiophores.
2. **Tuberculariales**: Fungi that form conidia on compact masses of mycelium, often resembling tubercles.
3. **Stilbellales**: Fungi that form conidia in coremial structures or in tight bunches on erect conidiophores
4. **Agonomycetales**: Includes the fungi which do not produce conidia, form sclerotial bodies i.e., modification of mycelium, reproduction is by random fragmentation of hyphae.

PRESERVATION OF DISEASED PLANT 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:

- **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.
- **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.
- **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.

STAINING AND SLIDE PREPARATION

Slide preparation

1. Begin by preparing a clean glass slide and cover slip. Place a drop of water in the center of the slide.
2. Carefully add the specimen to the water drop. Use dissecting needles to properly align the specimen on the slide. If necessary, tear and tease apart the specimen using the needles to ensure proper arrangement.
3. Gently place the cover slip over the preparation. Start by placing one edge of the cover slip on the slide so it contacts the water drop. Then, using the tip of a dissecting needle, carefully lower the cover slip into position. When done correctly, this method will help avoid air bubbles under the cover slip.

Fungal Stain:

Lactophenol Cotton Blue: This stain is a general-purpose staining and mounting agent used for observing fungal structures. Its components include:

Phenol (pure crystals)	20 gm
Lactic acid	20 gm
Glycerine	40 gm
Water	20 ml.
Cotton Blue	In traces (0.5%)

Mounting Agent:

Gelatin	1.0 gm
Glycerine	7.0 gm
Water	6.0 ml
With the addition of phenol	1%

Purpose of the Stain:

1. Facilitates accurate observation of microorganisms under the microscope.
2. Differentiates between host tissue and microorganisms.
3. Aids in identifying various parts of the microorganism.

Precautionary Measures:

1. Avoid using excessive or overly thick material on the slide, as only very thin specimens can be effectively studied with a compound microscope.
2. Ensure that the cover slip lies flat on the slide.
3. The specimen and the area beneath the cover slip must be flooded with the mounting medium. Ensure there is no water on the rest of the slide or on top of the cover slip to prevent distortion or interference.

PREPARATION OF POTATO DEXTROSE AGAR MEDIA

Materials required: Following ingredients in different quantities are used.

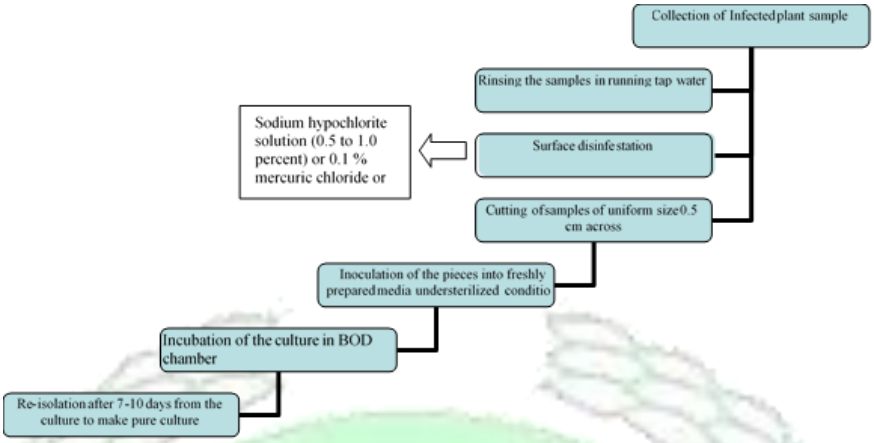
Peeled potato slices	-	200g
Dextrose	-	20g
Agar- agar	-	20g
Distilled water	-	1000 ml

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.

Medium is sterilized in an autoclave at 1.1kg/cm2 pressure for 20 minutes at temperature of 121.6°C. Thus the medium is ready for use.

ISOLATION OF 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 above.

KOCH POSTULATES

Four steps of Koch Postulates:

1. The suspected causal agent must be present in every diseased organism examined.
2. The suspected causal agent must be isolated from the diseased host organism and grown in pure culture.
3. When a pure culture of the suspected causal agent is inoculated into a healthy susceptible host, the host must reproduce the specific disease.
4. The same causal organism must be recovered again from the experimentally inoculated and infected host *i.e.*, the recovered agent must have the same characteristics as the organism in step 2.

Fungal DNA isolation

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, mix properly 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 I hr at -20°C.
8. Precipitate 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:

- 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.
- Pipette the following PCR reagents in the following order into a 0.2 ml thin walled PCR tube: Sterile Water, 10X PCR buffer, dNTPs, MgCl2, 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).
- 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.
- 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.
- 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.
- 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.