E-Practical Manual

Fundamentals of Plant Pathology

Course No. APP 211

B. Sc. (Hons.) Horticulture

Credit Hours: 3(2+1)

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Syllabus

Theory

Introduction to the science of Phytopathology, its objectives, scope and historical background. Classification of plant diseases, symptoms, signs, and related terminology. Parasitic causes of plant diseases (Fungi, Bacteria, Viruses, Phytoplasma, Protozoa, Algae and flowering parasitic plants), their characteristics and classification. Non-parasitic causes of plant diseases. Infection process. Survival and dispersal of plant pathogens. Plant disease epidemiology, forecasting and disease assessment. Principles and methods of plant disease management. Integrated plant disease management. Fungicides classification based on chemical nature, commonly used fungicides, bactericides and Nematicides.

Practical

Familiarity with general plant pathological laboratory and field equipments. Study of disease symptoms and signs and host parasite relationship. Identification and isolation of plant pathogens. Koch's postulates. Preparation of fungicidal solutions, slurries, pastes and their applications.

Name of the student .....

Id No.: .....

Batch: .....

Session: .....

Semester: .....

Course Name: .....

Course No.: .....

Credit: .....

Published: 2023

CERTIFICATE

This is to certify that Mr. /Ms. ..... Id No.: ..... has completed the practical of course .....Course No. ..... as per the syllabus of B. Sc. (Hons.) Horticulture ..... semester in the year ..... in the respective laboratory/field of college.

Date: Course Teacher

Index

Practical No. 1

Objective: To get familiar with general Plant Pathological laboratory equipment's.

The students in batches will visit the laboratory of Plant Protection 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 laboratory equipment's available in the Plant Protection **Laboratory:**

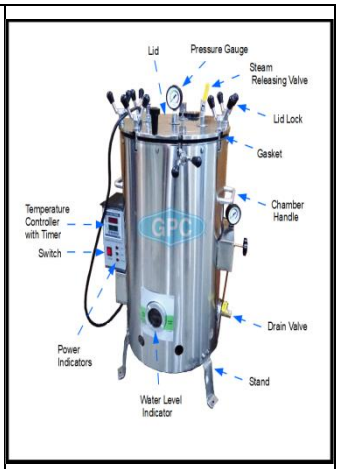
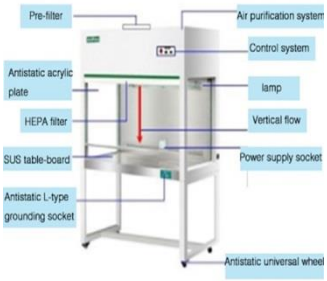
(a) Laboratory appliances/tools

(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)	
(xiii)		(xiv)	
(xv)		(xvi)	

2. Label the following laboratory equipment's and state its principle and functions.

Autoclave:	
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 labelled diagram of a compound microscope and indicate all the important parts

Functions of each part:

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Precautionary measures that need to be taken while handling the 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: Prepare Potato Dextrose Agar medium. Describe quantity and procedure 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 diseases plant tissues.

Isolate and identify plant pathogens from infected plant sample

Materials required: .....

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Procedure for isolation: .....

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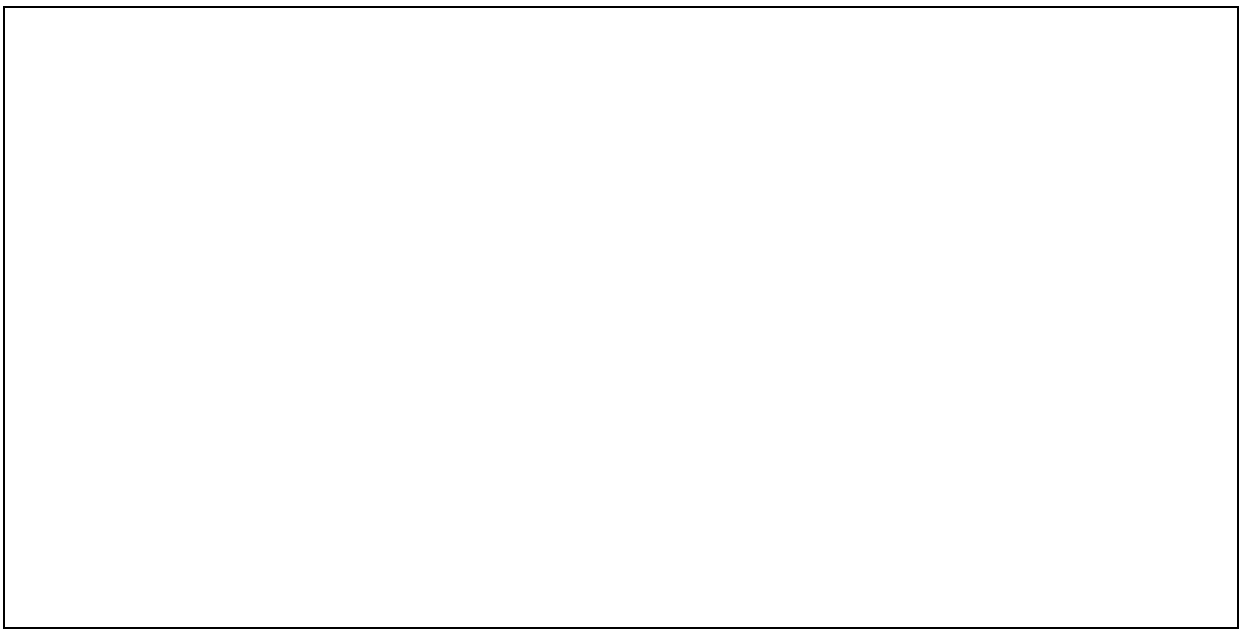
Steps for isolation of pathogen from plant tissues – flow chart



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

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

Material required: .....

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Types of mycelium: .....

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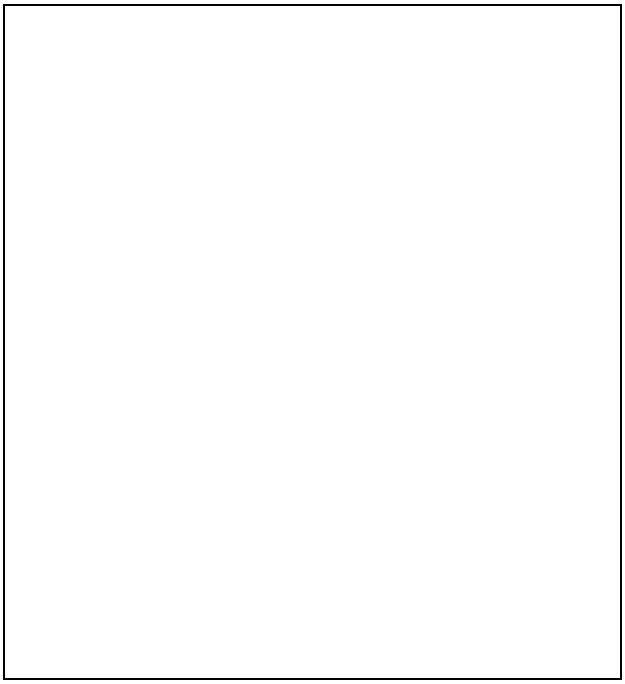

Types of mycelium: .....

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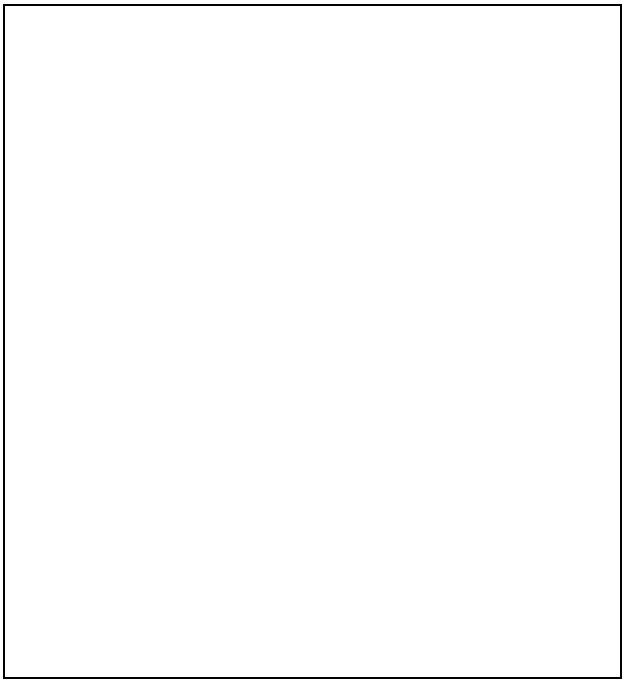

Practical No. 8

Objective: Identification of different types asexual fruiting bodies, sexual spores and ascocarps.

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. 9

Objective: Identification of plant disease symptoms

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

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Practical No. 10

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

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

1. Systematic position:

Genus: *Synchytrium* **Features:** …… …… …… …… …… …… …… ……	
Genus: *Pythium* **Features:** …… …… …… …… …… …… …… ……	
Genus: *Phytophthora* **Features:** …… …… …… …… …… …… ……	

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

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

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 labelled diagram of the spore along with conidiophores.

Systematic position	Systematic position
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.....	.....
.....	.....
.....	.....
.....	.....
.....	.....

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

Diagram	Diagram

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

Systematic position
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Characteristic	**Description**
Mycelium	.....
	.....
	.....
Sporangiophores	.....
	.....
	.....
Sporangia	.....
	.....
	.....
Oospores	.....
	.....
	.....

Diagram

Practical No. 11

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 labelled diagram of their spores.

Systematic position	**Systematic position**
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.....	.....
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.....	.....

Characteristic	**Description**
Mycelium	.....
	.....
	.....
Sporangiophores	.....
	.....
	.....
Sporangia	.....
	.....
	.....
Columella	.....
	.....
	.....
Alpanospores	.....
	.....
	.....
Zygospores	.....
	.....
	.....

Diagram	Diagram

Practical No. 12

Objective: identification of the plant pathogens of Phylum- **Basidiomycota**

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

Genus: *Uromyces*

Systematic position	Morphology	Fig.
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Genus: *Melampsora*

Systematic position	Morphology	Fig.
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Genus: *Ustilago*

Systematic position	Morphology	Fig.
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Genus: *Tilletia*

Systematic position	Morphology	Fig.
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.....	.....	

Genus: *Puccinia*

Systematic position	Morphology	Fig.
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Practical No. 13

Objective: Identification of the plant pathogens of Phylum-Ascomycota

1. Class: Eurotiomycetes

Genus: *Aspergillus*	**Genus:** *Penicillium*
Systematic position	**Systematic position**
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.....	.....
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Characteristics	***Aspergillus* spp.**	***Penicillium* spp.**
Mycelium		
	.....	.....
	.....	.....
	.....	.....
Foot cell		
	.....	.....
	.....	.....
	.....	.....
Conidiophore		
	.....	.....
	.....	.....
	.....	.....
Vesicle		
	.....	.....
	.....	.....
Sterigmata		
	.....	.....
	.....	.....
	.....	.....
Conidia		
	.....	.....
	.....	.....
	.....	.....
Perfect stage		
	.....	.....
	.....	.....
	.....	.....

Diagram	Diagram

2. Class: Sodariomycetes

Genus: *Fusarium*	**Genus:** *Claviceps*
Systematic position	**Systematic position**
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.....	.....
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Characteristics	***Fusarium* spp.**	***Claviceps* spp.**
Mycelium		
	.....	.....
	.....	.....
	.....	.....
Sporodochia		
	.....	
	.....	
	.....	
Conidiophore		
	.....	
	.....	
	.....	
Conidia		
	.....	.....
	.....	.....
Chlamydospore		
	.....	
	.....	
	.....	
Sclerotia		
		.....
		.....
		.....
Perfect stage		
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	.....	.....

3. Class: Hyphomycetes | Coelomycetes

Genus: *Pyricularia* | **Genus:** *Colletotrichum*

Systematic position | **Systematic position**

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Characteristics	***Pyricularia* spp.**	***Colletotrichum* spp.**
Mycelium		
	……	……
	……	……
	……	……
Conidia		
	……	……
	……	……
	……	……
Conidiophore		
	……	……
	……	……
	……	……
Acervuli		
	……	……
	……	……
Perfect stage		
	……	……
	……	……
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Diagram	Diagram

Class: Dothideomycetes

Genus: *Alternania*	**Genus:** *Helminthosporium*
Systematic position	**Systematic position**
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Characteristics	***Alternaria* spp.**	***Helminthosporium* spp.**
Conidiophore		
	.....	.....
	.....	.....
	.....	.....
Conidia		
	.....	.....
	.....	.....
	.....	.....
Perfect stage		
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Diagram	Diagram

Genus: Phyllosticta

Systematic position

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Genus: Cercospora

Systematic position

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Characteristics	***Phyllosticta***	***Cercospora***
Mycelium		
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	……	……
	……	……
Conidiophore		
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Pycnidia		
	……	……
	……	……
	……	……
Conidia		
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	……	……
	……	……
Perfect stage		
	……	……

Diagram	Diagram

Class: Letiomycetes

Genus: *Erysiphe*	**Genus:** *Sclerotinia*
Systematic position	**Systematic position**
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Characteristics	***Erysiphe***	***Sclerotinia***
Mycelium		
	.....	.....
	.....	.....
	.....	.....
Asexual stage	.....	.....
Conidiophore		
	.....	.....
	.....	.....
	.....	.....
Conidia		
	.....	.....
	.....	.....
	.....	.....
Sexual stage		
	.....	.....
Cleistothecia		Apothecia.....
	.....	.....
	.....	.....
	.....	
Appendages	.....	Sclerotia.....
	.....	.....
	.....	.....
Asci	.....	.....
	.....	.....
	.....	.....
Ascospores	.....	.....
	.....	.....
	.....	.....

Diagram	Diagram

Class: Taphrinomycetes

Genus: *Taphrina*

Systematic position

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Characteristics	***Taphrina***
Mycelium	
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Asci	
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Ascospores	
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Diagram

Practical No. 14

Objective: Staining and identification of plant pathogenic bacteria

1. Prepare smear of given material (bacteria) and perform Gram-staining and identify on the basis of Gram staining. Write the procedure of Gram's staining.

Material required: .....

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Procedure:

A. **Smear preparation:** .....

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B. **Gram staining:** .....

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Observations:

Sl. No.	Colour of stain	Gram reaction

Practical No. 15

Objective: To demonstrate sap transmission of viruses

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

Materials required: .....

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Procedure: .....

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Observations:.....

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Practical No. 16

Objective: To identify Phanerogamic plant parasites.

Identify the plant parasitic phanerogamic plants. Record their different host and parasitic nature

Practical No. 17

Objective: 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. **Cheshunt compound:** .....

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E. **Chaubattia Paste:** .....

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Precautionary measures: .....

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Practical No. 18

Objective: Calculation of fungicidal spray concentrations.

1. The recommended doses of the following fungicides for dry seed treatment are as follows: Vitavax Power 0.1% Thiram 0.2% Captan 0.25%

Calculate the required amount of each fungicide foe 5 kg seed.

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

Material required: ……………………………………………………………………………

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Doses of fungicides: Mancozeb @ 0.2%, Hexaconazole @ 0.1% COC @ 0.3%

Note: For one hectare spray the water requirement is 800 litres.

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Practical No. 19

Objective: Methods of application of fungicides and their safe use

1. Write down different methods of application of fungicides.

Appendices

General plant pathological laboratory equipment

a. Laboratory appliances/tools:

1.	Autoclave	6.	Hot-Air Oven	11.	Scissor	16.	Sprit lamp
2.	Freeze	7.	BOD incubator	12.	Cork-borer	17.	Forceps
3.	Hot plate	8.	Pan (different size)	13.	Needle	18.	Rotary shaker
4.	Knife/Blade	9.	Scalpel	14.	Bearing blender	19.	Glass marker
5.	Inoculating needle	10.	Laminar air flow	15.	Gel Electrophoresis	20.	Centrifuge

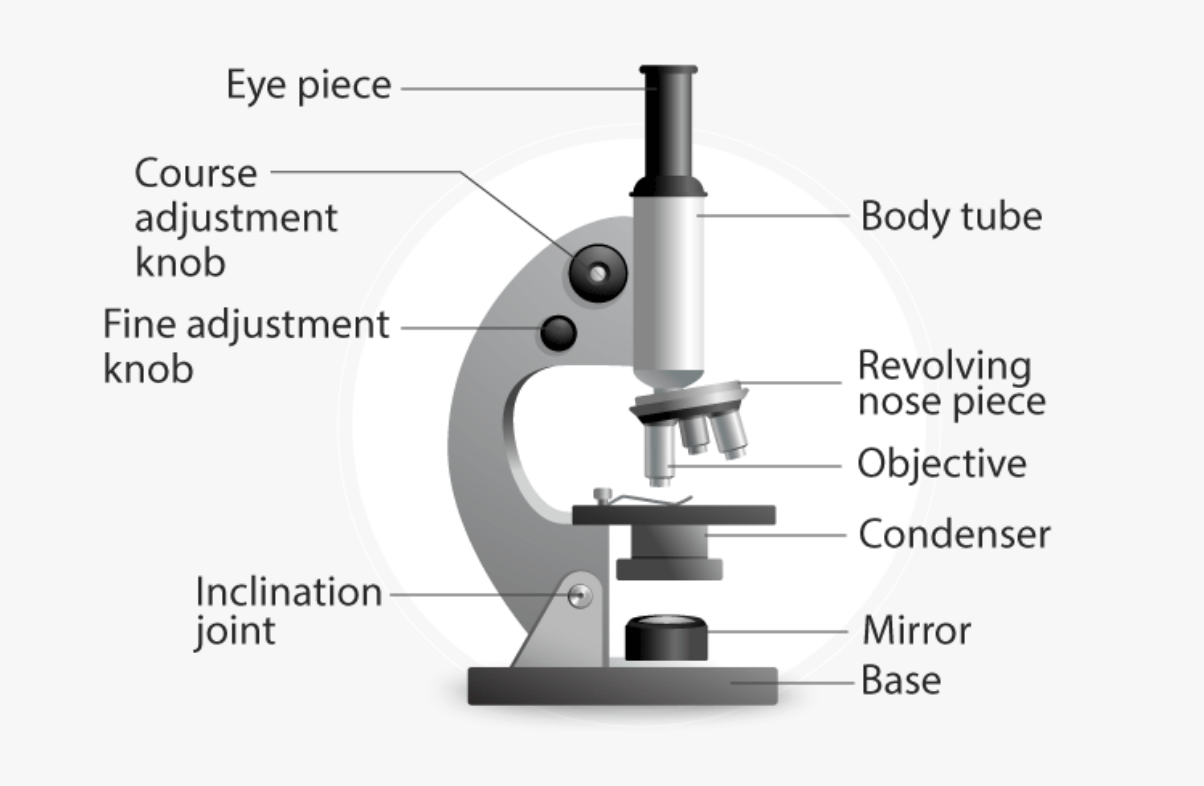
b. Glass-wares:

1.	Conical Flask	5	Beaker	9	Slides
2.	Measuring cylinder	6	Pippete	10	Watch glass
3.	Petri-dishes	7	Culture tube	11	Dropping bottle
4.	Cover slip	8	Nematode counting dish	12	Baermann funnel

c. Miscellaneous

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.	Sieves	8	Rubber bands	12	Mortar and pestle

Microscope



Compound Microscope

Types of microscopes:

1. Simple microscope (Magnifying glass)
2. Student Compound Microscope
3. Stereoscopic Microscope
4. Electron Microscope (SEM & TEM)
5. Simple dissecting Microscope (Monocular)
6. Compound Microscope (Binocular)
7. Phase Contrast Microscope

Compound Microscope: A compound microscope consists of more than one lenses fitted one above the other at a proper distance (160 mm) in a cylindrical tube. An object magnified by one lens (Objective) is further magnified by another lens (eye piece).

Different parts of a Compound Microscope:

1. **Eyepiece:** It is a lens that fit into the top of body tube. It is also called ocular lens. It is usually marked with 6x, 10x or 15x that means it can magnify the object 6, 10 or 15 times.
2. **Drawtube:** This is a small cylindrical tube on the top of which eyepiece is fitted.
3. **Body Tobe:** It is a hollow cylindrical tube attached to upper end of the arm on which it can be moved up and down with the help of course adjustment knob.
4. **Arm:** It is a curved structure used for holding the microscope.
5. **Course adjustment knob:** This is used to locate the object by objective.
6. **Fine adjustment knob:** Mostly fitted below the course adjustment knob. It is used when object is viewed either under high power or under low power for getting sharp and distinct view.
7. **Inclination joint:** It is the point where microscope with the stage and body with two lenses can be bent to a comfortable angle for smooth and strain free observation. This point lies close to junction stage and arm.
8. **Nose piece:** It is disc like body fitted at lower end of the body tube. It has provision for three lenses. It can be removed also.
9. **Objectives:** They are the lenses of different magnifications, screwed in the nose piece. Objective is also marked with 6x, 10x, 40x, 100x etc.
10. **Stage:** A flat rectangle or square plate with round aperture in the centre of the stage.
11. **Clip:** Two clips on either side of the aperture on the stage for holding the slide.
12. **Mechanical device:** Slide is fitted in it, which can be moved forward, backward, right and left to locate the object.

13. **Diaphragm:** It is a circular plate with several holes of different diameter and is attached undemeath the stage. It can also be rotated so as to bring the hole of the diaphragm in front of the hole in the stage. It regulates the quantity of light towards body tube.
14. **Condenser:** It is used to regulate intensity of light.
15. **Pillars:** These are the two vertical structures to give the support on the base of microscope.
16. **Mirror:** It is spherical reflecting mirror, which can be adjusted to direct the light through diaphragm, stage and lenses. It is fitted in the mirror holder.
17. **Foot or Base:** It for the base of microscope.

Precautionary measures:

1. Closing one eye while using the other to look through a monocular microscope tends to the tire certain eye muscles. Learn to keep both eyes open.
2. Another source of eyestrain results from imperfect focusing. Keep the hand on the fine adjustment and continually making slight changes in focus to study different parts of the field can obviate this.
3. Clean the lenses with tissue paper.
4. Never use ordinary tissue paper or cloth which might contain grit.
5. Never rub the lenses heavily it may scratch the lens.
6. Do not remove lenses from their mounts or unscrew the objectives form the nosepiece.

Collection and preservation of Plant Disease samples

Preservation means killing or restricting the growth of an organism in or on the substrate on which it grows.

1. Dry Preservation:

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

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

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

2. **Wet Preservation:** Washed fresh diseased specimen 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

percent formalin in the glass jars. All mounted are preserved specimens must be labelled with as much of the following information as far as possible.

1. Name of the host plant
2. Name of place from where collected
3. Name of the disease causing organism
4. Date of collection
5. Name of the collector

Size of Specimen: A specimen should be 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 sheet, 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 individuals may be placed on each sheet.

Preparation of Potato Dextrose Agar Medium

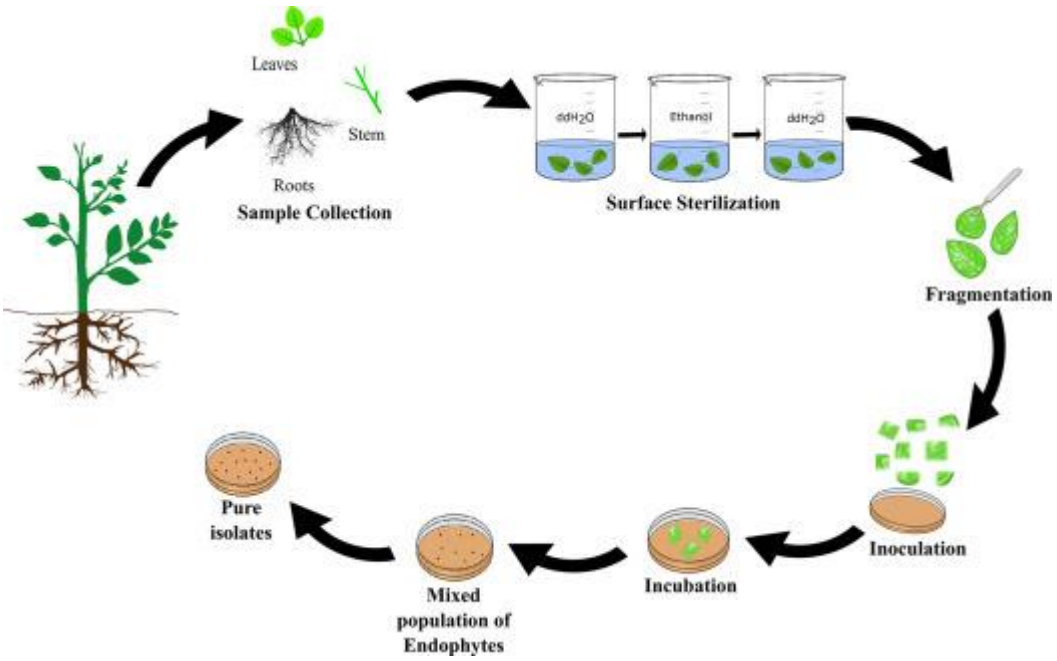
Materials required: Peeled potato 200g, Dextrose 20g, Agar-agar 20g, Distilled water 1000ml.

Method:

- Potato (peeled) are boiled until soften in 500ml of water
- Filtered with muslin cloth
- Agar – agar is melted in 500ml distilled water
- Potato juice is added to the melted agar
- Total volume is make 1000ml by adding required distilled water
- Dextrose is added in this mixture and shaken well
- Medium is sterilized in an autoclave at 15lbs^{psi} for 15-20 minutes at temperature of 121.6^{0}C. Thus the medium is ready for use.

Isolation of Plant Pathogens from disease 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 surface of such tissues, however are usually contaminated with saprophytic organism. The steps of isolation of the pathogen have been given in the flowchart.



Koch's Postulates

Steps od Koch's Postulates:

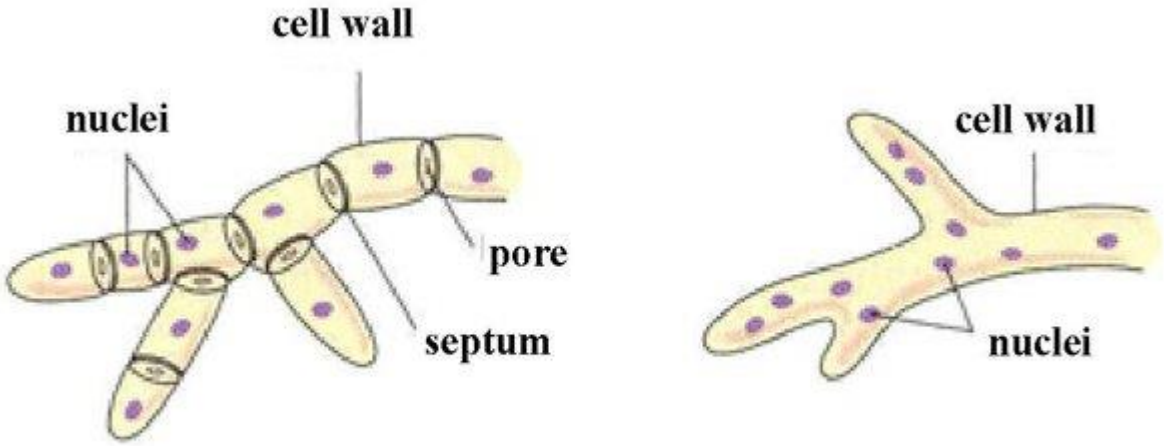
1. The suspected causal agent must be present in every diseased organism examined.
2. The suspected causal agent must be isolated from diseased host plant and grown in pure culture.
3. When a pure culture of suspected causal agent is inoculated into a healthy susceptible host, the host must reproduce 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 as the step 2. (the 4th step was appended by E. F. Smith, 1905)

Different structure of fungi

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

Aseptate mycelium: When the hyphae are undivided cross-walls (septa) it is known as aseptate mycelium. This type of mycelium is found in lower fungi.

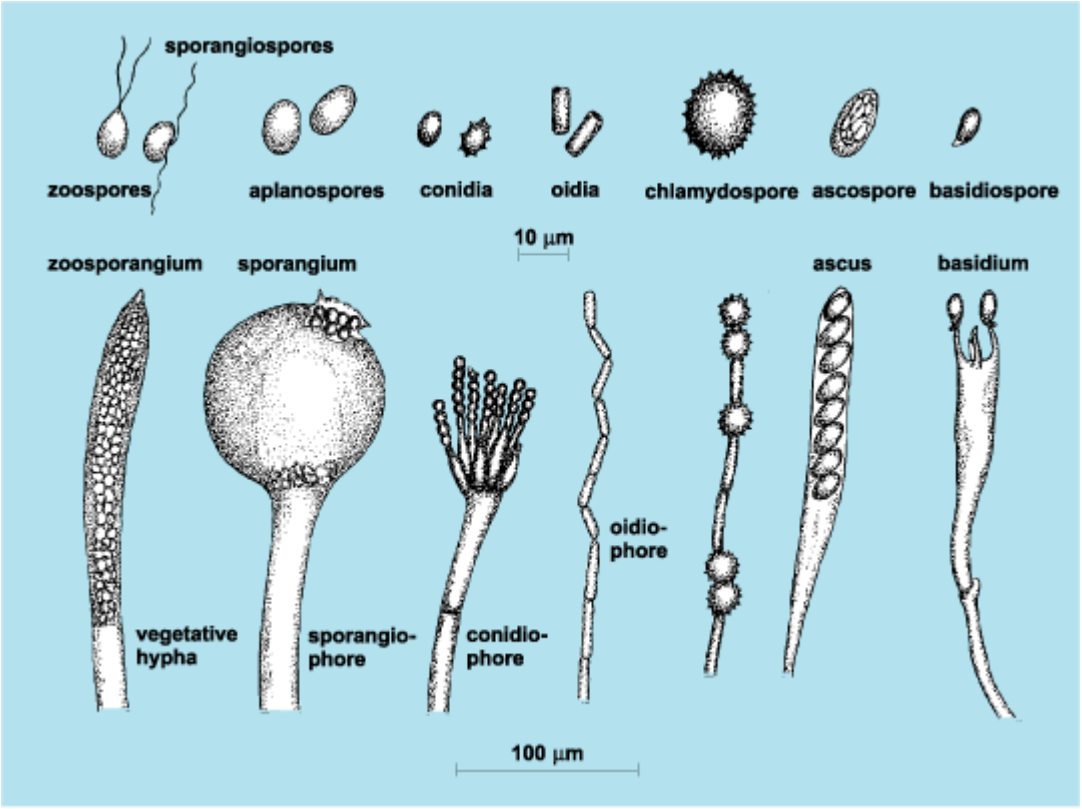
Septate Mycelium: When the mycelium is divided by cross walls (septa) at certain intervals, it is known as septate mycelium. In the septa there is a minute hole, which is known as "septal pore". This type of mycelium is found in higher fungi groups.



(a) septate hypha (b) nonseptate hypha

Types of sexual spores: Asexual spores are those in which sex is not divided. Generally, five types of asexual spores are produced in fungi.

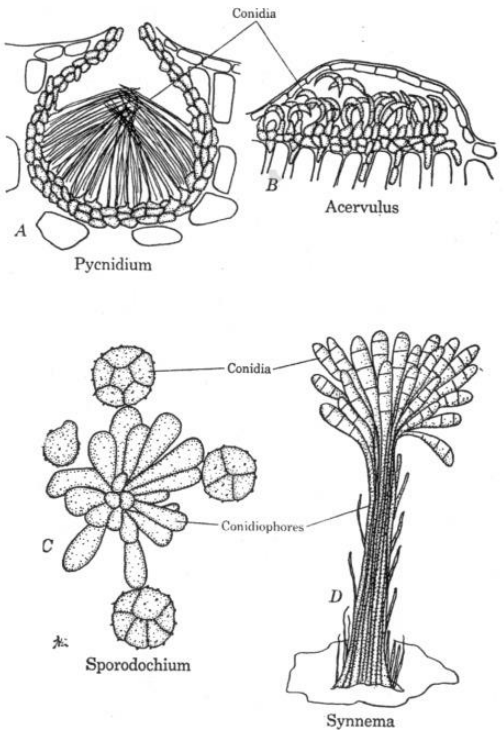
1. **Arthrospores (Oidia):** It is formed in chain (basipetal) on short conidiophores, single celled, barrel shape or drum shaped.
2. **Chlamydospores:** It is formed singly or in chains, which may be terminal or intercalary, provided with an envelope.
3. **Blastospores:** Spores formed by process of budding, which are single celled, first formed in chains but latter separated from each other.
4. **Conidia:** formed at the tip or side of the hyphae (conidiophores) may be formed singly or in chains, quite variable in shape, size, septation, colour and also in ornamentation.
5. **Zoospores:** Pear or kidney nematode, single, naked, motile (flagellate), produced in sporangium (zoosporangium).
6. **Aplanospores:** Oval or spherical in shape, single celled, non-motile (aflagellate) and produced mostly in columellate sporangium.



TYPES OF ASEXUAL FRUITING BODIES, SEXUAL SPORES AND ASCOSPORES

Asexual fruiting bodies:

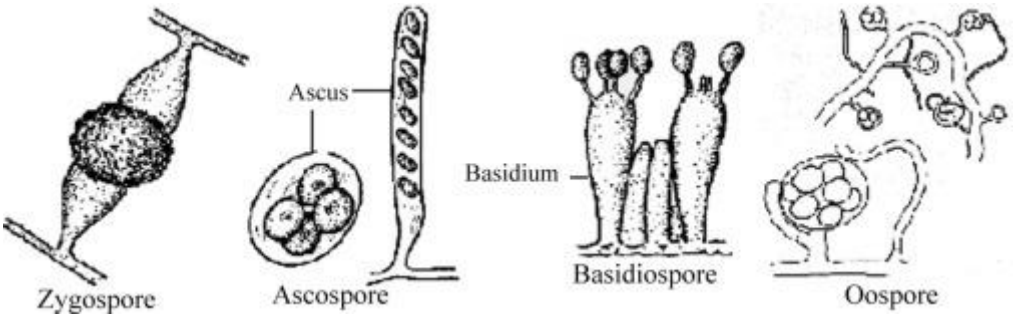
1. **Pycnidia:** These are spherical or flask shaped structures in which the conidia are produced. They have the natural opening known as ostiole through which the conidia are liberated. This type of structure is produced in order Spaeropsidales of sub division Deuteromycota.
2. **Acervuli:** These are 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 this structure the conidiophores are grouped together at the base and free towards apex.



Types of sexual spores

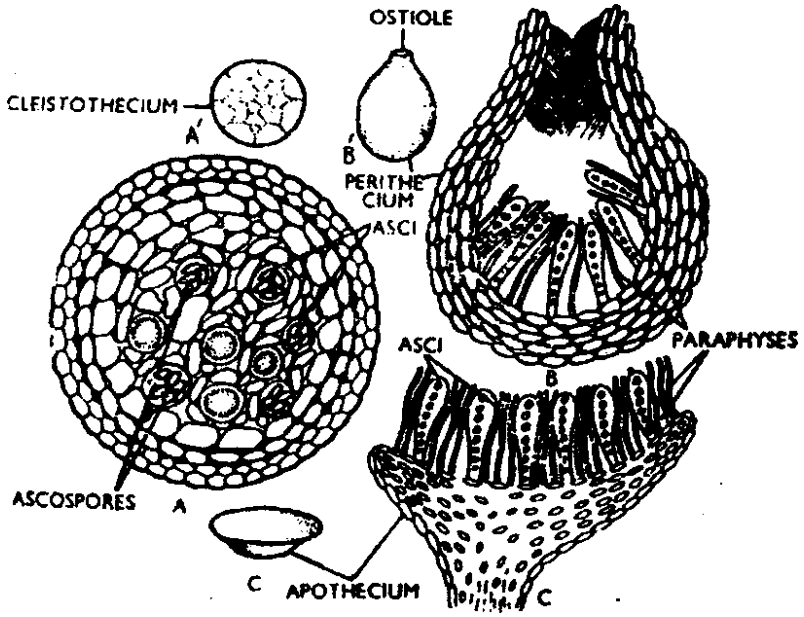
Four types of sexual spores are formed in fungi, which are produced by various methods and they form the bases of the classification of the fungi in different sub divisions.

1. Oospores: It is thick walled sexual spore that develop from a fertilised oospore in some algae fungi and oomycetes.
2. Zygospores: Black in colour, rough walled, warty in appearance provided with suspensors. They are formed by gametangial copulation (zygogamy), characteristics of sub division Zygomycota.
3. Ascospores: Produced in asci, definite in number (2-8). They are firmed by spermatization/somatogamy, characteristics of sub division Ascomycota.
4. Basidiospores: Borne on the basidium, definite in number (usually 4). They are formed by spermatization/somatogamy, characteristics of sub division Basidiomycota.



Types of Ascocarps

1. **Cleistothecia:** Spherical in shape, black in colour, hard structure and without any natural opening. Asci come out by tearing or braking of the cleistothecium. Cleistothecia are also provided with appendages.
2. **Perithecia:** Flask shaped with natural opening known as ostiole sometime having long neck. Asci are produced in perithecium at basal region. Paraphyses may also be present in between the asci.
3. **Apothecia:** The ascocarp, which produces its asci in an open disc or cup shaped structure, is called apothecium. It is exposed and from the layer of asci in a "hymenium" among them paraphyses may also be present.



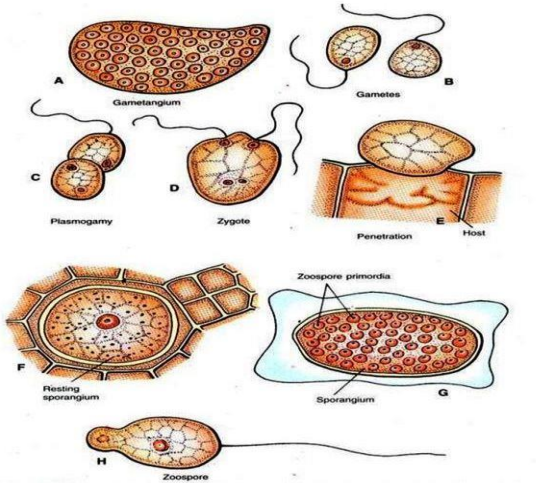
Types of disease symptoms produced due to infection by pathogen

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. **Mildew:** In which the pathogen are seen as a growth (mildew) on green surface of the host. This growth appears as white, gray, brownish or purplish patches of varying size. It is two types' powdery mildew and downy mildew.
4. **Rust:** This disease appears as relatively small pustules of spores, usually breaking through the host epidermis. The pustules may be dusty or compact and red, brown, yellow, black or orange in colour.
5. **Scab:** The roughened or crust-like lesion or to a freckled appeared on diseased organs.

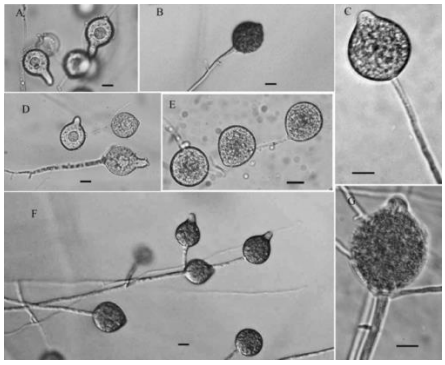
6. **Exudations:** In bacterial and some fungal diseases like bacterial blight of rice, fire blight of pome fruit and citrus gummosis the bacterial ooze as well as gum exudates appears on infected organs.
7. **Damping off:** Destruction of the seedling near the soil surface, resulting in toppling of seedling over on the ground.
8. **Phyllody:** It is a metaplastic disease in which all the floral parts converted into leaf-like structures.
9. **Die-back:** It also a result of necrosis of terminal tissues of twigs in which the twigs and branches start drying from the tip to backwards.
10. **Water-soaking:** It is a water-soaked, translucent appearance of the tissues caused water moving from the host cells into intercellular spaces due to damage to cell walls by enzymes and toxins of the pathogen.
11. **Leaf curl:** It is the curling of leaves due infection of pathogen excessive growth of tissues. It may be upward or downward curling.
12. **Necrosis:** This condition denotes that the death of host tissues or organs occurred as a result of parasitic activity.

Phylum Chytridiomycota and Oomycota

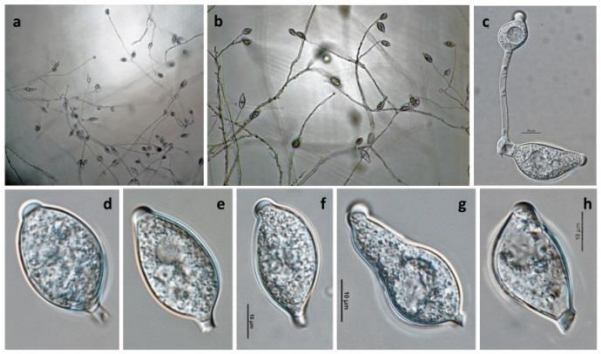
1. **Genus *Sychitridium*:** The species of this genus produces galls on different plant parts, particularly on the root. The most important genus *Synchytrium edobioticum* which causes wart disease in potato. On the potato tubers the warts are more common and typical sometimes covering the whole tuber and larger than the tuber itself.



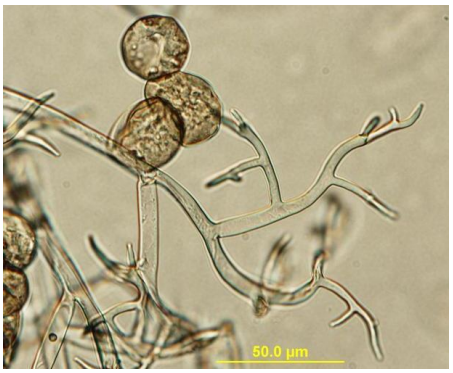
2. **Genus *Pythium*:** Mycelium aseptate, branched, cottony white: Sporangiophores different from vegetative hyphae, erect, simple and bearing sporangia singly; Sporangia, spherical or globose sometimes filamentous or toroid; oospores, thick walled, spherical usually smooth and three layered and plerotic; ex. *Pythium aphanidermatum*, *Pythium ultimum*, *Pythium graminicolum* causes damping off disease.



3. **Genus *Phytophthora*:** Mycelium are aseptate, branched, cottony white; sporangiophres are indeterminate growth, zig-zag, sympodially branched, noduate. Sporangia are single celled, lemon shaped with short papilla. Oospores are spherical, smooth walled, aplerotic. Important species is *Phytophthora infestans* causing late blight disease in potato and tomato.

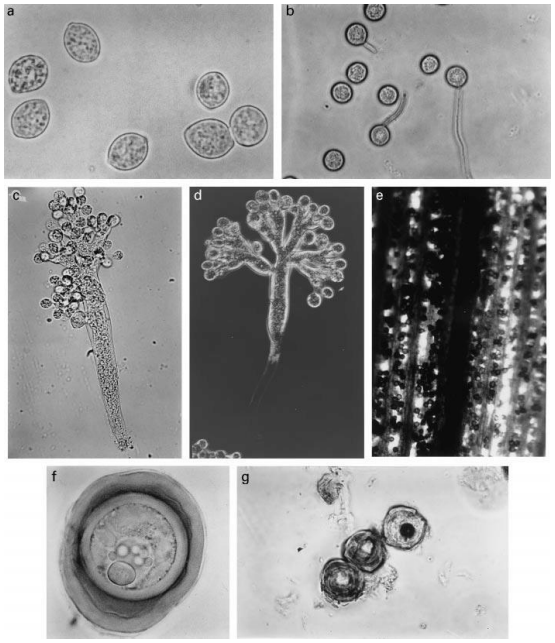


4. **Genus *Peronospora*:** Mycelium coenocytic, branched, hyphae are colourless (hyaline) endophytic and intercellular. Conidia are single celled, spherical, or oval shape and borne singly. **Sterigmata** dichotomously branched at acute angle. Oospores are spherical and reticulate. Conidiophores are pointed, long and bearing single conidia. It is arise from stomata opening. The important genera are *Peronospora paracitica* (downy mildew of crucifers), *Peronospora pisi* (downy mildew of pea), *Peronospora tabacinia* (downy mildew of tobacco).

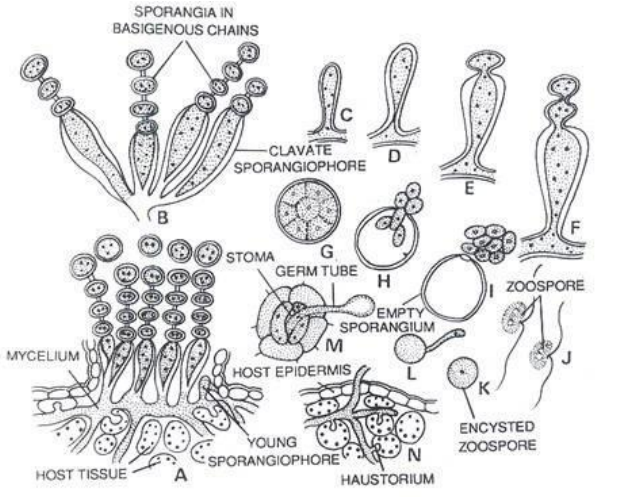


5. **Genus *Sclerospora*:** Mycelium coenocytic, branched, hyaline, endophytic and imtercelluar; Sporangiophores arises from stomata, short and broader towards apex. Dichotomously or trichotomously branched and last branched is changed in sterigmata. Sterigmata short, swollen and borne single

sporangia. Sporangia are single celled and papilate sometimes. Oospores are irregular in appearance because the sporangial wall shrinks and touches the oosporic wall at several places. Important species is (*Sclerospora graminicolal*) causing green ear disease of Bajra.



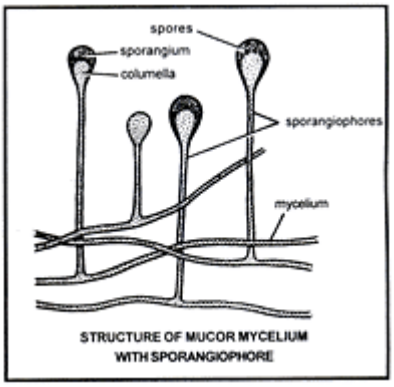
6. **Genus *Albugo*:** Mycelium aseptate, branched, hyaline, intercellular with knob shaped haustoria. Sporangiophores club shaped (clavate), simple forms palisade layer below the epidermis bears sporangia in besipetal chains. Sporangia are single celled globose. Oospores rough, warty and yellow in colour. Albugo candida is important genera causing white rust/blister in cruciferous crops.



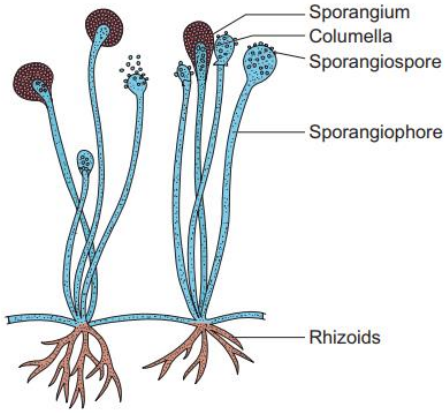
Phylum Zugomycota

1. **Genus *Mucor*:** Mycelium aseptate, branched, cottony white without stolons and rhizoids. Sporangiophores simple arises singly and bears single sporangia. Sporangia are spherical or globose, smooth walled, gargle collumelate and multi spored. Columella is sterilized and dome shaped. Aplanospores are oval

or spherical and single celled. Zygospore rough walled, warty and black in colour. Ex. *Mucor mucedo* and *Mucor basiliformis*.

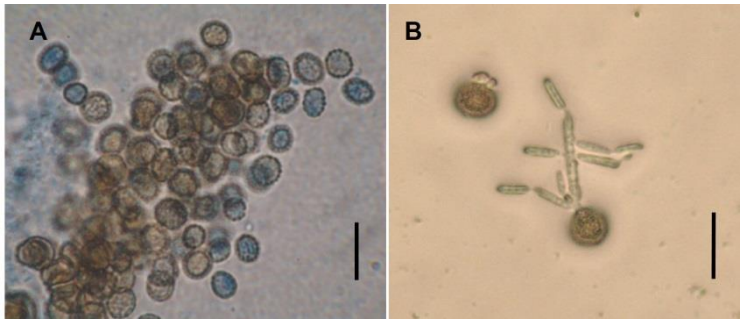


2. **Genus *Rhizopus*:** Characters are same like Mucor except the formation of stolons and rhizoids. Ex. *Rhizopus stolonifers*

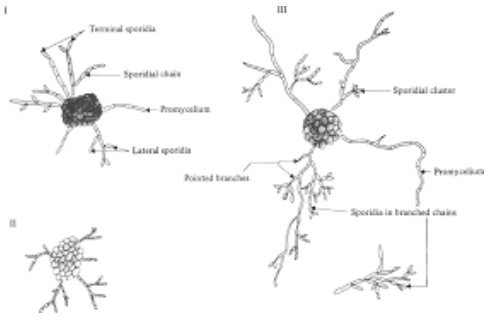


Phylum Basidiomycota

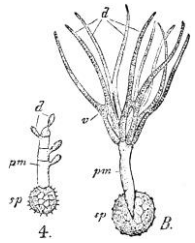
1. **Genus *Sphacelotheca*:** Sorus conical or cylindrical covered with peridium and filled with black spores. Columella are slender or curved made up with host tissues in *Sphacelotheca sorghi.* Teliospore are round to oval, dark brown in mass but olive brown in single and smooth walled. Ex. *Sphacelotheca sorghi* (grain smut of jowar), *Sphacelotheca cruenta* (loose smut of jowar), *Sphacelotheca reiliana* (head smut of jowar).



2. **Genus *Tolyposporium*:** Sori formed in various parts of the host are but more common in ovaries. The spores are covered by a membrane of host origin. The spore balls may consist of only one type of spores or differentiated into an outer layer of coloured sores and inner mass of hyaline spores.

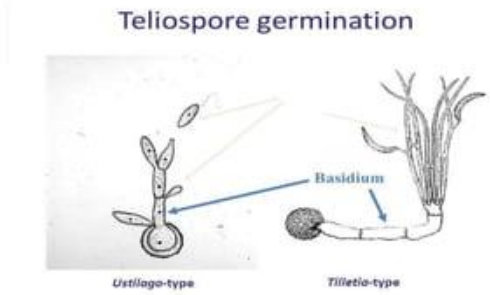


3. **Genus *Tilletia*:** The disease is caused by Tilletia is "Bunt". Teliospores on germination form hollow promycelium bearing 8 filiform terminal sporidia. Sporidia form H shaped structure as results of fusion of compatible sporidia.



4. **Genus *Neovosia*:** It bears numerous filiform basidiospore. Teliospore bears a long pedicel like appendages called *apiculus*. It causes Karnal bunt of rice disease.

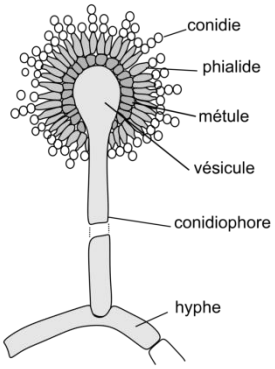
5. **Genus *Ustilago*:** Teliospore is without peridium, black in colour and covered with host origin membrane. Spores are small less than 20 microns. It causes loose smut of wheat disease.



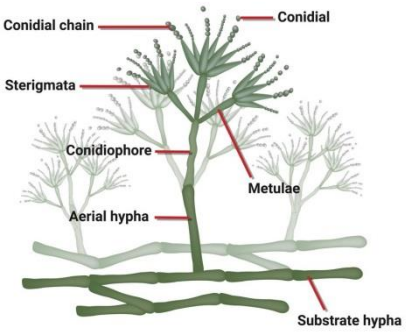
Phylum Ascomycota

Class: Eurotiomycetes

1. **Genus – *Aspergillus*:** Mycelium well developed, branched, septate, hyaline and submerged. Conidiophores arise from foot cells, aseptate, simple and terminating into vesicle. Two rows of sterigmata are formed on the vesicle. Primary sterigmata flat and secondary are bottle shaped. Conidia borne on secondary sterigmata in long besipetal chains. Perfect stage is *Eurotium*.



2. **Genus *Penicillium*:** Mycelium septate branched, hyaline and substratum. Conidiophores septate branched without vesicle and foot cells. Peg like sterigmeta is formed. Conidia are borne on sterigmata in long besipetal chains. They are globose to ovoid, smooth walled and resemble as glass beads. Ex. *Penicillium notatum.*

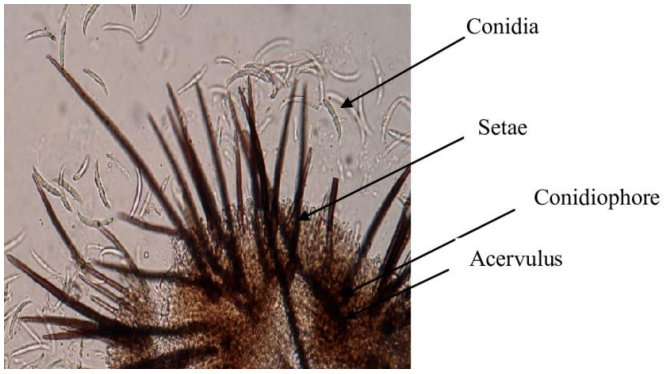


Class: Sordariomycetes

1. **Genus- *Fusarium*:** Mycelium branched, septate, pinkish brown in colour. Sporodochia spherical, oval or ovate. Conidiophore aseptate or septate, short bearing single conidia. Two types of conidia are found microconidia (usually single or bi-celled) and macroconidia (varied from 2-7 celled, sickle shaped and notched at the base. Chlamydospore formed on mycelium and macroconidia. Ex. *Fusarium oxysporum* (wilt), *Fusarium udam* (wilt in pigeonpea).
2. **Genus - *Cleviceps*:** Mycelium septate, branched destroying ovary tissues and replacing it by cottony white mycelial mat forming conidiophores bearing at the tip. Conidia are minute, oval and single celled forming honey dew stage. Sclerotia black, hard variable in shape. Perithecia has flask like ostiole. Several asci are formed in perithcium it may elongate cylindrical in shape. Eight ascospores are formed in each ascus.
3. **Genus - *Pyricularia*:** *Pyricularia* forms hyaline or pale grey bi-celled pyriform conidia singly at tip of the conidiophores, conodiophores are simple; erect septate and hyaline or grey.

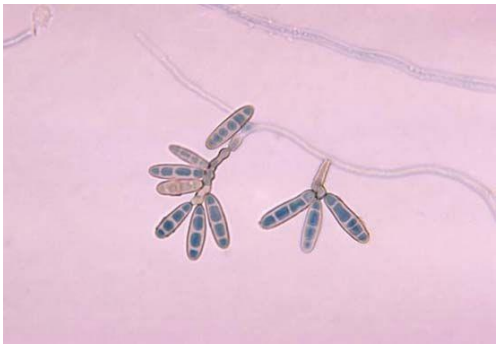
4. **Genus – *Colletotrichum*:** Mycelium septate, branched and light brown in colour. Ascervuli are cushion shaped with sterile hair like black structure

called setae. Conidiophore aseptate, short and unbranched. Conidia are single celled and falcate shape.

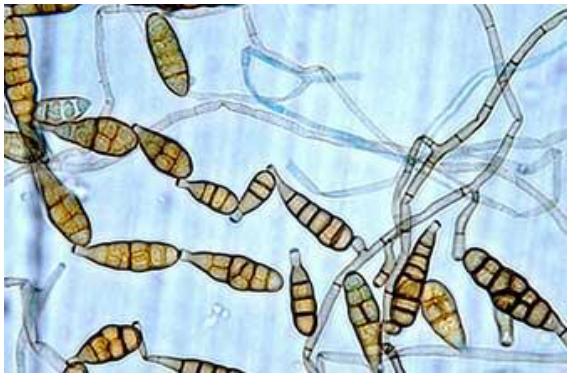


Class: Dothideomycetes

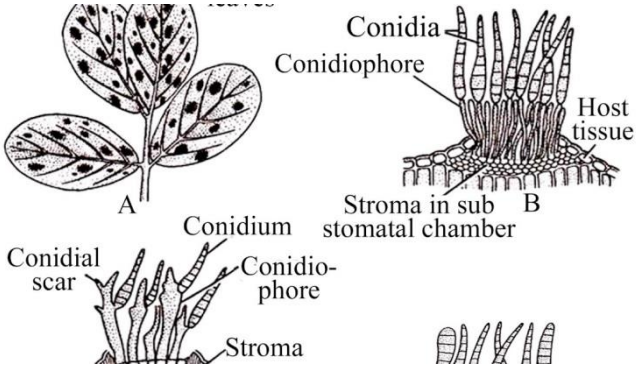
1. **Genus – *Helminthosporium*:** Mycelium typically dark, bearing conidia on simple, erect septate conidiophores. The conidia are obclavate, brown, thick-walled with 8-10 transverse septa. Conidia re produced singly from a pore at the tip and on lateral side of the upper part of conidiophores. Ex. *Helmithosporium oryzae* (leaf spot of rice).



2. **Genus – *Alternaria*:** Conidiophores are short and dark remerge through stomata from the dead centre of the spots. Conidia are beaked, muriform and dark in colour. Important genera are *Alternaria solani* (early plight of potato), *Alternaria brassicae* (leaf blight of crucifers) and *Alternaria triticina* (leaf blight of wheat).

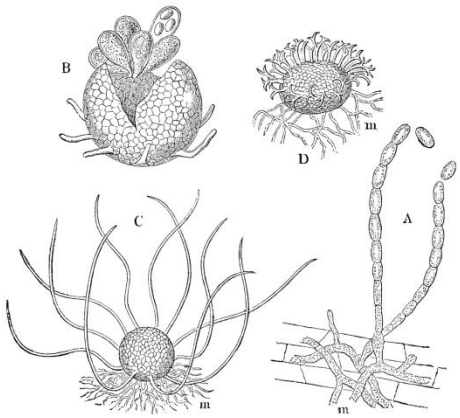


3. **Genus – *Cercospora:*** Conidiophores are 1-2 septate, unbranched, geniculate develops on dense, globular, brown to black stroma. They emerge in dense fascicle by rupturing the epidermis. Conidia are obclavate to cylindrical, light coloured and 1-7 times septate. Important spp. are *Cercospora arachidicola* and *Cercospora personata* (tikka disease of groundnut).

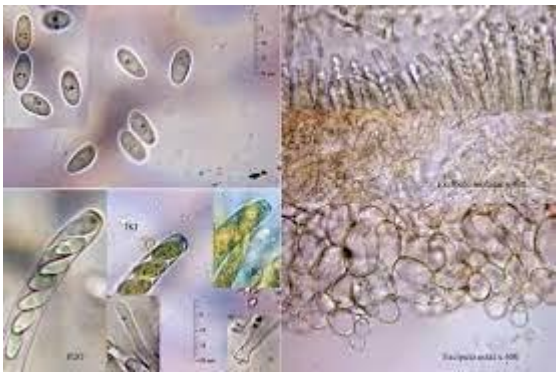


Class: Letiomycetes

1. **Genus – *Erysiphe*:** Mycelium septate branched, hyaline and ectoparasitic. Conidiophore arises singly short, septate, straight and simple. Conidia are single celled, barrel shape and arise in besipetal chains. Sexual fruiting bodies cleistothecia are spherical in shape without any natural opening and appendages are myceloid type. Several asci are formed in cleistothecia with 2 – 8 ascospores (hyaline, spherical or oval shape). The important spp. are *Erysiphe graminis tritici* (powdery mildew of wheat), *Erysiphe poligoni* (powdery mildew of pea) and *Erysiphe cichoracearum* (powdery mildew of cucurbits).

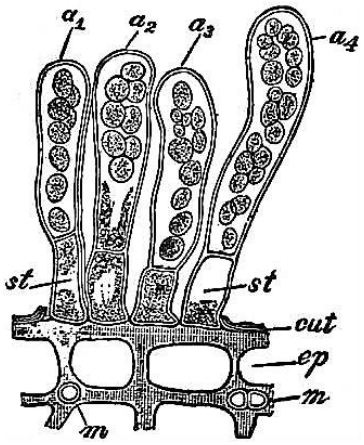


2. **Genus - *Sclerotinia*:** Mycelium septate and white in colour. Conidiophores are septate and branched. Conidia are oval to lemon shape, single shape and formed in chains. Sclerotia hard and black in colour. Clavate, slightly thickened at apex with paraphysis asci are formed on stalked apothecia. Each ascus has 8 single celled and elliptical to elongated ascospores. Imp. spp. *Sclerotinia sclerotiorum* (root rot or white disease).



Class: Taphrainomycetes

Genus – *Taphrina*: Mycelium septate, bi-nucleate cell and intercellular to sub cuticullar. Naked asci are formed with eight ascospores in each ascus. Important spp. *Taphrina deformans* (peach leaf curl).



Phanerogamic Plant Parasites

Over 3000 species of flowering plant obtain nutrition as parasite. In many cases the damage caused by these parasites is only slight or the attacked host plants are of little economic importance. But there are many examples where these flowering plants attack valuable crops and threes causing considerable damage.

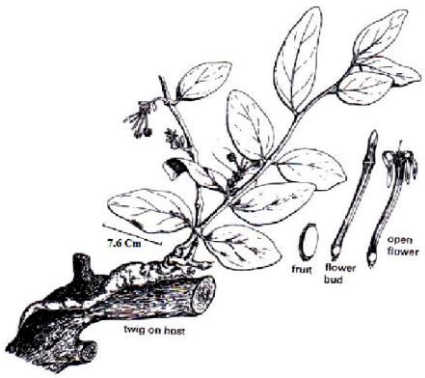
The common plant parasitic flowering plants can be grouped as follows:

1. **Stem parasites**
   i. Total parasite – *Cuscuta* sp. (Dodder)
   ii. Semi parasite – *Dendrophthoae* sp. (Loranthus)
2. **Root parasites**
   i. Total parasite – *Orobanche* sp. (Broom rape)
   ii. Semi parasite – *Striga* sp. (Witch's weed)

Dodder (*Cascuta* spp.): It is achlorophyllus, leafless, twining parasitic seed plant, which attaches it's yellow, orange or pink, thread like stem to stem or other parts of cultivated or wild plants/crops. Leaves are representing by minute scales. A minute root like organs (Haustoria) sends to the host cortex which serves 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.

Loranthus (*Dendrophthoae* spp.): It is the common parasites of fruit plants. They attack on areal parts of fruits trees. It is devoid of true root system of its own and hence, is dependent on host for water and minerals. Leaves are leathery and evergreen and possess chlorophyll. Flowers are long

tubular in shape greenish white or red colour and borne in cluster. Infected areas of host become swollen.



Broomrape (*Orobanche* spp.): Total root parasite, affecting crops likes tobacco, brinjal, tomato, cabbage, cauliflower, turnip and many other Solanaceous and Cruciferous crops. The parasite consists of stout, fleshy stem. 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. *Orobanche rasoma* is an important species.

Whitch's weed (*Striga* spp.): it is a semi root parasites of sugarcane, cereals, maize and many millets in India. The parasite is 15-30 cm toll with bright green, slightly hairy stem and leaves. Leaves are narrow long and in opposite pairs. The flowers are usually small and brick red or scarlet. The seed are borne in capsule and very small. *Striga asiatica* is an important species.

Staining and identification of Plant Pathogenic bacteria

1. Smear preparation:
   i. Take a clean grease free dry slide.
   ii. Sterilize the inoculation loop on the flame of burner.
   iii. Put a loopful of culture on slide and make a smear at the centre of slide.
   iv. Dry the smear by gentle heat on flame or air dry.
   v. Fix the dry smear by passing the slide on burner flame 3-4 times. Put the smear side up.
2. Gram staining procedure:
   i. Place the slide on staining rods.
   ii. Apply the crystal violet on smear and leave it for 30-60 sec.
   iii. Wash with tap water.
   iv. Flood the smear with Gram's iodine solution and leave for 30-60 sec.
   v. Drain off of the iodine by the gentle stream of tap water.
   vi. Decolorize with 70% ethyl alcohol.
   vii. Gently wash the slide with slide with running tap water and drain completely.
   viii. Counterstain with safranin for 20-30 sec.
   ix. Wash the slide with distilled water and blot dry with absorbent paper and observe under the microscope.

   Gram positive bacteria appear dark purple colour and Gram negative bacteria appears pale to dark red colour.

Calculation of fungicidal spray solutions

Preparation of fungicidal solutions

Bordeaux mixture: preparation of 1% Bordeaux mixture

Procedure: For 1% solution take 5:5:50 ratio or copper sulphate 5gm, lime 5g and water 500ml.

Since 250ml water contains 2.5g of copper sulphate, therefore 1 ml water contain 2.5/250, so that 100 ml water will contain 2.5/250 x 100= 1%

Exercise: Dry and wet seed treatment of seed with Vitavax power @ 0.1%.

Material required: Balance, weight box, seed treating drum or wooden container.

Procedure: The appropriate fungicide is weighed on balance and put into a seed treating drum. Seed is also put into the drum and thoroughly mixed by rotating.

Calculation: five kg seed to be treated with vitavax power @ 0.1%

Since 100g seed required 0.1g fungicide, therefore, 1g seed required 0.1/100. Therefor 5kg seed or 5000g seed needs 0.1/100 x 5000= 5g fungicides.

Exercise: To prepare fungicidal (Indofil M45) solution for 1 ha area @ 0.2% per hectare.

Materials required: Balance, weight box, plastic drum, fungicide, sprayer and water.

Procedure: The appropriate amount of fungicide weighed and fungicidal solution is prepared by adding the required amount of water. This is then sprayed over the crops.

Calculation:

Since 100cc water requires 0.2g fungicide, therefore 1cc water requires 0.2/100 and 1000cc water would require 0.2/100 x 1000 = 2g.

Therefor 1000 litre water needs 1000 lit. × 2g = 2000gms Indofil M 45

Methods of application of fungicides and their safe use

Fungicides are generally used either as seed treatment, soil drenching, spraying and dusting. Among these foliar spraying and seed treatment are carried out over throughout the world. At present dusting is mostly discarded and soil drenching followed in selective measures and in the case of nematode management.

A. Seed treatment:

It is very much essential because a large number of pathogenic fungi and bacteria are either carried outside or inside the seed i.e. they are externally seed borne or internally seed borne. When the seed germinate the pathogen also become active and cause pre emergence damping off or seed decay. Post emergence damping off also seen. Seed treatment inhibits the Rhizosphere microflora. The aim of seed treatment is destroy the seed or soil borne fungi and bacteria causing seed decay, seedling blight and smuts etc. It is the cheapest method of control of disease.

Two methods of seed treatments are applied:

1. **Physical method:** In this method hot water and solar treatment are used to control the loose smut of wheat.
2. **Chemical method:** In this method a combination of fungicides is used to control the plant diseases. This is because there is no ideal fungicide available at present which could control a number of pathogens of a particular crop.

B. Soil treatment: This is two types.

1. **Physical method:** In this use of heat in form of steam hot water or electricity.
2. **Chemical method:** In this case volatile and non-volatile chemicals are used. This also classified in following groups.
   - **i. Soil Drenching:** Fungicidal solutions are made with water as per recommended concentrations and drench to the soil surface before or after plant emergence.
   - **ii. Broadcasting:** The non-volatile fungicides are mixed with soil or fertilizers and applied over the filed uniformly by hand. It should be

mixed properly with suitable implements. This method used for large quantity of fungicides.

iii. **Furrow application:** In this method the fungicides are used as dust or granules form at time of sowing. Comparatively lesser quantity is required as broadcasting method.

iv. **Fumigation:** It is method that used for nematode control. Fumigator chemicals are used for this method.

v. **Spraying:** This is the method which is commonly adopted. Spraying is done on leaf, stem and fruits. Spraying is two types i.e. high and low volume. When spray involves large quantity of liquid/unit area they termed as high volume. Six hundred (600) liters and above per ha are considered to be of high volume category. While, in the case of low volume category it cover one ha area or 100 liters or less quantity of water. In high volume spray the drop size ranged from 0.5 to 3 mm whereas in low volume spray the droplet size varied from 15 to 40 micron.

vi. **Dusting:** Dust is applied on leaves, stem and fruit as an alternative of spraying. Dry powder is dusted by means of dusters for covering the plant/crop surface.

Fungicides and their formulations

Fungicide: The word is derived from Latin word the fungus and *caedo* means to kill. Therefore, a fungicide is any material that has ability to kill the fungus i.e. heat, chemical, UV-rays, light etc.

Fumigants: The volatile chemicals are applied to confined spaces or into soil, which produces gas that destroys microorganisms and work as soil sterilizing agents. The most common chemicals are methyl bromide, methane, allyl alcohol, carbon disulphide, chloropicrin and tetrachlorethane.

Eradicant: 1. (Curative) a fungicide use to control disease after infection. 2. A fungicide applied to a substratum in which the fungus is already is present.

Protective: A fungicide used to protect an organism against infection by a fungus.

Fungicidal residue: Fungicide or its ingredients present on or in the plant.

Systemic fungicide: A fungicide which absorbed through a plant surface and is translocated from the site of application to others plant parts.

Fungistatic: Certain chemicals may temporarily inhibit fungus spore germination without killing them.

Fungistasis (Mycostasis): Dobbs and Hinson (1953) the phenomenon in which the fungal propagules are restricted to a certain extent in their ability to grow or germinate.

Formulations of fungicides:

1. **Wettable powder:** It is a very common formulation of fungicides which is used for spray mixture. The modern wettable powders are water dispersible and have the ability to wet easily and disperse well in water.
2. **Dust formulations:** Usually contain 1:10% active ingredients for direct application in dry forms. These are manufactured in such ways that are high 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 kaolin, pyrophylite, bentonite, calcium silicate, hydrated silica etc.
3. **Water dispersible powder:** The active ingredient is incorrect, usually at the rate of 30-80% with a finely ground inert dust. Such as kaolin a wetting and suspending agent. The commonly used suspending agents are sodium lignin sulphonate, methyl celluloses, polyvinyl acetate and aluminium silicate. In addition, spreader-sticker is sometime desirable, especially on plant with glossy or waxy leaves.
4. **Granules:** This is the formulation of fungicide with inert material formed particles about the size of course 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 t soil and seed treatments. Such formulations usually contain high percentage of active ingredient similar to wettable powders. They are mixed with water for final use and required agitation.
5. **Solutions:** The formulations in which active ingredient or a combination of active ingredient and a solvent is dissolved in water solutions. It has the advantage of requiring no agitation after formulation is added in water.
6. **Suspensions or slurries:** These are the formulations in which a dry form of active ingredient is mixed with a liquid. Such formulation usually contains a high percentage of active ingredients similar to wettable powder. They are mixed with water for final use and required agitation. These are mostly used as seed dresser.

Preparation of fungicidal solutions

1. **Bordeaux mixture:** One kg of copper sulphate powder is 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 tendency to plants. It is cheap and utility to control a wide range of diseases like downy mildew, bacterial cancer etc. It is non-toxic to human and cattle.

   Demerits: Phytotoxic on certain plant like paddy, apples, peach etc. it causes delay in ripening of fruits. The preparation is not very easy

under field conditions. It's corroding action on metallic containers of spraying equipment.

2. **Bordeaux paste:** Bordeaux paste has same constituents as like Bordeaux mixture, but it is in the form of paste the quantity of water used 10 percent of Bordeaux mixture. For preparation of Bordeaux paste used 1 kg of copper sulphate and 1 kg of lime and 10 litre of water. It is used as wound dresser and it protect from fungal infection.
3. **Burgundy mixture:** It is also prepared as same way of Bordeaux mixture but sodium carbonate used instead of lime. So it is called as Soda Bordeaux. It was developed in Burgundy (France), 1887 by Mason. One kg of sodium carbonate and one kg copper sulphate mixed in 100 litre of water.
4. **Cheshunt compound:** It is a compound usually prepared by mixing of 2 parts of copper sulphate and 11 parts of ammonium carbonate. This formula suggested by Bewely in 1921. Both are well powdered and mix thoroughly and stored in air tight container for 24 hrs before being used. The ripened mixture is used by dissolving it in water at the rate of 3g/litre. Mixture is dissolve in 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 U. B. Singh in 1942 at Govt. Fruit Research Station, Chaubattia in Almora. It is usually prepared in glass containers by mixing of 800g copper carbonate and 800g red lead in a litre of raw linseed oil or lanolin. This paste is usually applied to pruned parts of apple, pear and peaches to control the several fungal diseases. The advantage of this paste is not easily washout with rain water.