FUNDAMENTALS OF PLANT PATHOLOGY

PRACTICAL MANUAL

Course No. APP-138 4(3+1)

For Undergraduate Agricultural Students

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Syllabus:

Practical: General plant pathological laboratory equipments, different parts of the Microscope, its handling and care, Collection and preservation of disease specimen, Preparation of media (Potato Dextrose Agar Medium), Isolation and purification of culture of plant pathogens from diseased Plant Tissues, Inoculation and Re-isolation of plant Pathogens, Identification of different types of mycelium and other fungal structures, Identification of different types of plant disease symptoms, Identification of the plant pathogens under the Phylum *Chytridiomycota* and *Oomycota, Zygomycota, Basidiomycota, Ascomycota,* Staining and identification of plant pathogenic bacteria, sap transmission of viruses, Phanaerogamic plant parasites, morphological features of plant parasitic nematodes, Sampling and extraction of nematodes from soil and plant material, preparation of nematode mounting, fungicides and their formulations, Calculation of fungicidal sprays concentrations.

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Batch .....

Session .....

Semester .....

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Date: Course Teacher

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Practical No. 1

Objective: To get familiar with general plant pathological laboratory equipment

The students in batches will visit the laboratory of Plant Pathology to acquaint with different appliances, tools, glass-wares, and other miscellaneous items, which they will be using in various exercises and experiments to be conducted.

1. Identify the flaboratory equipments available in the Plant Pathology Laboratory:

(a) Laboratory appliances / tools:

(i)		(ii)	
(iii)		(iv)	
(v)		(vi)	
(vii)		(viii)	
(ix)		(x)	
(xi)		(xii)	
(xiii)		(xiv)	
(xv)		(xvi)	
(xvii)		(xviii)	
(xix)		(xx)	

(b) Glass-wares:

(i)		(ii)	
(iii)		(iv)	
(v)		(vi)	
(vii)		(viii)	
(ix)		(x)	
(xi)		(xii)	

2. Label the following laboratory equipments and state its principle and functions.

Auto Clave: …….. …… …… …… …… …… …… …… …… ……	
Laminar Air Flow: …….. …… …… …… …… …… …… …… ……	
BOD Incubator: …….. …… …… …… …… …… …… …… ……	
Hot Air Oven: …….. …… …… …… …… …… …… …… …… ……	

Practical No. 2

Objective: To get familiar with Microscope, its parts and handling

1. Draw a well labeled diagram of a Compound Microscope and indicate all the important parts. State the function of each parts.

2. State the precautionary measures that need to be taken while Handling Microscope.

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Practical No. 3

Objective: Collection and preservation of plant disease samples

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

Materials required: ……………………………………………………………………………………………..…..

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Procedure for wet preservation: ……………………………………………………………………………………

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2. Prepare herbarium of at least 20 samples of Plant diseases with all the details in it (Dry preservation):

Practical No. 4

Objective: Preparation of Potato Dextrose Agar medium

1. Prepare two 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. 5

Objective: Isolation and purification of plant pathogens from diseased plant tissues

Isolate and identify plant pathogens from infected plant sample

Materials Required: ……..……..

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Procedure for isolation: ……..……..

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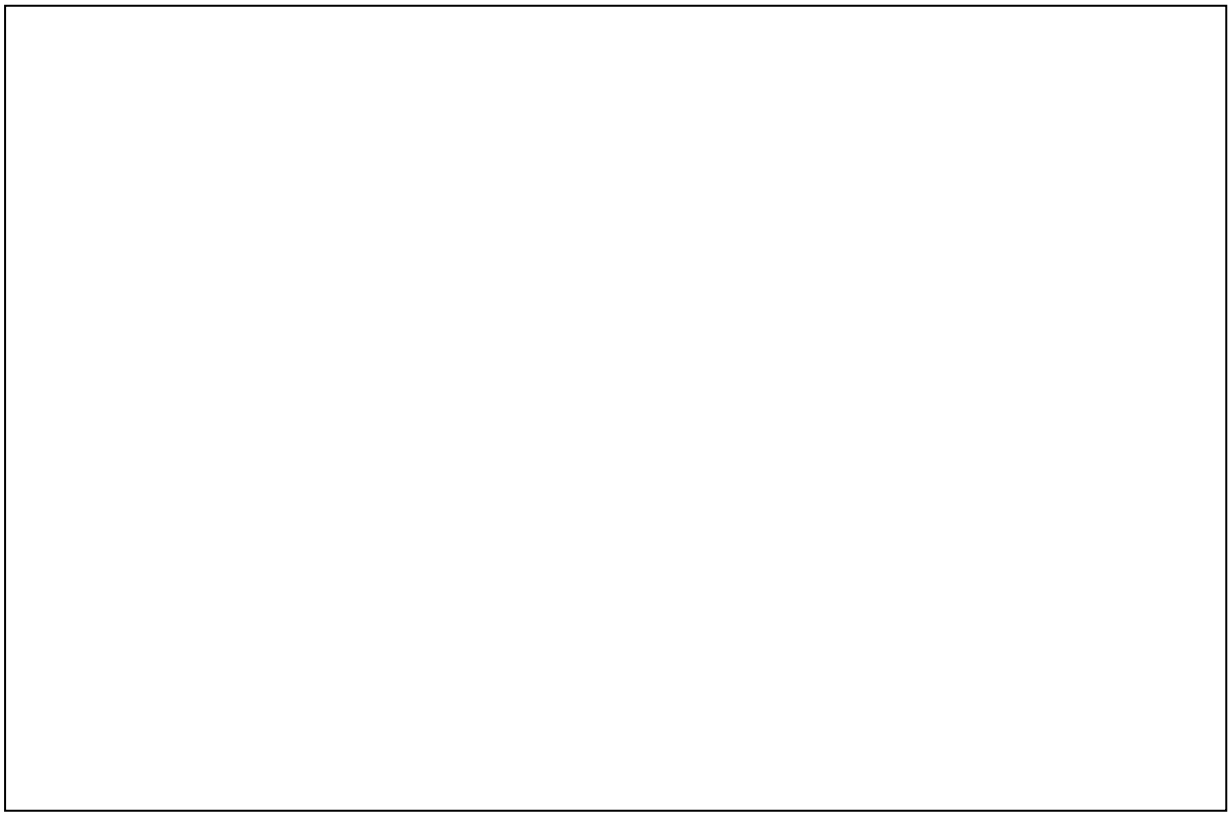
Steps of isolation of pathogen from plant tissues – Flowchart



Practical No. 6

Objective: Demonstration of Koch's Postulates

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. 7

Objective: Identification of different types of mycelium and other fungal structures

Identify and describe with well-labelled diagram of different types of mycelium and asexual spores

Materials Required: ……..……..

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Types of Mycelium: ……

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Types of Asexual Spores: ……

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2. Identify different asexual fruiting bodies and ascocarps provided in the slides and draw the structures observed under the microscope and describe its characteristics.

Ascocarps:……..……...

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Characteristics: ………

Practical No. 8

Objective: Identification of plant disease symptoms

Visit the University Research Farm and describe different symptoms you observed in the field.

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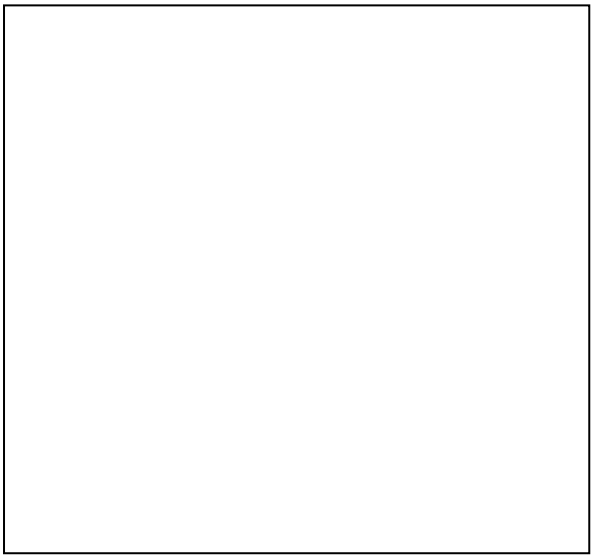

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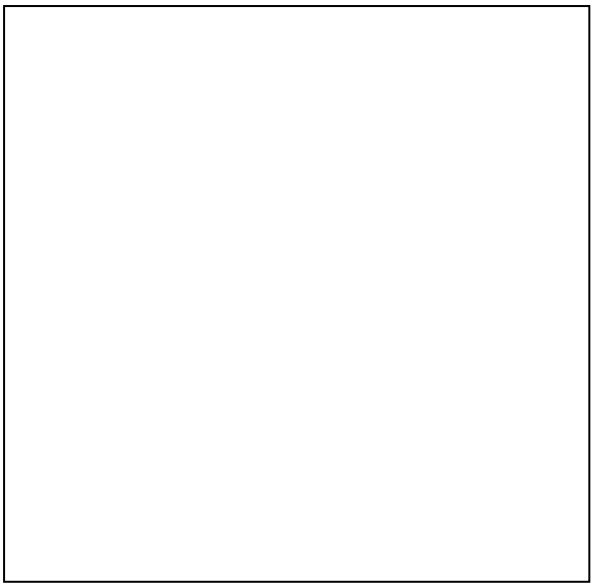

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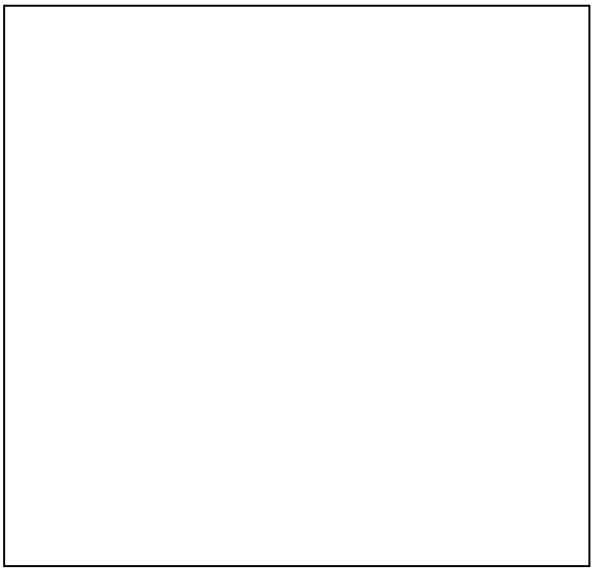

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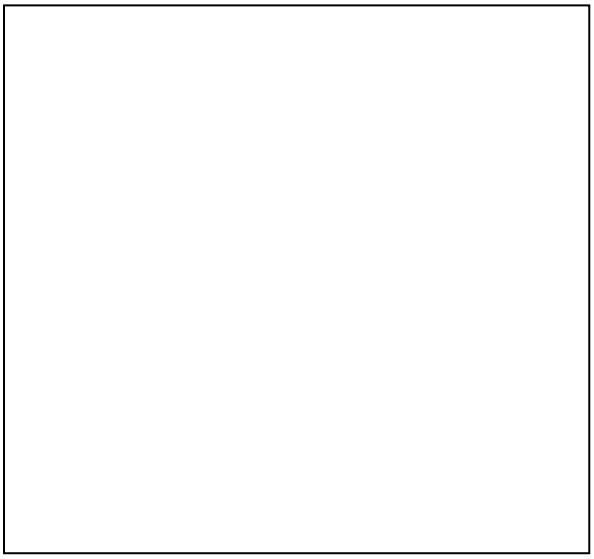

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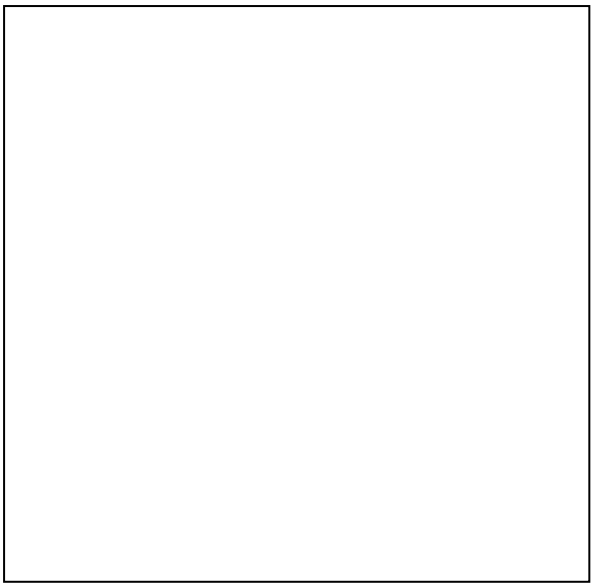

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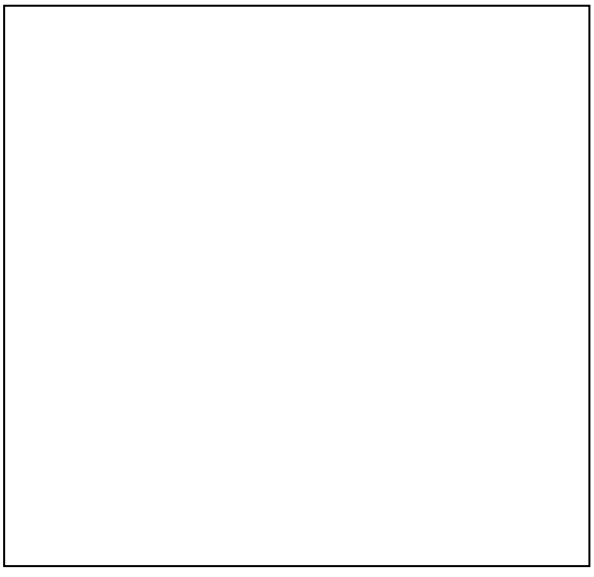

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Practical No. 9

Objective: Identification of plant pathogens of Phylum *Chytridiomycota* and *Oomycota*

Note: The students need to observe the slides, state the systematic position of the fungal genera, draw and record the features while describing the genera given.

Systematic Position:

Genus: *Synchytrium*

Features:

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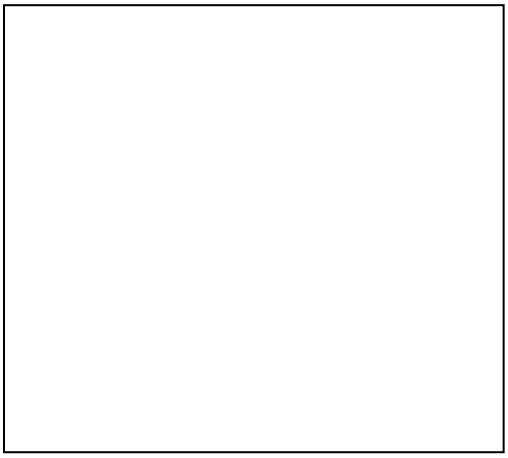

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Genus: *Pythium*

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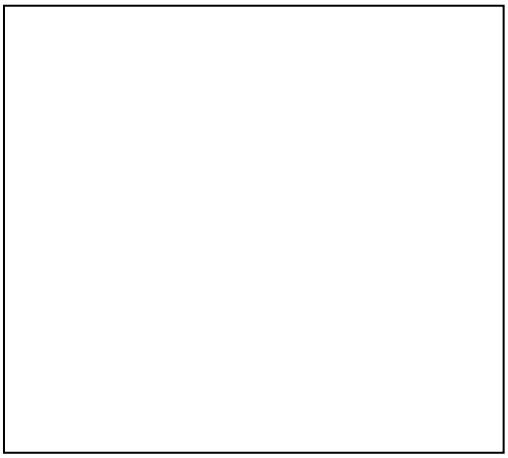

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Genus: *Phytophthora*

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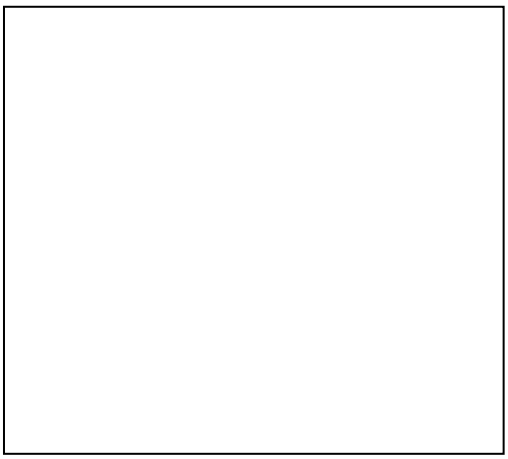

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2. Record the characteristic differences in morphology of *Pythium* and *Phytophthora* and draw a neat and labeled diagram of the spores.

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

Diagram	Diagram

3. 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
……	……
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……	……
……	……
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……	……

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

Diagram	Diagram

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

SYSTEMATIC POSITION:

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Characteristic	**Description**
Mycelium	……
	……
	……
Sporangiophores	……
	……
	……
Sporangia	……
	……
	……
Oospores	……
	……
	……

Diagram

Practical No. 10

Objective: Identification of the plant pathogens of Phylum *Zygomycota*

1. 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**
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……	……
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……	……
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Characteristics	***Mucor***	***Rhizopus***
Mycelium	……	……
	……	……
	……	……
Sporangiophores	……	……
	……	……
	……	……
Sporangia	……	……
	……	……
	……	……
Columella	……	……
	……	……
	……	……
Aplanospores	……	……
	……	……
	……	……
Zygospores	……	……
	……	……
	……	……

Diagram

Diagram

Practical No. 11

Objective: Identification of the plant pathogens of Phylum *Basidiomycota*

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

Genus: *Uromyces* ***Features:***

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Genus: *Melampsora* ***Features:***

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Genus: *Ustilago* ***Features:***

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Genus: ***Tilletia***

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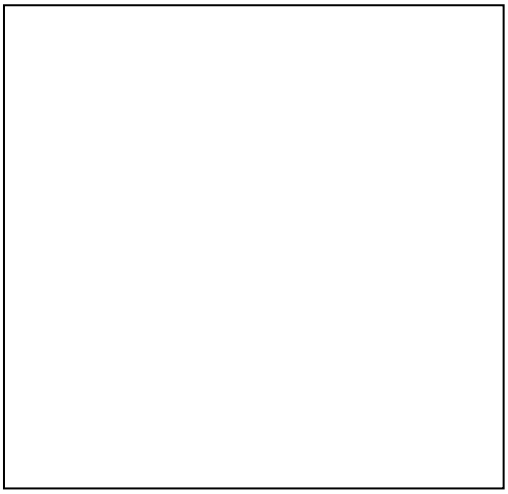

Features:

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Genus: ***Puccinia***

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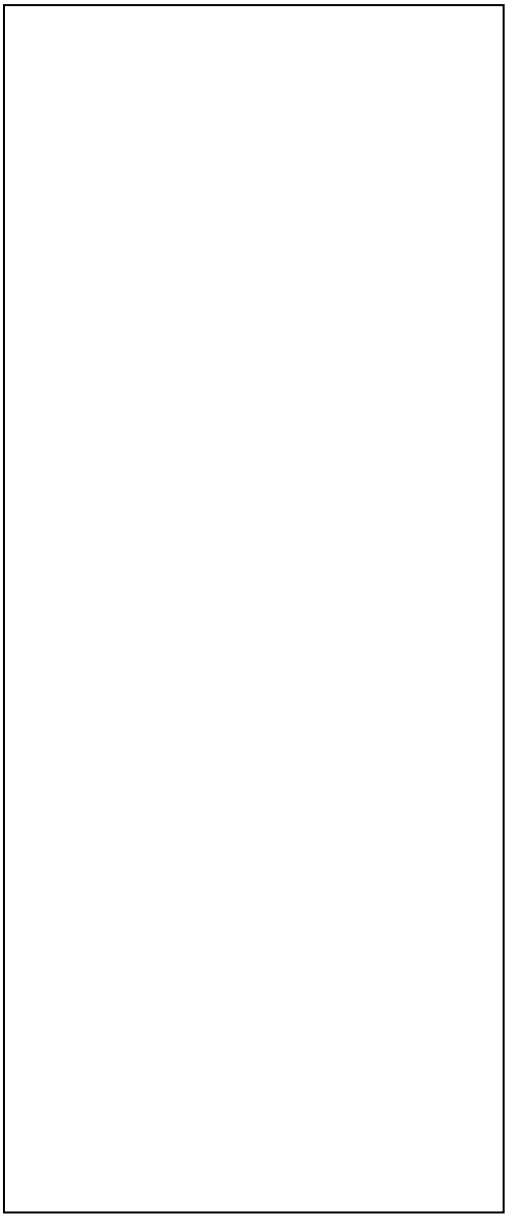

Features:

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Practical No. 12

Objective: Identification of the plant pathogens of Phylum Ascomycota

Class: Eurotiomycetes

SYSTEMATIC POSITION (*Aspergillus*)	**SYSTEMATIC POSITION (*Penicillium*)**
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Characteristics	***Aspergillus***	***Penicillium***
Mycelium	……	……
	……	……
	……	……
Foot Cell	……	……
	……	……
	……	……
Conidiophore	……	……
	……	……
	……	……
Vesicle	……	……
	……	……
	……	……
Sterigmata	……	……
	……	……
	……	……
Conidia	……	……
	……	……
	……	……
Perfect Stage	……	……

Diagram

Diagram

Class: Sodariomycetes

SYSTEMATIC POSITION (*Fusarium*)	**SYSTEMATIC POSITION (*Claviceps*)**
…… …… …… …… …… ……	…… …… …… …… …… ……

Characteristics	***Fusarium***	***Claviceps***
Mycelium	……	……
	……	……
	……	……
Sporodochia	……	
	……	
	……	
Conidiophore	……	
	……	
	……	
Conidia	……	……
	……	……
	……	……
Chlamydospores	……	
	……	
	……	
Sclerotia		……
		……
		……
Perfect Stage	……	

Diagram	Diagram

SYSTEMATIC POSITION (*Pyricularia*)	**SYSTEMATIC POSITION (*Colletotrichum*)**
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……	……
……	……
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Characteristics	***Pyricularia***	***Colletotrichum***
Mycelium		……
		……
		……
Conidiophore	……	……
	……	……
	……	……
Conidia	……	……
	……	……
	……	……
Acervuli		……
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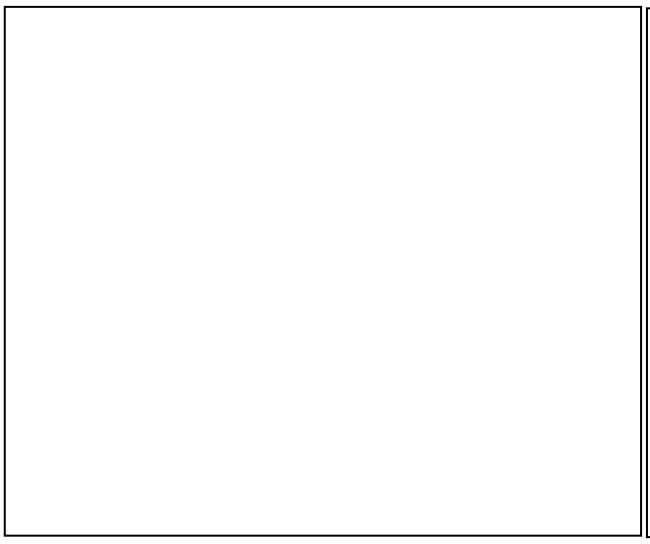
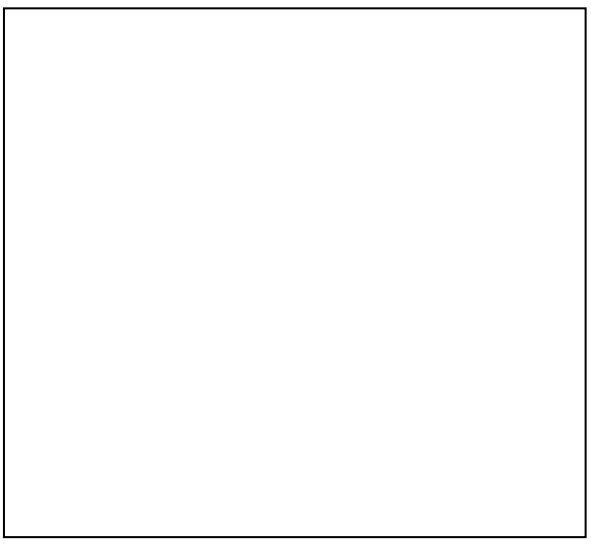
		……
Perfect Stage	……	……





Dothideomycetes

SYSTEMATIC POSITION (*Helminthosporium*)	**SYSTEMATIC POSITION (*Alternaria*)**
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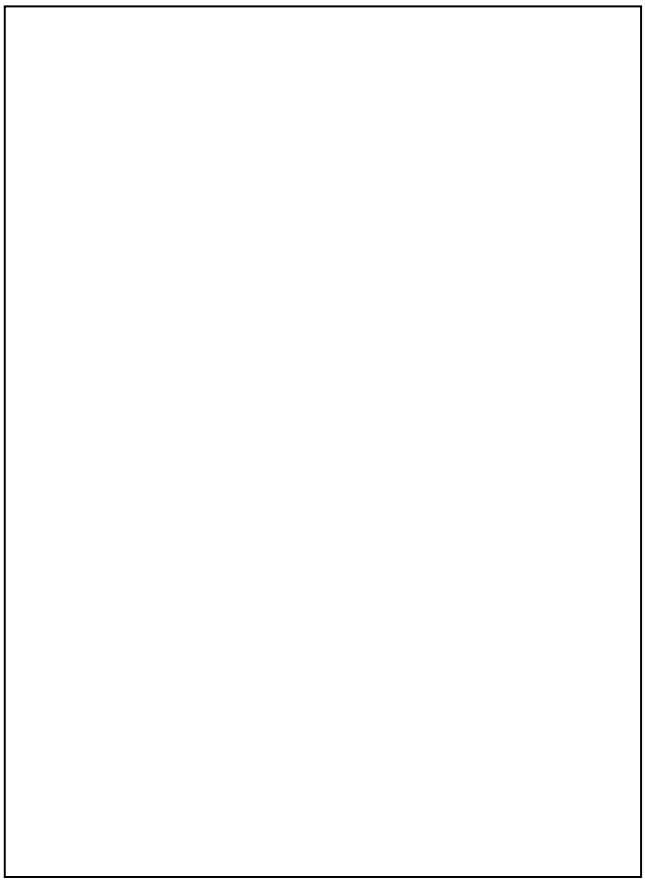
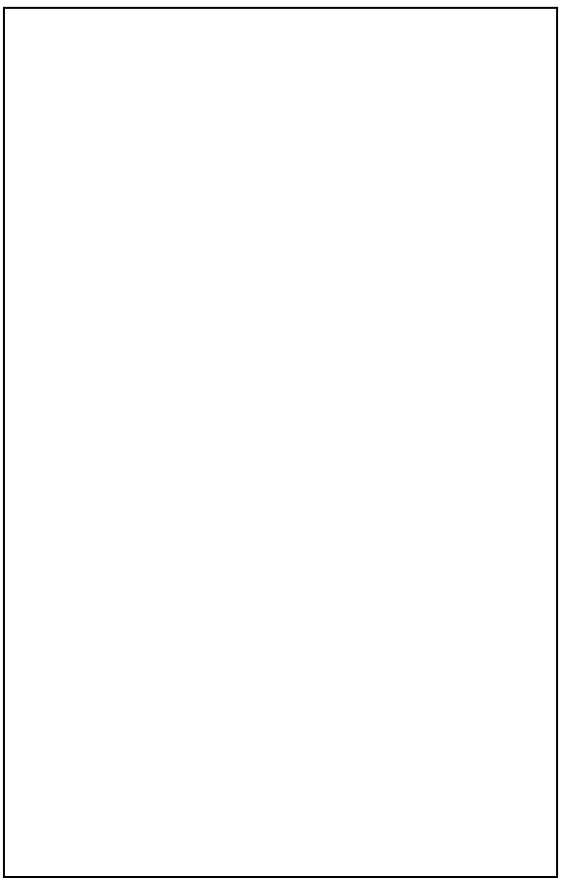
Characteristics	***Helminthosporium***	***Alternaria***
Conidiophore	……	……
	……	……
	……	……
Conidia	……	……
	……	……
	……	……
Perfect Stage	……	……





SYSTEMATIC POSITION (*Phyllosticta*)	**SYSTEMATIC POSITION (*Cercospora*)**
……	……
……	……
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Characteristics	***Phyllosticta***	***Cercospora***
Mycelium	……	
	……	
	……	
Conidiophores	……	……
	……	……
	……	……
Pycnidia	……	
	……	
	……	
Conidia	……	……
	……	……
	……	……
Perfect Stage	……	……

Class: Letiomycetes

SYSTEMATIC POSITION (*Erysiphe*)	**SYSTEMATIC POSITION (*Sclerotinia*)**
……	……
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Characteristics	***Erysiphe***	***Sclerotinia***
Mycelium	……	……
	……	……
	……	……
Asexual Stage	……	
Conidiophores	……	……
	……	……
	……	……
Conidia	……	……
	……	……
	……	……
Sexual Stage	……	
Cleistothecia	……	Apothecia ……
	……	……
	……	……
Appendages	……	Sclerotia……
	……	……
	……	……
Asci	……	……
	……	……
	……	……
Ascospores	……	……
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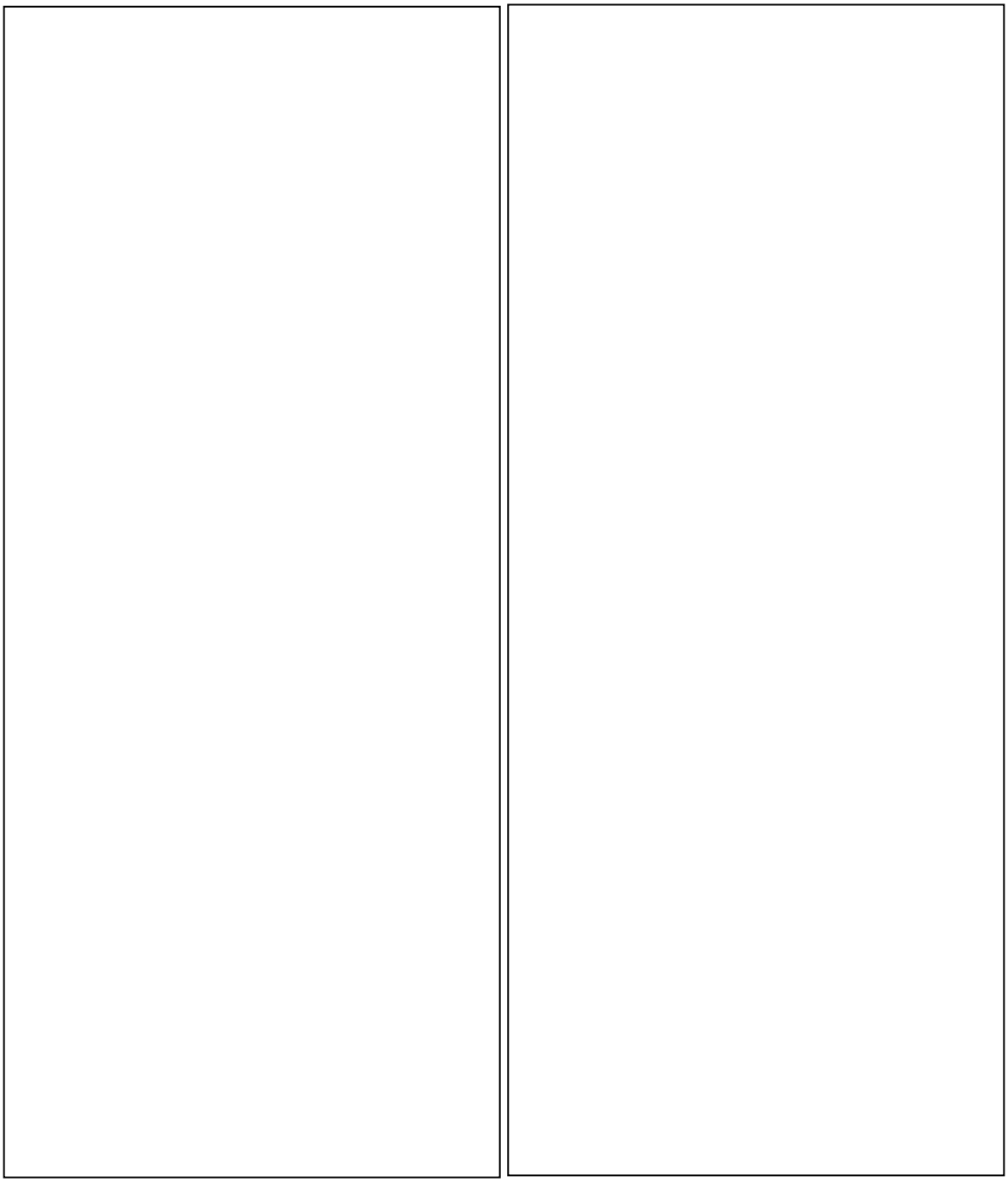
	……	……



Class: Taphrinomycetes

SYSTEMATIC POSITION (*Taphrina*)

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Characteristics	***Taphrina***
Mycelium	……
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Asci	……
	……
	……
Ascospores	……
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Practical No. 13

Objective: Staining and identification of plant pathogenic bacteria

1. Prepare smear of given bacterial samples and perform gram-staining and identify on the basis of gram staining. Write the step by step procedures of gram-staining.

Materials Required: ……

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Procedure:

A. Smear preparation: ……

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B. Gram-staining: ……

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Observation:

Sl.No.	Color of the stain	Gram-reaction

Practical No. 14

Objective: To demonstrate sap transmission of viruses

Perform transmission of virus through sap using *tomato leaf curl virus* and note the symptoms.

Materials Required: ……

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Procedure: ……

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Observations: ……

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Practical No. 15

Objective: To identify Phanaerogamic plant parasites.

Identify the plant parasitic phanaerogamic plants. Note their different host and parasitic nature.

Practical No. 16

Objectives: To get familiar with different morphological features of plant parasitic nematodes

1. Draw a neat and labeled diagram indicating different morphological features of typical male and female plant parasitic nematode.

Male

Female

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Practical No. 17

Objective: Sampling and extraction of nematodes from soil and plant material

Extract Nematodes from the soil using **Cobb's sieving and Gravity Method (Decanting and sieving Method)**

Materials Required: ……

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Procedure: ……

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Preparation of nematode mounting

Prepare nematode mounts using the extracted nematode from previous experiment.

Materials Required: ……

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Procedure: ……

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Practical No. 18

Objectives: To get familiar with different fungicides and their formulations

1. Write the constituents of the following fungicides:

A. **Bordeaux mixture:** ……

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B. **Bordeaux paste:** ……

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C. **Burgundy mixture:** ……

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D. **Chestnut compound:** ……

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E. **Chaubattia Paste:** ……

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Precautionary measures: ……

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Practical No. 19

Objective: Calculation of fungicidal spray concentrations.

1. The recommended doses of the following fungicides for Dry seed treatment are as follows:

Agrosan G.N. 0.3% Thiram 0.2% Captan 0.2%

Calculate the required amount of each fungicide for treating 8 Kg seed.

Calculation

2. Prepare fungicidal solution for spraying of 1 hectare area of different crops. The doses for different fungicides are given below:

Requirements: 1. Balance 2. Weight Box 3. container 4. Fungicide **5.** Sprayer

Doses:

Sulfex 0.3% **Indofil M 45 0.2%** **Benlate 0.1%** **Water 1 litre**

Note: For 1 hectare of spray, the water requirement is 1000 litre.

Calculation

Annexures

GENERAL PLANT PATHOLOGICAL LABORATORY EQUIPMENTS

(a) Laboratory appliances / tools:

1.	Autoclave	6.	Hot-air oven	11.	Scissor	16.	Sprit Lamp
2.	Freeze	7.	Incubator	12.	Cork-borer	17.	Forceps
3.	Hot Plate	8.	Pan (different sizes)	13.	Needle, Inoculating needle	18.	Rotary shaker
4.	Knife / Blade	9.	Scalpel	14.	Bearing Blander	19.	Glass marker
5.	Inoculating needles	10.	Laminar flow	15.	Gel electrophoresis	20.	Centrifuge

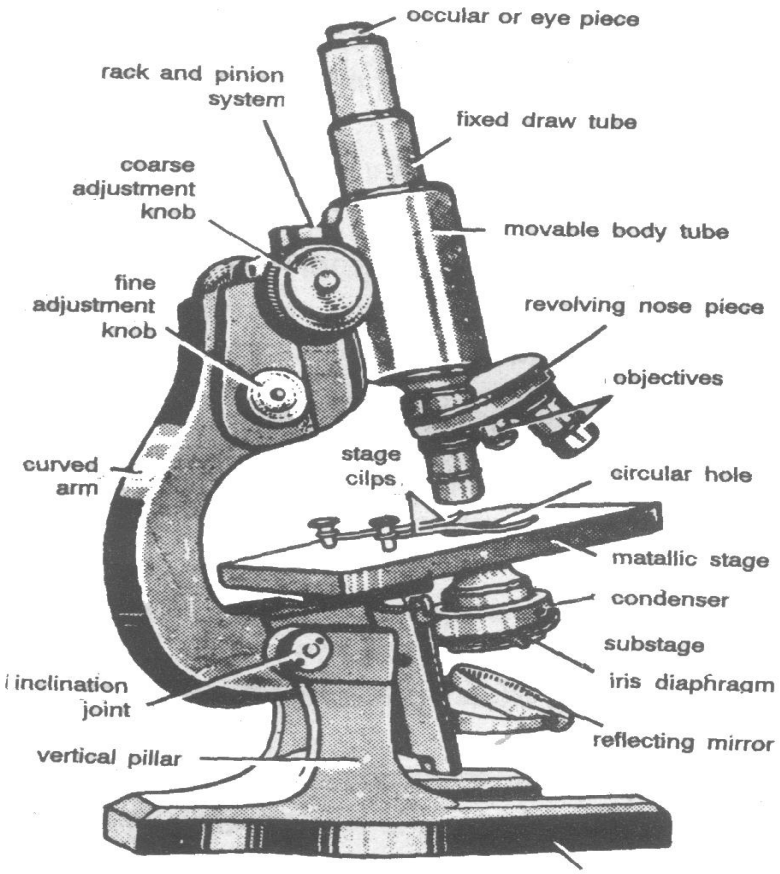
(b) Glass-wares:

1.	Conical flask (different sizes)	5.	Beaker (different sizes)	9.	Slides
2.	Measuring cylinder (different capacity)	6.	Pippet (different volume)	10.	Watch glass
3.	Petridishes	7.	Culture tubes	11.	Dropping bottle
4.	Cover-slip	8.	Nematode counting dish	12.	Bearman funnel

(c) Miscellaneous items:

1.	Cotton	5.	Blotting paper	9.	Washing brush
2.	Aluminium foil	6.	Wash bottle	10.	Washing powder
3.	Trays	7.	Thread	11.	Wire basket
4.	Sieve of different sizes	8.	Rubber bands	12.	Mortar and pestle

MICROSCOPE



Compound Microscope

COLLECTION AND PRESERVATION OF PLANT DISEASE SAMPLES

1) Dry Preservation:

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 h 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.

POTATO DEXTROSE AGAR MEDIUM

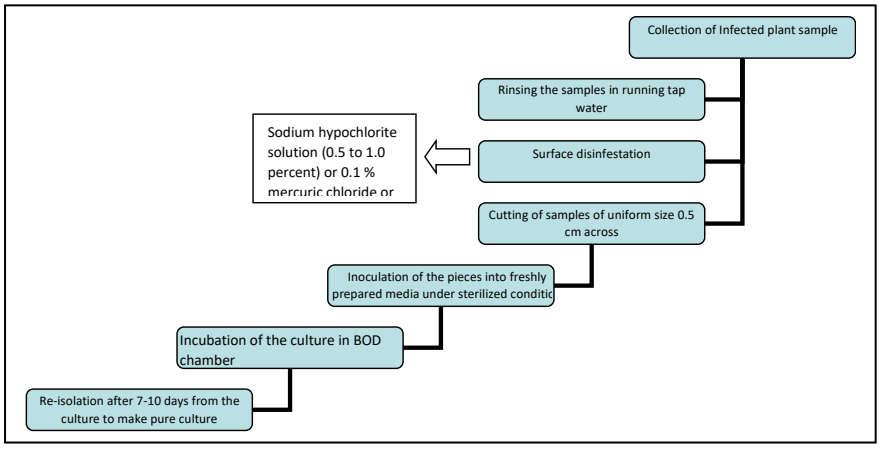
Materials required: Peeled potato slices (200g); Dextrose (20 g); Agar- agar (20 g); Distilled water (1000 ml)

Method:

- Potato slices are cooked in 500 ml of water.
- Then filtered with the help of muslin cloth.
- Agar-agar is melted in 500 ml of water.
- Potato juice is added to the melted agar.
- Volume is made 1000 ml by adding required water.
- Again lit is filtered through muslin cloth.
- Dextrose is added in this mixture and shaken well.
- 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.

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:



KOCH POSTULATES

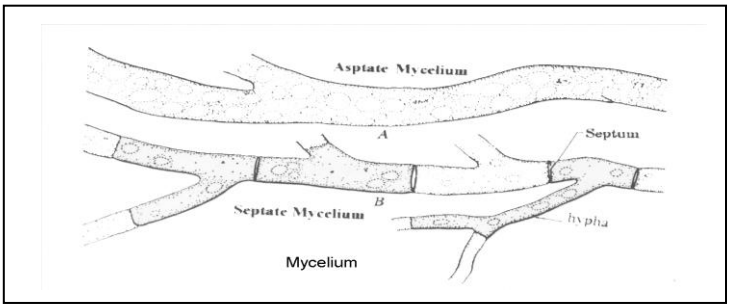
Four steps of Koch Postulates:

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.

DIFFERENT STRUCTURES OF FUNGI

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.

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.

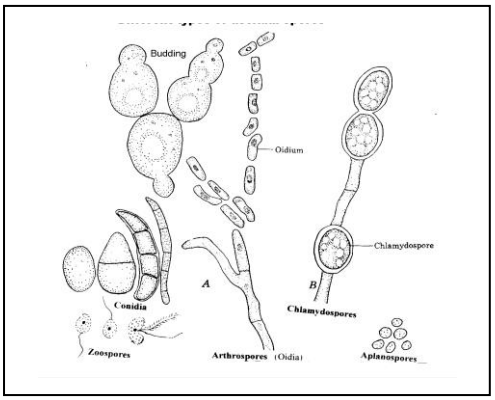


Types of Asexual Spores: Asexual spores are those in which sex is not involved. Generally five types of asexual spores are produced in fungi.

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.
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.

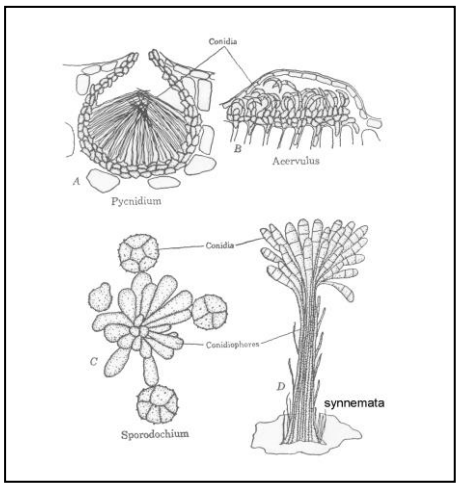
5. **Zoospores:** Pear or kidney shaped, single shaped, naked, motile (flagellate), produced in sporangium (zoosporangium).
6. **Aplanospores:** Oval or spherical in shape, single celled, non-motile (aflagellate) and produced mostly in collumellate sporangium.



TYPES OF ASEXUAL FRUITING BODIES, SEXUAL SPORES AND ASCOCARPS

Asexual fruiting bodies:

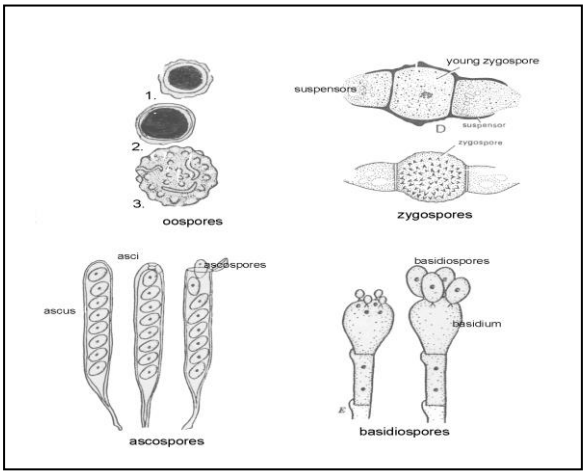
1. **Pycnidia:** These are spherical or flask shaped structures in which the conidia or produced. They have the natural opening known as ostiole through which the conidia are liberated. This type of structure is produced in order Spaeropsidales of sub division Deuteromycotina.
2. **Acervuli**: These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



TYPES OF SEXUAL SPORES

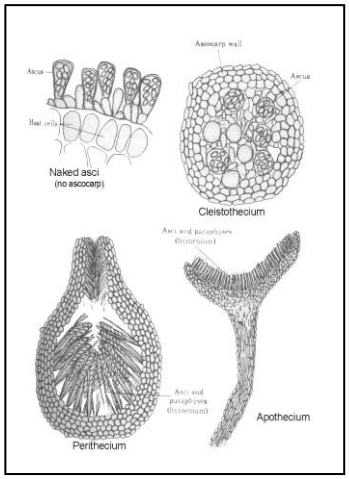
Four types of sexual spores are formed in fungi, which are produced by various methods and they form the bases for the classification of fungi in different sub-divisions.

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



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**", sometime 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.



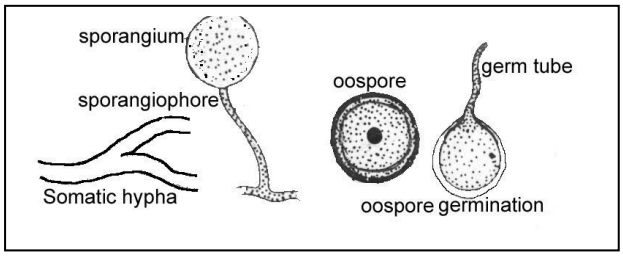
TYPES OF DISEASE SYMPTOMS PRODUCED DUE TO INFECTION BY PATHOGEN

1. **Blights:** A disease characterized by general and rapid killing of leaves, flowers and stems.
2. **Chlorosis**: When repression of colour is partial i.e., normally green tissues are yellow or when yellow colour is uniform and unbroken in leaves infected by plant pathogen.
3. **Mosaic:** Patches of normal green tissues alternate with yellow areas resulting in mottling, spotting, flecking, striping or blotching against the normal background tending to have a clearly defined boundary delineated by the veins.
4. **Vein-clearing:** is a kind of sub-type of mosaic where tissues close to veins turn yellow and remaining lamina surface remains green.
5. **Vein-banding:** is a kind of sub-type of mosaic where tissues close to veins remain green and rest of the lamina surface turns yellow.
6. **Leaf curl:** is curling of the leaves as a result of over growth on one side of the organ.
7. **Phyllody:** it is a metaplastic symptom where all the floral parts develop into leaf-like structures.
8. **Canker:** A necrotic, often sunken, lesion on a stem, branch, or twig of a plant.
9. **Anthracnose:** A disease that appear as black sunken leaf, stem or fruit lesions, caused by fungi that produced their asexual spores in an acervulus.
10. **Damping off:** Destruction of the seedlings near the soil line, resulting in seedlings falling over on the ground
11. **Mottle:** A symptom in which small but numerous areas of discolouration, commonly chlorotic, irregularly shaped and without sharply defined boundaries, standout against a background of a different tint.
    Yellows: Because of the reduction in chlorophyll synthesis the presence of carotene and xanthophylls becomes evident even in young leaves leading to yellowing.

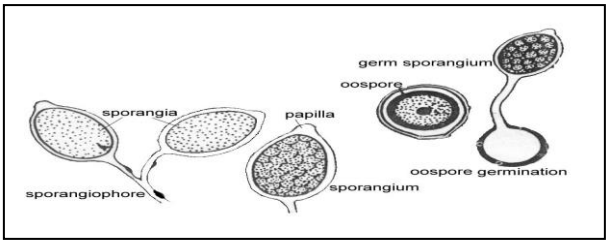
PHYLUM CHYTRIDIOMYCOTA AND OOMYCOTA

1. **Genus *Synchytrium:*** All the species of the genus produce galls on different parts of the plants, particularly on the roots. The imp-ortant species is *Synchytrium endobioticum* which causes was disease of potato. On the potato tubers, the wards are more typical and conspicuous, sometimes covering the whole tuber larger conspicuous, sometimes covering the whole tuber and larger than the tuber itself.

2. **Genus – *Pythium*** (Damping off): **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)



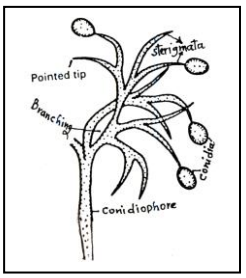
3. **Genus – *Phytophthora:* Mycelium (**Aseptate, coenocytic, branched); **Sporangiophores (**of indeterminate growth, zig-zag, sympodially branched, noduate (with nodular swellings); **Important species (***P. infestans* (Late blight of potato); **Sporangia (**Single celled, lemon shaped and papillate); **Ooospores (**Spherical in shape, smooth walled and aplerotic).



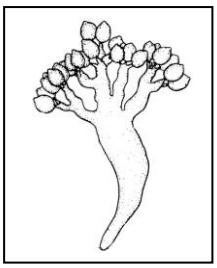
Differences between *Pythium* and *Phytophthora*

S.N	Point of differences	*Pythium*	*Phytophthora*
1.	Haustoria	Absent	Rudimentary
2.	Sporangiophore	Of determinate growth	In determinate growth
3.	Sporangia	Spherical	Lemon shaped and papillate
4.	Vesicle	present	Normally absent
5.	Zoospore formation	In the vesicle	In the sporangium
6.	Oospore	Plerotic type	Aplerotic type
7.	Germination of Oospore	By germ tube	By germ sporangium

4. **Genus – *Peronospora*** (Downy mildew): **Mycelium (**Aseptate, coencocytic, branched, hyaline, endophytic and intercellular); **Conidia (**Single celled, spherical or oval in shape and borne singly); **Branching (Sterigmata-**Dichotomous at acute angles. Last (ultimate) branch is changed into the sterigmata); **Ooospores** (Long and pointed and bearing conidia singly); **Conidiophores (**Spherical and reticulate in Peronospora parastitica (downy mildew of Crucifers).; Arise from the stomatal openings. They are slender, long, 2/3 portion unbranched and only 1/3 portion is branched; **Important species (***Peronospora parastitica* (downy mildew of Crucifers), *P. tabacinia* (downy mildew of tobacco). *P. pisi* (downy mildew of pea).



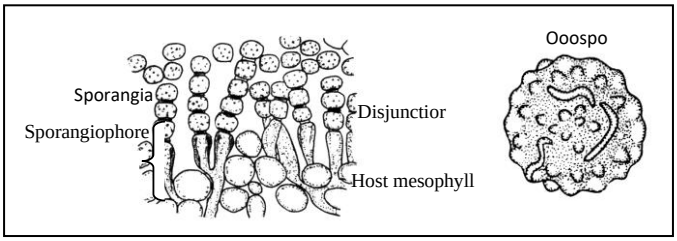
5. **Genus–*Sclerospora:* Mycelium (**Aseptate, coencocytic, branched, hyaline, endophytic and intercellular); **Sporangiophores (**arise from the stomatal openings. They are short and broader towards apex); **Branching –** Dichotomous or even trichotomous. Last branch is changed into the sterigmata); **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 (***Sclerospora graminicola*, which causes green ear disease of Bajra).



Differences between *Peronospora* and *Sclerospora*

S. N	Point of differences	*Peronospora*	*Sclerospora*
1.	Conidiophore / Sporangiophore	Long and slender	Short and broader at apex
2.	Branching	Dichotomous	Dichotomous or even trichotomous
3.	Sterigmata	Long and pointed	Short and swollen
4.	Conidia / sporangia	Conidia are formed	Sporangia are formed
5.	Oospore	Spherical/regular in appearance	Irregular in appearance

6. **Genus – *Albugo*** (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); **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).



PHYLUM ZYGOMYCOTA

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"); **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. stolonifera).*

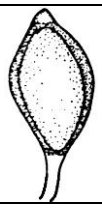
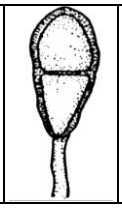
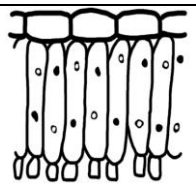
Differences between *Mucor* and *Rhizopus*

S. No.	Point of differences	*Mucor*	*Rhizopus*
1	Stolon	Absent	Present
2	Rhizoids	Absent	Present
3	Sprangiophores	Arise single	Arise in groups from rhizoids
4	Aplanospores	Simple	Striate (marked with lines)

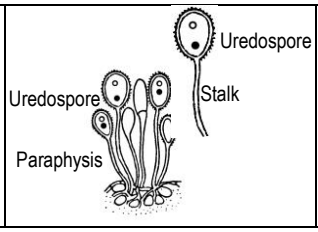
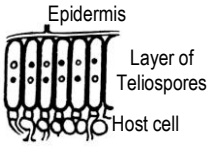
PHYLUM BASIDIOMYCOTA

1. **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).
2. **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 species (*T. penicillariae* (smut of bajra), *T. ehrenbergii* (long smut of jowar)
3. **Genus- *Tilletia*:** 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 elliptial 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 sugarcane).

Teliospores of Rust Fungi

Uromyces		***Puccinia***		***Melampsora***	
• Teliospores are stalked • They are single celled • Apex of teliospores is thickened		Teliospores are bicelled They are stalked		Teliospores single celled, They are sessile and cylindrical in shape Form layer below the epidermis	

Difference between Uredial and Telial stage

Uredial Stage 1. Epidermis ruptured Uredospore 2. Uredospores stalked 3. Uredospores finely echinulate 4. Capitate paraphyses also present		**Telial Stage** 1. Epidermis intact (unbroken) 2. Teliospores sessile 3. They are single celled, cylindrical in shape 4. Teliospores form layer below epidermis	

Phylum Ascomycota

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. Primary sterigmata are flat. Secondary sterigmata are bottle shaped); **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*).
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*).

Differences between *Aspergillus* and *Penicillium*

Point of differences	***Aspergillus***	***Penicillium***
1. Foot cell	Present	Absent
2. Conidiophores	Simple, aseptate	Septate and branched
3. Vesicle	Present	Absent
4. Sterigmata	Two rows	One row
5. Conidia	Echimulate (spiny)	Smooth
6. Perfect stage	*Eurotium*	*Talaromyces*

Class –Sordariomycetes

1. **Genus –** ***Fusarium*****: 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 (**Microconidia – 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) :** 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*****: 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 sp.** *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*).

Dothideomycetes

1. **Genus –** ***Helminthosporium*****: 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 sp. (***H. gramineum* (stripe disease of barley) and *H. oryzae* (brown spot of paddy); **Perfect stage (***Cochliobolus* and *Pyrenophora*).

2. **Genus – *Alternaria*: Conidiophores (**Septate, simple or sometimes 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 sp. (***A. solani* (early blight of potato), *A. brassicae* (Alternaria blight of crucifers), *A. triticina* (Leaf blight of wheat); **Perfect stage** (*Pleospora*).

3. **Genus – *Phoma*:** *Phoma* is similar to *Phyllosticta*; infect we call the same fungus as *Phyllosticta* when it occurs on leaves and *Phoma* when it occurs on the stem or other parts.

4. **Genus – *Phyllosticta*: Mycelium (**Well developed, branched and septate); **Pycnidia (**They are mostly flask shaped, dark, having natural opening known as "Ostiole". Conidia are produced in pycnidia); **Conidiophores (**Short and simple); **Conidia (**Single celled, spherical or oval in shape, hyaline and come out in "Cirrhus" from the ostiole); **Important species (***P. cajani* (leaf spot of pigeonpea); **Perfect Stage (***Mycosphaerella*).

5. **Genus – *Cercospora*: 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 sp**. (*C. personata* and *C. arachidicola*, which cause tikka disease of groundnut); **Perfect stage (***Mycosphaerella*).

Class: Letiomycetes

1. **Genus – *Erysiphe* (Powdery mildew): Mycelium (**Septate, branched, hyaline, ectophytic); **Asexual stage (Conidiophores–** Arise singly, short, septate, straight and simple); **Conidia (**Single celled, barrel shaped, hyaline and formed in basipetal chains); **Sexual stage (Cleistothecia-** Spherical in shape, black, hard, without any natural opening (closed) and provided with appendages); **Appendages (**Many, hypha like (myceloid); **Asci (**Several in a cleistothecium, clavate, with 2, 4 or 8 ascospores); **Ascospores (**Usually spherical or oval, single celled, hyaline and formed in asci); **Important species (***E. graminis tritici* (powdery mildew of wheat), *E. polygoni* (powdery mildew of pea), *E. cichoracearum* (powdery mildew of cucurbits).

2. **Genus - *Sclerotinia*: 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).

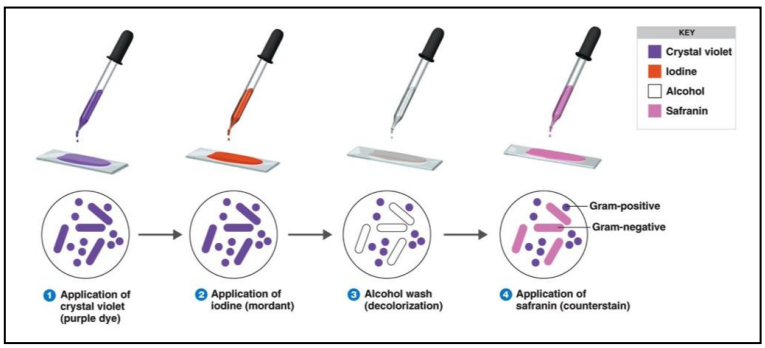
Class: Taphrinomycetes

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 having 8 ascospores); **Ascospores (**Eight in number, mostly located at upper portion of asci, single celled, round or ovoid); Important sp. (*T. deformans* (Peach leaf curl; *T. pruni* (plum pocket).

STAINING AND IDENTIFICATION OF PLANT PATHOGENIC BACTERIA

A. Smear preparation:

1. Take a grease free dry slide.
2. Sterilize the inoculating loop on a flame of a Bunsen burner.
3. Transfer a loopful of culture (or the specimen) by sterile loop and make a smear at the center. Smear should not be very thin or very thick.
4. Allow the smear to dry in the air.
5. Fix the dry smear by passing the slide 3-4 times through the flame quickly with the smear side facing up.



B. Gram-staining procedure:

1. Place the slides on the staining rods.
2. Cover the smear with crystal violet stain and leave for 1 minute.
3. Wash carefully under running tap water.

4. Flood the smear with Gram's iodine solution and leave for 1 minute.
5. Drain off the iodine Wash the slide for the again in a gentle stream of tap water.
6. Flood the slide with the decolorizing agent then wait for 20-30 seconds. This can also be done by adding a drop by drop to the slide until the decolorizing agent running from the slides runs clear.
7. Gently wash the slide under running tap water and drain completely.
8. Counterstain with safranin for and wait for about 30 seconds to 1 minute.
9. Wash slide in a gentile and indirect stream of tap water until no color appears in the effluent and then blot dry with absorbent paper.
10. Observe under microscope.

Gram Positive: Dark purple (*Bacillus, Nocardia, Clostridium, Propionibacterium, Actinomyces, Enterococcus, Cornyebacterium, Listria, Lactobacillus, Gardnerella, Mycoplasma, Staphylococcus, Streptomyces, Streptococcus etc.*)

Gram Negative: Pale to dark red (*Escherichia, Helicobcater, Hemophilus, Neisseria, Klebsiella, Enterobacter, Chlamydia, Vibrio, Pseudomonas, Salmonella, Shigella).*

TRANSMISSION OF VIRUSES

Sap transmission

1. Young TLCV affected leaves of tomato plants are washed with tap water and dried with blotting sheet
2. Leaves are weighed. Usually 10g leaf is preferred.
3. For Preparation of standard extract potassium phosphate buffer volume equal to the weight of leaves (V/W, 1:1), is added into a mortar and leaves are ground with the pestle. (So, 10ml buffer is added).
4. After thorough grinding, the whole leaf pulp is passed through double layers of muslin cloth to get filtered standard extract of the leaves. This is best accomplished by pressing the juice (extract) through the muslin cloth used to hold the extract. The expressed juice (sap), which will contain the infectious principle, is used as inoculum.
5. Only young rapidly growing plants of urdbean showing distinct primary leaf stage (7-8 days after emergence) should be selected for inoculation.
6. Carborandum powder is slightly sprinkled on the leaves of test plants. This can be best done using a small sterilized cotton swab previously just touched with the carborandum powder separately taken in test tube or Petri dish.
7. The primary leaves of the test plants are inoculated by rubbing the sap over the leaf surface with quick, gentle strokes. Best result is observed if primary leaves are inoculated both sides i.e., upper surface as well as down surface.
8. Keep inoculated plants with proper label in the glasshouse for observations.
9. Keep regular watch and make observations every alternate day. ULCV will be seen transmitted systemically on the third trifoliate first after about 13-16 days after inoculation.

PHANAEROGAMIC PLANT PARASITES

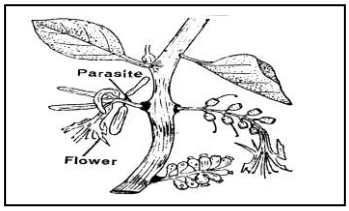
Some of the species of higher plants are known to live parasitically on other plants. These parasitic plants produce flowers and seeds also. They attack valuable crops and trees causing considerable damage. Some of these parasites attack roots of host while others parasitise stem. Some are devoid of chlorophyll (total paraistes) and entirely depend on host for nutrition while others have chlorophyll (semi parastic) but no true roots and obtain water and mineral constituent of food from the host.

The common parasitic flowering plants can be grouped as follows:

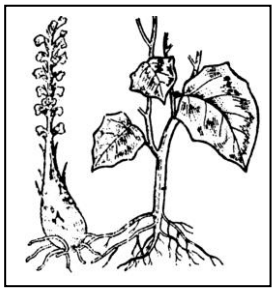
I. Stem parasites	**II. Root parasites**
a) Total parasite - *Cuscuta* sp. (Dodder)	a) Total parasite - *Orobanche* sp. (Broom rape)
b) Semi-parasite - *Dendrophthoae* sp. *(*Loranthus*)*	b) *Semi-parasite - Striga* sp.(Witch's weed)

DODDER *(Cuscuta* sp.*)*- Stem Total parasite: This is a non-chlorophyllous, leafless, twining parasitic seed plant, which attaches its yellow, orange or pink, thread-like stem to stem or other parts of (cultivated or wild plants). Leaves are represented by minute scales. It sends minute root like organs (haustoria) to the host cortex, which serve as an anchor as well as organs of food absorption. It bears tiny, white, pink or yellow flowers in cluster. Clover, berseem, flax and many oilseed crops are commonly attacked. The common is am important species which Cuscuta gronovii attacks garden, ornamental and hedge plants.

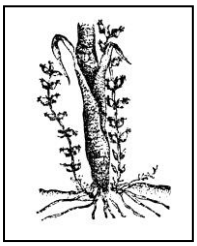
LORANTHUS (*Dendrophthiae* sp.)- Stem Semi-parasite: It is a common parasite of fruit trees. The parasite attacks aerial parts of host trees. It is devoid of a true root system of its own and hence, is dependent on host for water and mineral. Leaves are leathery and evergreen and possess chlorophyll. The stem is thick, erect or flattened at the nodes and appear to arise in cluster at the point of attack. Flowers are borne in clusters. They are long and tubular in shape and greenish-white or red in colour. The infected area of



host becomes swollen and forms attachment disc. *Dendrophthae falcate*, is an important and species.



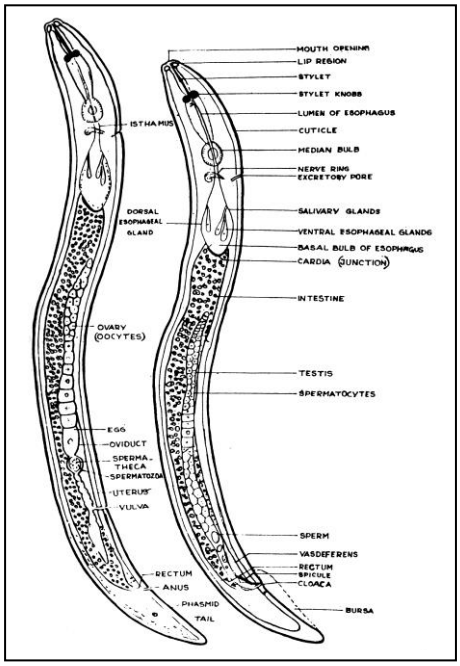
BROOMRAPE *(Orohanche* sp.): Root Total parasite: It is a total root parasite affecting tobacco, brinjal, tomato, cabbage, cauliflower, turnip and many other Solanaceous and Cruciferous plants. The parasite consists of stout, fleshy stem, 15-20 cm tall. Stem is pale yellow or brownish-red in colour and covered by small, thin and brown scaly leaves. Flowers appear in axil of scales and are white and tubular. A large number of parasitic stems may be seen. *Orobanche ramosa is* an Import species



WITCH'S WEED (*Striga* sp.): Root Semi parasite: Witchweed is a well-known semi-parasite of sugarcane, cereals, maize and millets in India. The parasite is a small plant, 15-30 cm tall with bright green, slightly hairy stem and leaves. Leaves are narrow, long and in opposite pairs. The flowers are small and usually brick red or scarlet, although some may be yellowish-red, yellowish or almost white. The seeds are borne in a capsule and are very minute to see with naked eye. Infected roots bear a large number of witch's weed haustoria, which are attached to root to feed on it. *Striga asiatica* is an important species.

PLANT PARASITIC NEMATODES

Morphology: Adult plant parasitic nematodes are elongated worms ranging in length from about 0.30mm to over 5.0mm. The anterior end tapers to a rounded or truncated lip region, the body proper is more or less cylindrical, and the posterior and tapers to a terminus which may be pointed or hemispherical Proportions of the elongated body vary greatly. Females have greatly expanded bodies, sometimes nearly spherical, but always with a distinct neck. The adult males are always slender worms. Plant parasitic nematodes have no appendages. The mouth of a nematode is at the ANTERIOR end, and the terminus is at the POSTERIOR end. The excretory pore, vulva, and anus are on the VENTRAL side; and the opposite side is called DORSAL. The right and left sides are called LATERAL. The cuticle is attached to several other layers of tissue, which are separated laterally, dorsally and ventrally by chords. These contain nerves, excretory organs, etc., and separate four bands of muscles, which move the body.



A. **Alimentary canal –** The alimentary canal starts at the mouth and ends at the and ends at the anus. It includes the oesophagus, intestine, intestine, and rectum

B. **Stylet –** In plant parasitic nematodes of the "Tylenchida" group, the mouth contains a stylet or mouth spear, a hardened, hollow, culticular structure similar to a hypodermic needle. Muscles are attached to three knobs at the posterior end of stylet and extend forward. They are used to pull the stylet forward so that it projects from the mouth opening and can be used to pierce plant cells. The food of the nematode is taken through the stylet.

C. **Oesophagus –** A slender tube is attached to the posterior end of the stylet. This is the oesophageal tube leading to the median bulb, which in turn is attached by means of another slender tube to the intestine. Posterior to the median bulb, the oesophagus contains three glands, one dorsal and two subventral, each with a nucleus. Three glands may form a terminal bulb to which the intestine is attached, or may form a lobe lying alongside the intestine. In either case, the dorsal gland has a duct leading anteriorly through the median bulb and connecting with the oesophageal tube. The connection is called the dorsal gland orifice.

D. **Dorsal Gland Orifice –** This in most species of plant parasitic nematodes is located behind the stylet at a distance seldom exceeding the stylet length and generally much closer. At this point there is an opening into the oesophageal tube and often an abrupt bend in it.

E. **Median bulb –** The median bulb contains a "valve" to which muscle fibres are attached. In cross-section, this structure is tri-radiate. When activated by muscles, it functions as a pump, sucking food through the stylet and forcing it into intestine.

F. **Intestine –** It is a simple tube with walls one cell thick. It functions as a storage organ and is usually filled with globules of fatty substances. Posteriorly it narrows to a rectum, which terminates at the anus.

G. **Excretory system –** Nematodes have an excretory system, but in the plant parasites, the only part usually seen is a section of the excretory tube leading to the excretory pore.

SAMPLING AND EXTRACTION OF NEMATODES FROM SOIL AND PLANT MATERIAL

Plant-parasitic nematodes can be extracted from soil and plants materials in many different ways. Some methods are more effective than others for particular types of nematodes or for special kinds of plant materials. The method effectively depends upon the type of nematodes or kind of plant materials. For extraction of nematodes from plants, roots, tubers, bulbs, leaves, stem, crowns etc. are used. Baermann Funnel or mist extraction is the most effective techniques.

Baermann Funnel:

1. This method is an excellent system of separating specimens in roots and also soil and condensing them for examination.
2. Place about a handful of root (5-10g)/ soil (50 g) on a two-layered tissue paper on top of wire screen.
3. Place the envelope root/soil sample on a Baermann funnel in the rack.
4. Fill the funnel up to the rim with water
5. Collect 10-20 ml of suspension after 24-48 hours.
6. Nematodes are ready for counting and identification.

Advantages: The technique is simple and the equipment is inexpensive. Recovery of active nematodes from very small samples is fairly good.

Disadvantages: Lack of aeration in the water reduces the movement of nematodes, thus hindering their recovery. Recovery of active nematodes from large samples is poor. The funnel capacity is less, hence may be too small to be a representative.

Decanting and sieving Method

Materials: 20-mesh sieve (833 µm aperture); 200-mesh sieve (74 µm aperture); 325-mesh sieve (43 µm aperture); Coarse sieve (1 cm aperture); Two stainless steel bowls or plastic buckets; 250 ml beaker; 600 ml beaker; Coarse spray water bottle

Procedure

1. Review Potential extraction errors
2. Mix the soil sample and pass through a coarse sieve to remove rocks, roots etc
3. Take a 600 cc sub-sample of soil and pack lightly into a beaker for uniformity
4. Place soil in one of the buckets or pans half-filled with water.
5. Sieving and decanting process (various combinations of the following):

a. Mix soil and water by stirring with hand or paddle. Allow to stand until water almost stops swirling
b. Pour all but the heavy sediment through a 20-mesh sieve into a second bucket and discard the residue in first bucket. Discard the material retained on the sieve
c. Stir the material present in second bucket; allow to stand until water almost stops swirling
d. Pour all but the heavy sediment through a 200-mesh sieve into the first bucket, discard the residue in the second bucket.
e. Backwash the material retained on a 200-mesh sieve, which includes large nematodes, into a 250 ml beaker.
f. Stir the material in the first bucket. Allow to stand until water almost stops swirling.
g. Pour all but the heavy sediment through a 325-mesh sieve into a second bucket; discard the residue preset in the first bucket.
h. Backwash the material retained on a 325-mesh sieve, which includes small to mid-sized nematodes and silty material, into a 250 ml beaker.
i. Sample present in the 250 ml beaker will probably be too dirty for direct viewing. So, it may be placed on Baermann Funnel or subjected to sucrose-centrifugation. This combined procedure allows the extraction of nematodes from larger volumes of soil.

Advantages: The method is not dependent on nematode movement; sluggish nematodes are recovered. It allows the recovery of most nematodes from large soil samples. Nematodes are available for direct examination in less than half an hour.

Disadvantages: The method requires expensive sieves and an experienced worker. Nematodes are difficult to see because of fine particles.

NEMATODE MOUNTING

Step1. Killing and fixing nematodes: Collect live nematode specimens in distilled or deionized water in a small beaker or watch glass. Concentrate the nematodes in a minimal volume of water and add equal volume of hot (90C) fixative solution and buffered formalin (Humason, 1972) to it. Nematodes may be killed with heat before adding fixative, though adding hot fixative directly is also effective. Buffered formalin provides very good fixation. Leave the specimens in the fixative for 1-2 days. Nematodes may be stored in buffered formalin indefinitely; it does not clear characters. Buffered formalin solution is prepared as follows:

Formalin (ca 40% formaldehyde)-10.0 ml; Water-90 ml; Sodium acid phosphate-0.4 g; Anhydrous disodium phosphate-0.65 g

Step II. Processing Specimens to glycerin

1. Prepare the following two solutions and keep them at room temperature

Seinhorst I solution: 20 parts 95% ethanol; 1 parts glycerin; 79 parts water

Seinhorst II solution: 95 parts 95% ethanol; 5 parts glycerin

Place fixed nematodes in a BPI dish. Draw-off excessive fixative and concentrate the nematodes in a small volume. Add 6-8 ml of Seinhorst solution I to the nematode suspension. (A very small quantity of rose Bengal, acid fuchsin, or aqueous picric acid may be added to the solution to stain the nematodes. This is optional. Place the open BPI dish in a larger closed glass container with 95% ethanol at the bottom, and place in oven at 35-40C for at least 12 hours. This removes most of the water in the BPI dish. (Do not close or allow ethanol from the glass container to over-flow into the BPI dish.). Remove the dishes from oven and draw-off the excess Seinhost solution1 from the BPI dish using a pipette under a dissecting microscope to avoid loss of specimens. Add Seinhorst solution 2 to the BPI dish, place it in a partially covered Petri-dish and keep it in oven at 40C.
Several hours (at least 3 hours) later, draw-off excess solution from the BPI dish and repeat step 5. Keep the dishes in oven until all the alcohol has evaporated (at least 3 hours) and nematodes are in pure glycerin.

Step III Mounting nematodes

A. Temporary Mounts

1. Place a small drop of the fixative in the center of a clean glass slide.
2. Using a nematode pick under a dissecting microscope, pick up the desired specimens and place them in the fixative on the center of the slide.
3. Place the slide under the dissecting microscope, and arrange the nematodes in the centre of the slide and bottom of the drop.
4. Place glass wool (about 5mm in length) or glass microbeads in a triangular position near the edge of the drop.
5. Place a cover glass (18mm wide) gently over the drop using a forcep or supporting it with a needle. Draw off excess fixative carefully using filter paper.

Permanent Mounts

1. Fix a clean cover glass (25mm wide) in the center of a Cobb aluminium slide by supporting with appropriate size white cardboard pieces.
2. Place a small drop of anhydrous glycerin in the centre of the cover glass on the aluminium slide.
3. Pick up nematodes from the fixative, as in step 2 of (A), and place them in the glycerin drop.
4. Arrange the nematodes in the center of the slide and place glass wool as in steps 3-4 of (A).
5. Carefully place a cover glass (18mm wide) over the drop, and seal the edges of the cover glass as in steps of 5-6 of (A).
6. After the sealant has dried, a second coat of sealant may be added. Allow to dry, label the slides on the white cardboard, and examine under a compound microscope. Excess of glycerin on the slide is difficult to remove and can cause smudges, which interferes with the sealing process.
7. Store the slides in a flat position to avoid settling of nematodes towards the edge of the cover glass.
8. Use of aluminum slides enables viewing of the nematodes from both sides of the slides.

FUNGICIDES AND THEIR FORMULATIONS

Terminology:

Fungicide: The word is derived from the latin word *caedo* – to kill and the first term is fungus. Therefore, a fungicide is any agency that has the ability to kill a fungus e.g., heat, acid, UV-rays, light etc. However, in general the fungicide is defined as those chemicals capable of preventing infection of living plants by phytopathogenic fungi. Similarly this term could be applied in the case of Bacterial disease as Bactercides and in the case of Nematode infection it is Nematicides**.**
Fumigants: These are volatile chemicals applied into confined spaces or into a soil, which produces gas that destroys weed seeds and microorganisms and acts as a soil sterilant. The most common soil sterilants are methyl bromide, methane, allyl alcohol, carbon disulphide, chloropicrin and tetrachlorethane. They are packed in special pressure-resistant containers.
Fumigation: The application of a fumigant for disinfestation of an area.
Fungicidal Dispenser: An individual who has been certified to engage in the retail sale of fungicides for a licensed dealer.
Fungicidal: Killing fungal spores or mycelium. Applicable to physical agents such as heat, ultraviolet light, x-rays, gamma-radiation etc., as well as to chemicals that are lethal at low concentrations.
Fungicide Dealer: A person or firm holding a license to retail fungicides.
Fungicide, Applicator: An individual who provides services involving the use or application of fungicides.
Fungicide, Eradicant: (1) (curative fungicide) A fungicide used to control disease after infection has occurred. (2) A fungicide applied to a substratum in which the fungus is already present.
Fungicide, Protective: A fungicide used to protect an organism against infection by a fungus.
Fungicide, Residue: Fungicide remaining on or in a plant.
Fungicide, Systemic: A fungicide, which is absorbed through a plant surface and is translocated away from the site of application.
Fungistatic: Certain chemicals may temporarily inhibit fungus spore germination without being lethal. They are known as fungistatic. So, preventing the growth of a fungus without killing it.
Fungistat: A substance preventing the growth of a fungus without killing it.
Fungistatis (mycostasis): The prevention of fungal growth. The effect is reversible; if the inhibitor is removed or diluted, growth is resumed, cf. Fungicidal. In a broad sense the term can be applied to the non-germination of fungal spores due to the presence of auto-inhibitors or inhibitors from another organism or the substratum.

Formulations of fungicides:

1. **Wettable powder** is a very common formulation for most of the fungicides, which is used for spray mixtures. The modern wettable powders are water-dispersible, which have the quality to wet easily and disperse well in water. They are also called as Water-Dispersible Powders (WDP). The active ingredient is incorporated, usually at the rate of 30-80%, with a finely ground inert dust (filler) such as Kaolin, a wetting agent and a suspending agent.

2. **Dust formulations** usually contain 1-10% active ingredient for direct application in dry forms. They are manufactured in such a way that they are light enough to be carried by a slight breeze for a considerable distance. The finely divided particle of active ingredient is carried on a carrier particle. The commonly used carriers (diluents) are attapulgite, kaolin, talc, pyrophylite, diatomaceous earth, bentonite, calcium silicate, hydrated silica, calcium carbonate, magnesium carbonate, gypsum, lime etc.

3. **Water dispersible Powders (WDP).** The active ingredient is incorporated, usually at the rate of 30-80%, with a finely ground inert dust (filler) such as Kaolin, a wetting agent and a suspending agent. The commonly used suspending agents are sodium lignin sulphonate (Sulphite dye), methyl celluloses, polyvinyl acetate and aluminium silicate. In addition, spreader-sticker is sometimes desirable, especially on plants with glossy or waxy leaves. Agitation is generally necessary to keep uniform suspension.

4. **Granules (Pellets)** are the formulations of the fungicide with inert materials formed into particles about the size of coarse sugar. The granules normally contain 3-10% of the active ingredient. Due to their size, the granules do not drift but have limited application being confined to soil and seed treatments. Granules have the advantage they can be measured in dry form more easily and accurately than dusts or wettable powders. These are formulation in which a dry form of the active ingredient is mixed with a liquid. Such formulations usually contain a high percentage of active ingredient similar to wettable powders. They are mixed with water for final use and require agitation. These are mostly used as seed dressers in seed processing companies.

5. **Solutions** are formulations in which active ingredient or a combination of active ingredients and a solvent is dissolved in water solutions. This has the advantage of requiring no agitation after formulation is added in water.

6. **Suspension or slurries** are formulation in which a dry form of the active ingredient is mixed with a liquid. Such formulations usually contain a high percentage of active ingredient similar to wettable powders. They are mixed with water for final use and require agitation. These are mostly used as seed dressers in seed processing companies.

Preparation of fungicidal solutions

1. **Bordeaux mixture:** One kg of copper sulphate is powdered and dissolved in 50 litres of water. Similarly, 1 kg of lime is powdered and dissolved in another 50 litres of water. Then copper sulphate solution is slowly added to lime solution with constant stirring or alternatively, both the solutions may be poured simultaneously to a third contained and mixed well.

   Merits: Its natural tenacity to the plants. Its relative cheapness. Its utility in controlling wide variety of diseases. Somewhat non-toxic to human beings and cattle.
   Demerits: Its phytotoxic nature on certain plants like paddy, apples, peaches etc. It causes delay in ripening of fruits. The preparation is not very much practicable under field conditions. It's corroding action on metallic containers of spraying equipment. It is very much useful against a number of diseases like downy mildews, bacterial citrus canker etc.

2. **Bordeaux paste:** Bordeaux Paste consists of same constituents as that of Bordeaux mixture, but it is in the form of a paste as the quantity of water used is too little. It is nothing but 10 per cent Bordeaux mixture and is prepared by mixing 1 kg of copper sulphate and 1 kg of lime in 10 litres of water. The method of mixing solution is similar to that of Bordeaux mixture. Wound dresser used to protect the wounded portions, cut ends of trees etc., against the infection by fungal pathogens.

3. **Burgundy mixture:** It is prepared in the same way as Bordeaux mixture, except the lime is substituted by sodium carbonate. So it is called as 'Soda Bordeaux'. It was developed Burgundy (France) in 1887 by Mason. The usual formula contains 1 kg of copper sulphate and 1 kg of sodium carbonate in 100 litres of water. It is a good substitute for Bordeaux mixture and used in copper-sensitive crops.

4. **Cheshnut compound:** It is compound usually prepared by mixing 2 parts of copper sulphate and 11 parts of ammonium carbonate. This formula was suggested by Bewley in the year 1921. The two salts are well powdered, mixed thoroughly and stored in an air tight container for 24 hours before being used. The ripened mixture is used by dissolving it in water at the rate of 3 g/litre. Mixture is dissolved in a little hot water and volume is made up with cold water and used for spraying.

5. **Chaubattia Paste:** Chaubattia paste is another wound dressing fungicide developed by Singh in 1942 at Government Fruit Research Station, Chaubattia in the Almora. It is usually prepared in glass containers or chinaware pot, by mixing 800g of copper carbonate and 800g of red lead in litre of raw linseed oil or lanolin. This paste is usually applied to pruned parts of apple, pear and peaches to control several diseases. The paste has the added advantage that it is not easily washed away by rain water.