

PRACTICAL MANUAL

For

DISEASES OF FIELD AND HORTICULTURAL CROPS & THEIR MANAGEMENT-II

Course No. APP-339 Credit Hours 3(2+1)

For

B. Sc. (Agriculture) III Year (VI Semester)



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2019

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College of Agriculture
Rani Lakshmi Bai Central Agricultural University
Jhansi-284003**

Syllabus:

Identification and histopathological studies of selected diseases of field and horticultural crops given below. Field visit for the diagnosis of field problems. Collection and preservation of plant diseased specimens for herbarium.

Mango: anthracnose, malformation, bacterial blight and powdery mildew; **Citrus:** canker and gummosis; **Grape vine:** downy mildew, Powdery mildew and anthracnose; **Apple:** scab, powdery mildew, fire blight and crown gall; **Peach:** leaf curl.

Strawberry: leaf spot **Potato:** early and late blight, black scurf, leaf roll, and mosaic;

Cucurbits: downy mildew, powdery mildew, wilt; **Onion and garlic:** purple blotch, and Stemphylium blight; **Chillies:** anthracnose and fruit rot, wilt and leaf curl; **Turmeric:** leaf spot **Coriander:** stem gall **Marigold:** Botrytis blight; **Rose:** dieback, powdery mildew and black leaf spot.

Note: Students should submit 50 pressed and well-mounted specimens.

Name of Student

Roll No.

Batch

Session

Semester

Course Name :

Course No. :

Credit

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CERTIFICATE

This is to certify that Shri./Km.ID No.....
has completed the practical of course.....course
No. as per the syllabus of B.Sc. (Hons.) Agriculture/ Horticulture/ Forestry semester
in the year.....in the respective lab/field of College.

Date:

Course Teacher

CONTENTS

Sl. No.	Contents	Page. No.
1.	Preparation of temporary mounts (slides) and stain.	
2.	To collect and preserve plant diseased specimens for herbarium	
3.	To collect and preserve plant diseased specimens for wet preservation	
4.	Identification of Brown rust of wheat.	
5.	Identification of Loose smut of wheat.	
6.	Identification of Red rot disease of sugarcane.	
7.	Identification of White rust of mustard.	
8.	Identification of <i>Alternaria</i> leaf spot of Mustard	
9.	Identification of Early blight of potato	
10.	Identification of Late blight of potato	
11.	Identification of Wilt of chickpea	
12.	Identification of Wilt of cotton	
13.	Identification of Anthracnose of Mango	
14.	Identification of Powdery Mildew of Mango.	
15.	Identification of Mango Malformation.	
16.	Identification of Citrus Canker	
17.	Identification of Chilli Anthracnose	
18.	Identification of Black spot disease of rose	

PRACTICAL NO. 1

OBJECTIVE: Preparation of temporary mounts (slides) and stain.

ACTIVITY: Write the composition and use of Lactophenol-Cotton Blue. Mount the sample provided to you and stain it using Lactophenol-Cotton Blue.

MATERIALS REQUIRED:

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Composition:

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Use:

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Procedure:

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Precautionary measures:

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PRACTICAL NO. 2

OBJECTIVE: To collect and preserve dry plant disease specimens for herbarium

ACTIVITY: Collect ten disease samples from the nearby areas and prepare a herbarium with the following details in it:

	1	2	3	4	5
a. Name of the diseased plant sample					
b. Name of the disease					
c. Name of the causal organism					
d. Place of collection					
e. Date of collection					

	6	7	8	9	10
a. Name of the diseased plant sample					
b. Name of the disease					
c. Name of the causal organism					
d. Place of collection					
e. Date of collection					

MATERIALS REQUIRED:

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PREPARATION OF SPECIMEN:

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PRACTICAL NO. 3

OBJECTIVE: To collect and preserve plant disease specimens for wet preservation

ACTIVITY: Prepare FAA solution for preservation of plant disease sample. Collect disease sample and preserve in the glass bottle following wet preservation protocol.

MATERIALS REQUIRED:

PROCEDURE:

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PREPARATION OF FORMALIN ACETIC ACID ALCOHOL (F.A.A.):

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OBJECTIVE: Identification of Leaf or brown rust of wheat

ACTIVITY: Prepare a slide from the brown rust sample and observe under the microscope. Draw the diagram of the symptoms and microscopic features (uredospores and teliospores) as observed under the microscope.

MATERIALS REQUIRED:

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OBSERVATION TO BE MADE:

A. Macroscopic

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B. Microscopic

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PRACTICAL NO. 5

OBJECTIVE: Identification of Loose smut of wheat.

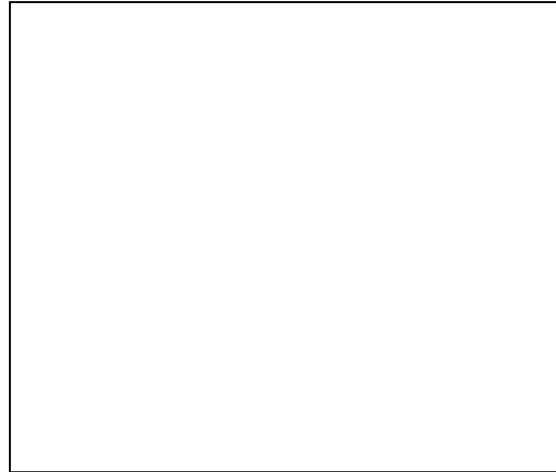
ACTIVITY: Identify the pathogen from the given sample. Prepare its slide, draw the diagram of the symptom, and smut spores as observed under the microscope.

MATERIALS REQUIRED:
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OBSERVATION TO BE MADE:

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B. Microscopic

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PRACTICAL NO. 6

OBJECTIVE: Identification of Red-rot disease of sugarcane.

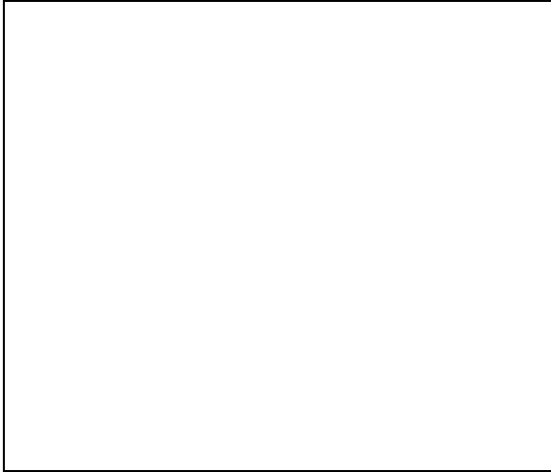
ACTIVITY: Cut T.S. of the infected sugarcane stem. Write the characteristic symptom and draw the symptom and spores as seen under the microscope

MATERIALS REQUIRED:
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OBSERVATION TO BE MADE:

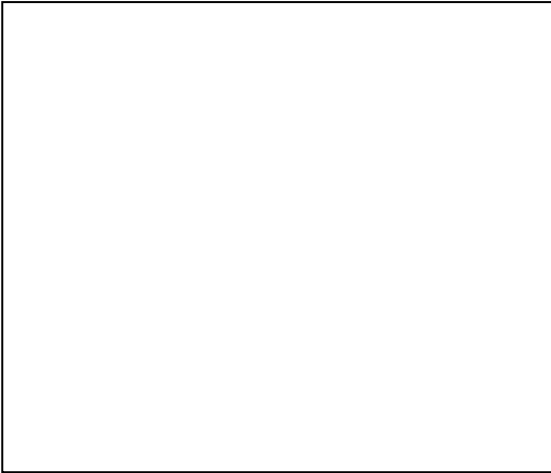
A. Macroscopic

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OBJECTIVE: Identification of White rust of mustard

ACTIVITY: Prepare a slide from leaf sample infected with white rust and observe under microscope and identify the pathogen. Draw the diagram of characteristic symptom and spores observed under the microscope.

MATERIALS REQUIRED:

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OBSERVATION TO BE MADE:

A. Macroscopic

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B. Microscopic

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OBJECTIVE: Identification of Alternaria leaf spot of Mustard

ACTIVITY: Prepare a slide from infected leaf and identify the pathogen. Draw the characteristic symptoms of the disease and spores as seen under the microscope.

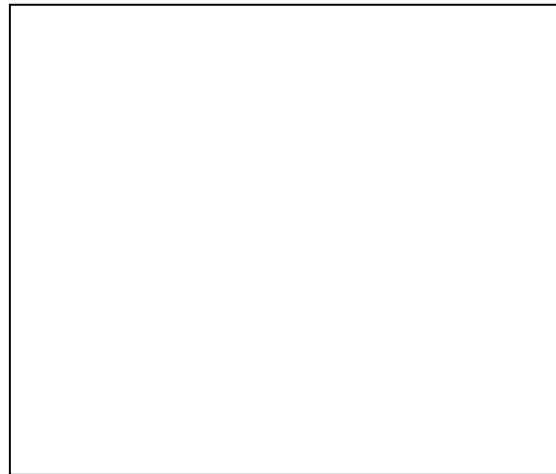
MATERIALS REQUIRED:

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OBSERVATION TO BE MADE:

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B. Microscopic

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OBJECTIVE: Identification of Early blight of Potato

ACTIVITY: Prepare a slide from infected potato leaf sample and observed under microscope and identify the pathogen. Draw the diagrams of characteristic symptom and spores seen under the microscope.

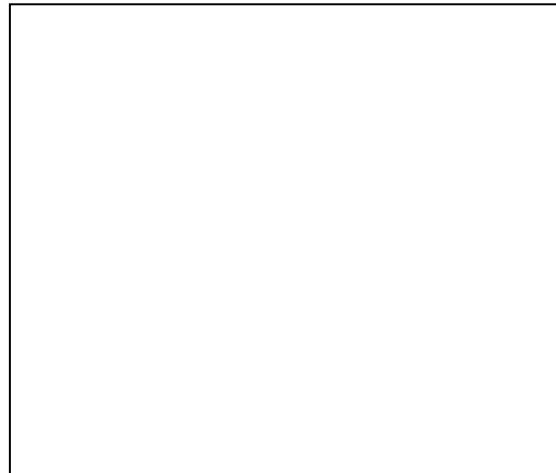
MATERIALS REQUIRED:

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OBSERVATION TO BE MADE:

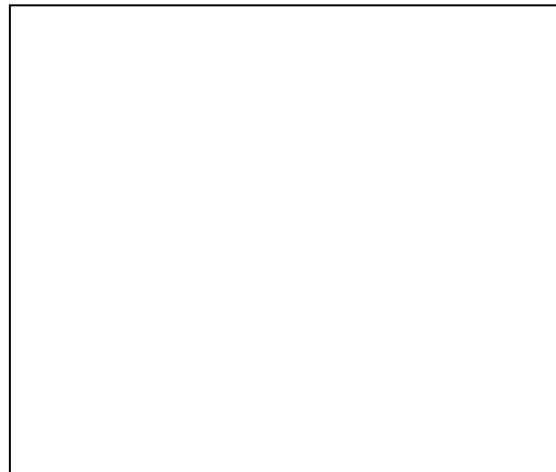
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B. Microscopic

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OBJECTIVE: Identification of Late Blight of Potato

ACTIVITY: Prepare a slide from infected potato leaf sample and observed under microscope and identify the pathogen. Draw the diagrams of characteristic symptom and spores seen under the microscope

MATERIALS REQUIRED:

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OBSERVATION TO BE MADE:

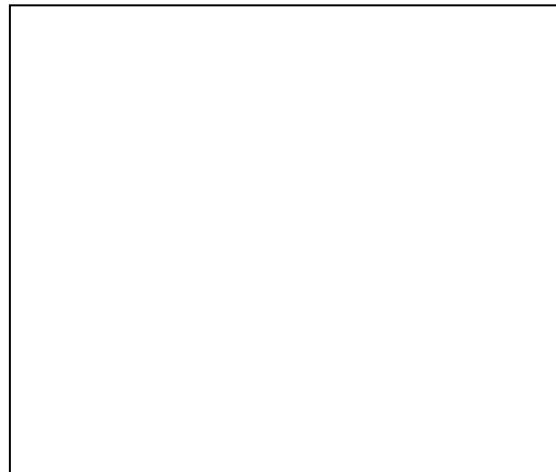
A. Macroscopic

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B. Microscopic

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OBJECTIVE: Identification of wilt of chickpea

ACTIVITY: Cut section of diseased root and prepare a temporary slide and observe under microscope.
Draw the diagram of characteristic symptom and spores seen under the microscope.

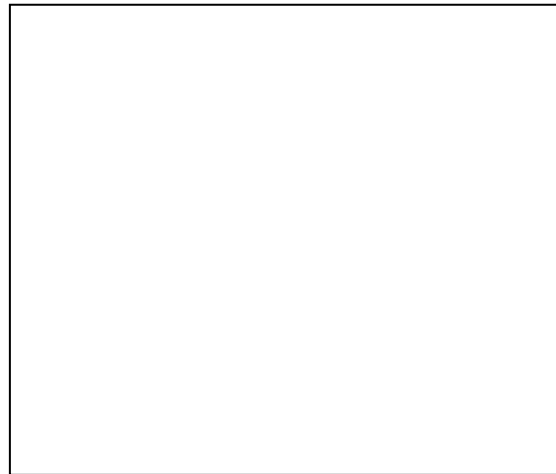
MATERIALS REQUIRED:

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OBSERVATION TO BE MADE:

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B. Microscopic

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PRACTICAL NO. 12

OBJECTIVE: Identification of wilt cotton.

ACTIVITY: Cut the fine section of diseased root and prepare a temporary slide. Draw the diagram of characteristic features seen under the microscope.

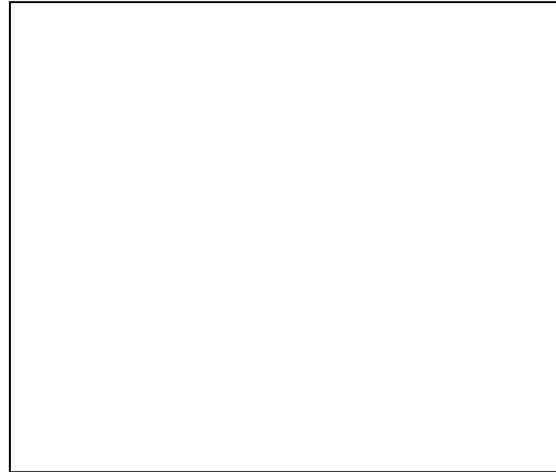
MATERIALS REQUIRED:

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OBSERVATION TO BE MADE:

A. Macroscopic

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B. Microscopic

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OBJECTIVE: Identification of Anthracnose of Mango

ACTIVITY: Cut section of diseased sample and prepare a temporary slide and observe under microscope. Draw the diagram of characteristic symptom and spores seen under the microscope.

MATERIALS REQUIRED:
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OBSERVATION TO BE MADE:

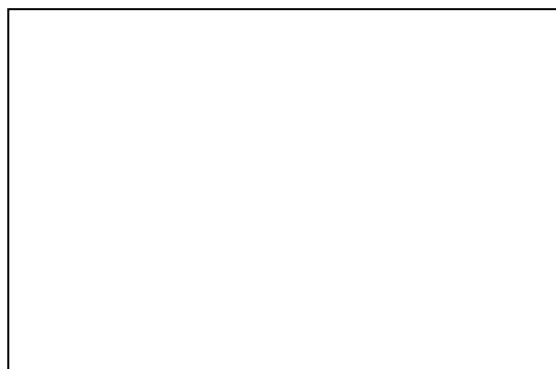
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B. Microscopic

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OBJECTIVE: Identification of Powdery Mildew of Mango

ACTIVITY: Prepare a temporary slide from given sample. Examine the slide under the microscope. Draw the diagrams of conidia and conidiophores as seen under microscope.

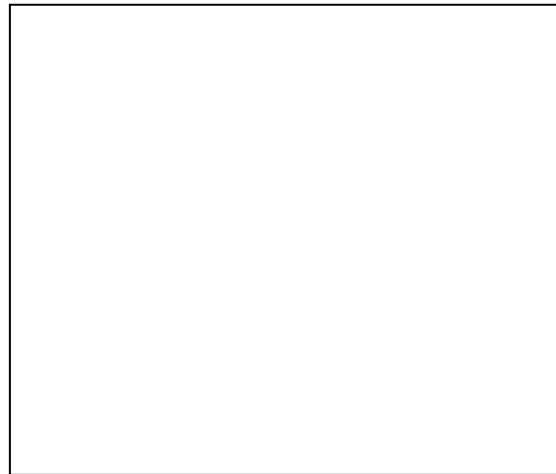
MATERIALS REQUIRED:

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OBSERVATION TO BE MADE:

A. Macroscopic

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B. Microscopic

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OBJECTIVE: Identification of Mango Malformation

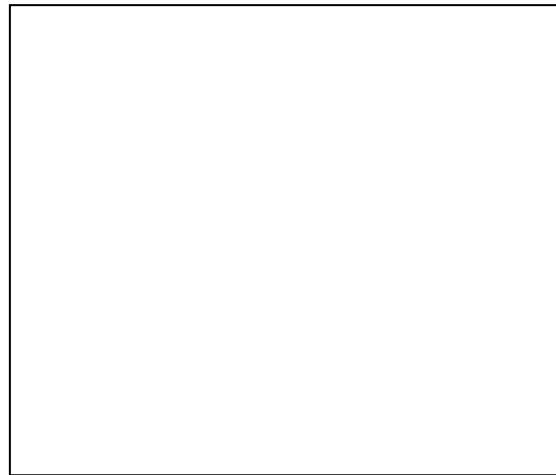
ACTIVITY: Prepare a temporary slide from the diseased material and examine under the microscope. Draw the diagrams of Macroconidia and Microconidia observed under the microscope.

MATERIALS REQUIRED:
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OBSERVATION TO BE MADE:

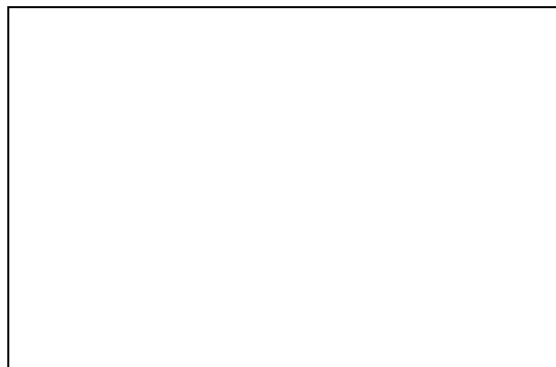
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B. Microscopic

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OBJECTIVE: Identification of Citrus Canker

ACTIVITY: Draw the diagram of the characteristic symptom of the disease

MATERIALS REQUIRED:

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OBSERVATION TO BE MADE:

A. Symptom

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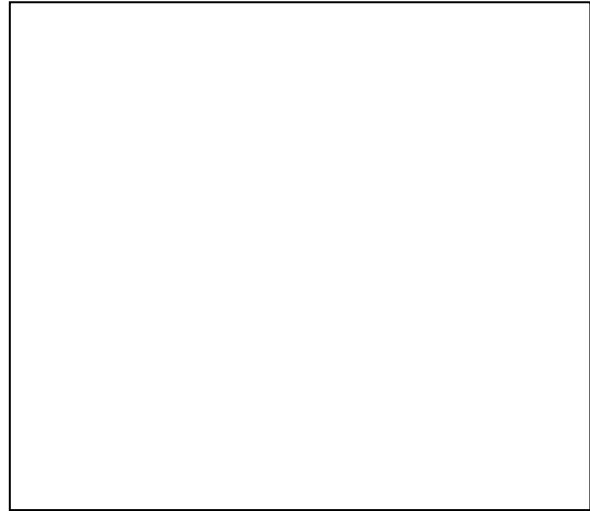
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B. Microscopic

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OBJECTIVE: Identification of Anthracnose and Fruit rot of chilli

ACTIVITY: Prepare a temporary slide by using cotton blue and examined under the microscope. Write characteristic symptoms of as seen under microscope.

MATERIALS REQUIRED:

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OBSERVATION TO BE MADE:

A. Macroscopic

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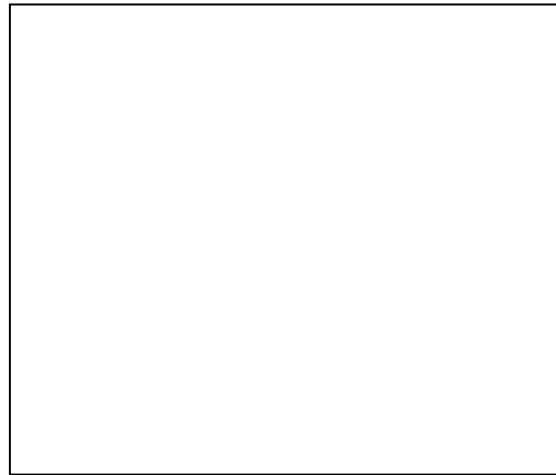
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B. Microscopic

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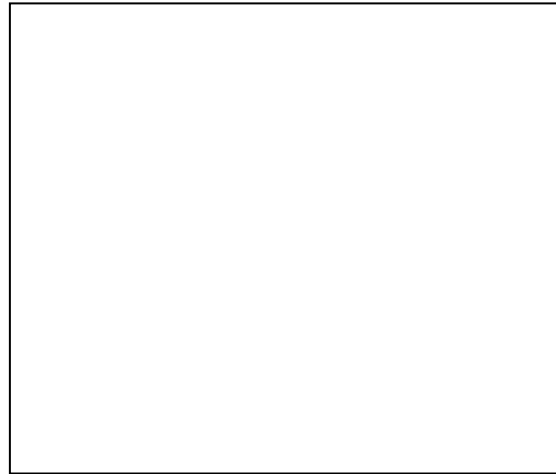
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OBJECTIVE: Identification of Black spot disease of rose

ACTIVITY: Prepare a temporary slide by using cotton blue and examine under the microscope. Draw the diagrams of conidia and conidiophores as seen under microscope.

MATERIALS REQUIRED:

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OBSERVATION TO BE MADE:

A. Symptom

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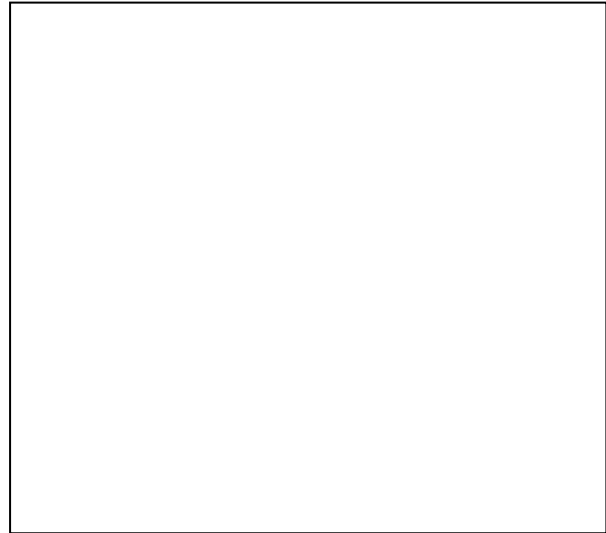
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B. Microscopic

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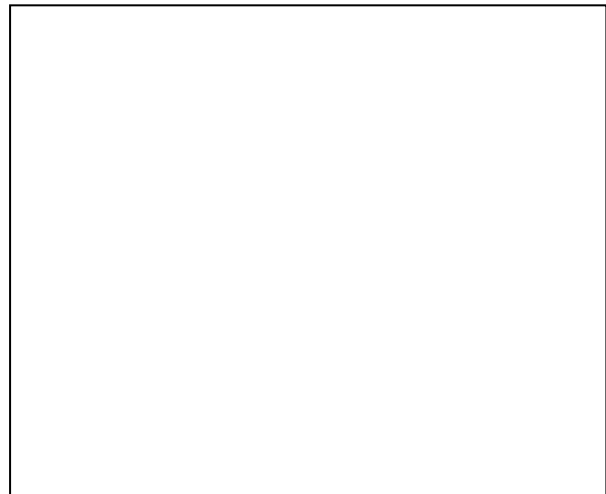
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Temporary mounts (slides) and staining

Materials Required: Glass slide, cover slip, dropper, specimen, Lactophenol cotton blue, needle, watchglass

Procedure: Temporary mounts of diseased plant parts or pathogens are frequently required for microscopic examination. To prepare a mount, the following procedure is followed:

1. First prepare a clean glass slide and cover slip and place a drop of water on the slide.
2. Add the specimen to the drop of water. The specimen is then, properly aligned on the slide with dissecting needles. In many cases, specimens must be torn and teased apart with needles.
3. The cover slip is then, placed on top of the preparation. This is done by placing one edge of the cover slip on the glass slide in contact with the drop of water. Using the tip of a dissecting needle, gently lower the cover slip into position. If this procedure is done correctly, the mount should be free of air bubbles.

Preparation of Fungal Stain:

Lactophenol Cotton Blue: It is used as a general purpose staining and mounting agent for the staining of the fungal structures. It has the following constituents:

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

Mounting Agent:

Glycerine jelly		
Gelatin	-	1.0 g
Glycerine	-	7.0 g
Water	-	6.0 ml

With the addition of phenol (1%).

Use of stain: 1. It helps in proper and correct study of the micro-organisms under the microscope. 2. It differentiates between the host tissue and the micro-organism and 3. It helps in the identification of the parts of the micro-organism.

Precautionary measures: 1. The most common error in making temporary mounts occurs from using too much on too thick material on the slide. Only very thin objects can be studied with the compound microscope. 2. The cover slip must lie flat. 3. The specimen and area under the cover slip must be flooded with the mounting medium. Avoid the presence of water on the rest of the slide or top of the cover slip.

Collection and preservation of plant diseased specimens for herbarium

Materials Required: Polythene bags, Newsprint paper, Pruning shear, knife, Scissors, Hand lens, Pencil, Ink markers, Plant press, Paper bags, Envelopes, blotting sheets methyl bromide

Procedure for Dry Preservation:

1. 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.
2. 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.
3. Disinfection and storage: The specimen folders are fumigated with methyl bromide vapours in fumigation chamber for 24-48 hrs before storage.

Preparation of 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.

Collection and preservation of plant disease specimens for wet preservation

Materials Required: Glacial acetic acid, copper acetate crystals, water, Ethanol, Formaldehyde

Procedure for Wet Preservation:

1. 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.
2. All mounted or preserved specimens must be labeled with as much of the following information as far as possible:
 - a. Host (name of the diseased plant)
 - b. Name of the disease Parasite (the name of the organism causing the disease)
 - c. Place where collected (nearest town and state is usually sufficient)
4. Date collected
5. Name of the collector

Preparation of Formalin Acetic Acid Alcohol (F.A.A.): It is a very good fixative and tissues could be left in it for a long period without any harm.

Composition

50% Alcohol	- 100 ml
40% Formaldehyde	- 6.5 ml
Glacial Acetic Acid	- 2.5 ml

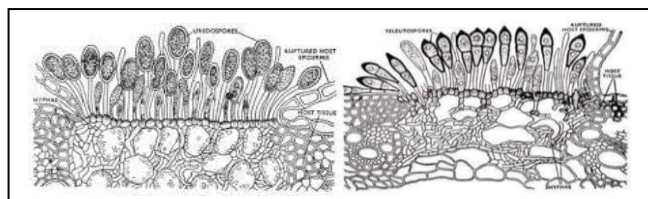
Identification of Leaf / brown rust of wheat

Materials Required: Diseased specimen, Microscope, Slides and cover slips, Forceps, Needle, Razor, Watch Glasses, Cotton blue, Brush.

Pathogen: *Puccinia recondita*

Microscopic

1. Uredia are seen on leaves being rare on leaf sheath and the stalk.
2. Uredia are never in rows and are scattered in small clusters or scattered irregularly over the lamina surface.
3. They are bigger in size as the uredia of yellow rust.
4. Teliospores are not seen.
5. Uredospores are brown and spherical.
6. Walls of the uredospores are minutely echinulate.



Identification of Loose smut of wheat

Materials Required: Diseased material, Slides, Cotton blue, Needle, Forceps and Microscope

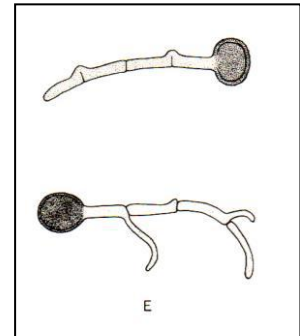
Pathogen: *Ustilago segatum tritici*

A. Symptom

1. Every head of the affected plant is converted into the black masses of spores.
2. There is no grain formation in affected ear.
3. Characteristic yellowing and chlorotic streaks turning into necrotic areas may be observed.

B. Microscopic: Prepare temporary slide of smut spores and observe under the microscope.

1. Spores are olivaceous brown, lighter on one side and darker on the other.
2. They are spherical or oval in morphology
3. The wall of the spores are echinulated specially on the darker side



Ustilago tritici, germinating teliospores

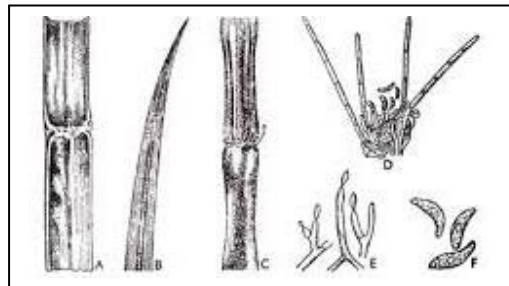
Identification of Red-rot disease of sugarcane

Materials Required: Diseased material, Slides, Cotton blue, Needle, Forceps and Microscope

Pathogen: *Colletotricum falcatum* Perfect Stage: *Glomerella tucumanensis*.

A. Symptom

1. Diseased canes are shrivelled.
2. The rinds are wrinkled.
3. Such canes are lighter in weight and easily broken.
4. The diseased cane is split open longitudinally and examine. The pith is seen to be red in colour along with scattered white mycelial growth of the fungus.



B. Microscopic: Prepare a temporary slide of the fungus and observe under microscope.

1. Conidia are sickled shaped.
2. Conidia are borne on small conidiophores in acervuli.

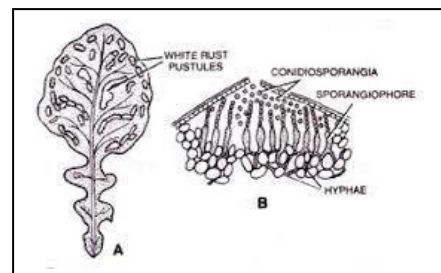
Identification of White rust of mustard

Materials Required: Diseased material, Slide, Cover slips, Microscope, Watch glass, Cotton blue, Razor, Forceps, Needle

Pathogen: *Albugo candida*

A. Symptom

1. Local infections are seen as localized pustules.
2. The pustules on leaves are raised, shiny and white in appearance.
3. The stem of infected plants thickened distorted and rolled.
4. The axis of the inflorescence (hypertrophied) and the stem are twisted and giving a zig-zag appearance.



B. Microscopic

The mycelium is ultra-cellular which produces knob-shaped haustoria in the host cell.

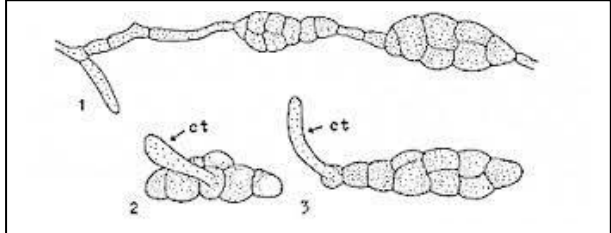
Identification of Alternaria leaf spot of Mustard

Materials Required: Diseased material, Slide, Cover slips, Microscope, Watch glass, Cotton blue, Razor, Forceps, Needle

Pathogen- *Alternaria brassicicola*, *A. brassicae*, and *A. raphani*

A. Symptom: Leaves with small, dark colored concentric spots, they enlarge and become circular with 1 mm in diameter. The spots coalesce to form bigger patch gives the blightened appearance to the leave producing peculiar target-board effect. Linear spots also appear on petioles, stems, pods & seeds.

B. Microscopic: Muricate conidia are produced in chains with transverse as well as longitudinal septa.



Identification of Early blight of Potato

Materials Required: Diseased material, Slide, Cover slips, Microscope, Watch glass, Cotton blue, Razor, Forceps, Needle

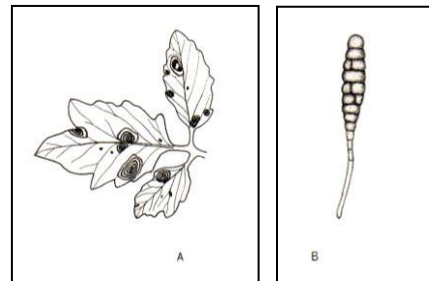
Pathogen: *Alternaria solani*

A. Symptom:

1. **Leaves:** Initially small, isolated pale brown spots on leaves near the ground. Fully developed spots are irregular, brown to dark brown in color, and with concentric rings inside the spot gives “target board” like effect. Spots coalesce to form large patches resulting in the leaf blight. Lowest leaves are attacked first and the disease progresses upwards.
2. **Stem:** Seedling stems are infected at or just above the soil line. The stem turns brown, sunken and dries (collar rot). If the infection girdles the stem, the seedling wilts and dies.
3. **Fruit:** Fruit can be infected at any stage of maturity. Fruit spots are leathery, black, with raised concentric ridges and generally occur near the stem. Infected fruit may drop from the plant.

B. Microscopic:

1. **Mycelium**– branched, septate and dark brown.
2. **Conidiophores**– simple, straight or curved, 1-3 septate and dark coloured.
3. **Conidia**– dictyospore, brown, obclavate with a beak, 3-8 transversely septate and 1-2 longitudinally or obliquely septate, conidia are produced acropetally in chains (catenulate) through the pores formed at the apex of the beak of conidia.



A. Early Blight, affected tomato leaf; B. *Alternaria solani*, conidium

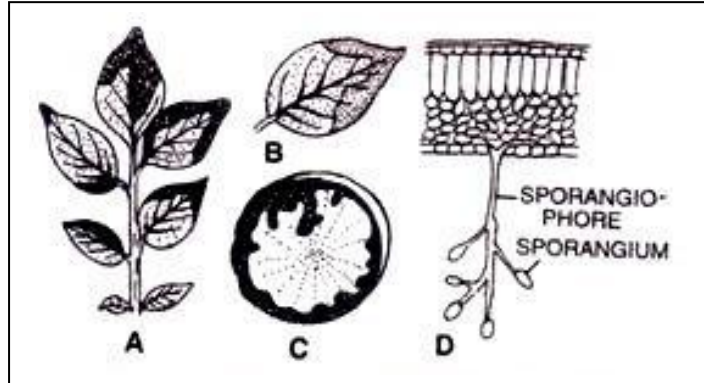
Identification of Late Blight of Potato

Materials Required: Diseased material, Slide, Cover slips, Microscope, Watch glass, Cotton blue, Razor, Forceps, Needle

Pathogen: *Phytophthora infestans*

A. Symptoms:

1. **Leaves:** Small faded green patches that turns into brown spots and downy growth of the pathogen is visible
2. **Stem:** Water soaked stripes
3. **Tuber:** Poor quality tuber, purplish brown spots, wet rot under humid conditions



B. Microscopic:

1. **Mycelium**– unbranched, aseptate and hyaline.
2. **Conidiophores**– sympodially branched, hyaline
3. **Sporangiospore**– hyaline thin walled, lemon-shaped,
4. distinctly papillate

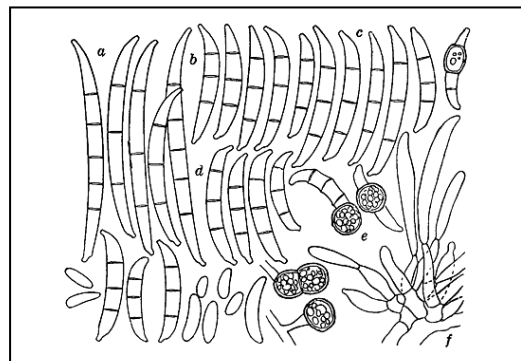
Identification of wilt of chickpea

Materials Required: Diseased material, Slide, Cover slips, Microscope, Watch glass, Cotton blue, Razor, Forceps, Needle

Pathogen: *Fusarium oxysporum* f.sp. *ciceris*

A. Symptoms:

1. Blackening of roots at the base of the stem.
2. Drooping of leaflets, yellowing, withering and drying of the leaf twigs from up wards to down words.
3. On split open of diseased plant root along with stem, black or dark brown streaks are seen in the middle portion of the root (Xylem vessels).
4. Plugging of xylem bundles by the fungal mycelium is seen.



B. Microscopic: Sickle shape macro-conidia and globular micro-conidia, Chlamydospores

Identification of wilt cotton

Materials Required: Diseased material, Slide, Cover slips, Microscope, Watch glass, Cotton blue, Razor, Forceps, Needle

Pathogen: *Fusarium oxysporum* f.sp. *vasinfectum*

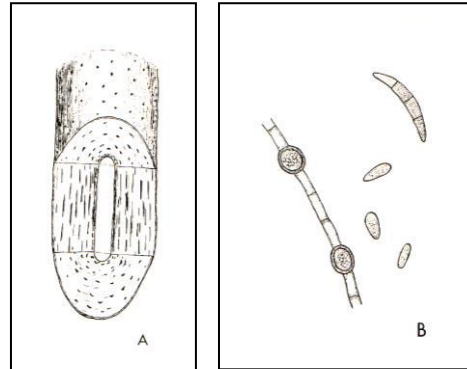
Observation:

A. Symptoms:

1. Vein clearing on cotyledons and first leaf is observed.
2. Wilting of seedling and adult plant as if they have suffered from water shortage even though there may be plenty of moisture in soil.
3. Yellowing withering and drying of leaves and in some plant drying of entire plant or some of its branches are seen.
4. Roots and the base of the stem show as the tissues are bleached.

B. Microscopic

1. The mycelium is hyaline and produces three types of spores.
2. Microconidia: small, elliptical, curved and unicellular.
3. Macroconidia: Long curved pointed at the tip and notched shape at the base with 3 to 4 septate.
4. Chlamydospores are oval or spherical single or in chain and terminal and intercalary.



A. *Fusarium* Wilt of Cotton, cut stem; B. *Fusarium oxysporum* f. *vasinfectum*, macroconidium, microconidia, and mycelium with intercalary chlamydospores

Identification of Anthracnose of Mango

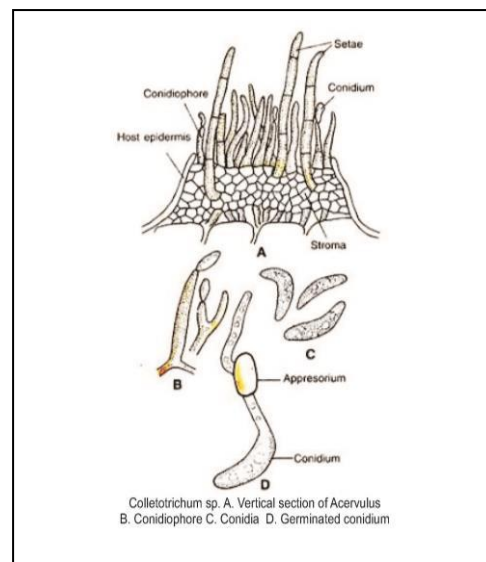
Materials Required: Diseased material, Slide, Cover slips, Microscope, Watch glass, Cotton blue, Razor, Forceps, Needle

Pathogen: *Colletotrichum gloeosporioides*

A. Symptoms

1. Leaves shows oval or irregular, greyish brown spot that may coalesce to cover large area of leaf.
2. Infected leaves often show 'shot hole' appearance.
3. The ripening fruits show black spot appearing on the skin that gradually become sunken and coalesce.

B. Microscopic: Conidia are barrel shaped, single celled, hyaline, small and elongated.



Colletotrichum sp. A. Vertical section of Acervulus B. Conidiophore C. Conidia D. Germinated conidium

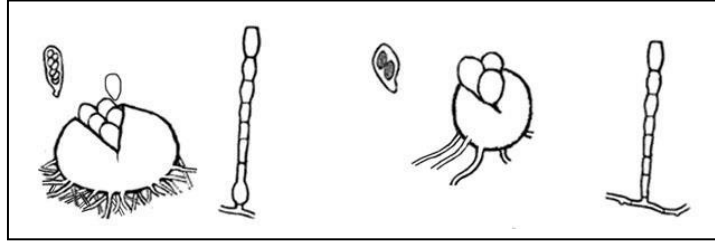
Identification of Powdery Mildew of Mango

Materials Required: Diseased material, Slide, Cover slips, Microscope, Watch glass, Cotton blue, Razor, Forceps, Needle

Pathogen: *Oidium mangiferae*.

A. Symptoms

1. White superficial powdery fungal growth on leaves, stalks of panicles, flowers and young fruits.
2. The affected flowers and fruits drop pre-maturely.
3. Young leaves are attacked on both the sides but it is more conspicuous on the lower surface.



B. Microscopic

1. Conidiophores are short and hyaline
2. Conidia are single celled, barrel shaped produced in chain.

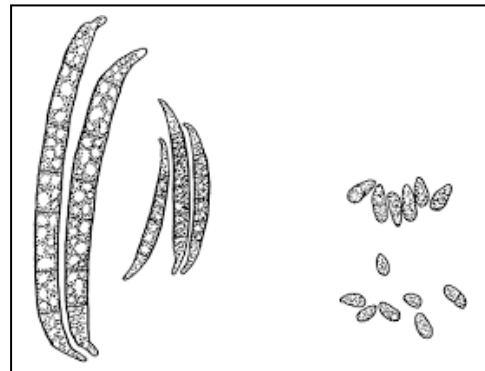
Identification of Mango Malformation

Materials Required: Diseased material, Slide, Cover slips, Microscope, Watch glass, Cotton blue, Razor, Forceps, Needle

Pathogen: *F. mangiferae* var. *subglutinans*, *F. sterilihyphosum*, *F. mexicanum*

A. Symptoms

1. Three types of symptoms: bunched top, floral malformation and vegetative malformation.
2. Bunched top: Shoots remain short and stunted giving a bunched top appearance.
3. Floral malformation: Malformed head dries up in black mass and persist for long time.
4. Vegetative malformation: Excessive vegetative branches of limited growth in seedlings. They are swollen with short internodes forming bunches of various size.



B. Microscopic

1. Microconidia are one or 2 celled, oval to fusiform and produced from polyphialides.
2. Macro conidia are 2-3 celled and falcate.

Identification of Citrus Canker

Materials Required: Diseased material, Slide, Cover slips, Microscope, Watch glass, Cotton blue, Razor, Forceps, Needle

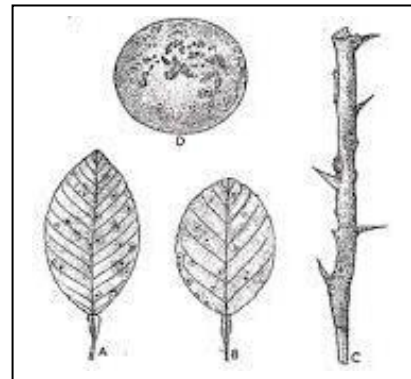
Pathogen: *Xanthomonas axonopodis* pv. *citri*.

A. Symptoms

1. Initially water soaked patches appear which slowly turn brown and produce corky raised spots which leads to yellow
2. halo.
3. Brownish corky out growth with cracks and bacteria oozing out during warm rainy season from cracks.

B. Microscopic

1. The bacterium is rod-shaped, gram-negative, and has a single polar flagellum.
2. Colonies on laboratory media are usually yellow due to 'xanthomonadin' pigment production.



Identification of Anthracnose and Fruit rot of chilli

Material Required: Diseased material, Slide, Cover slips, Microscope, Watch glass, Cotton blue, Razor, Forceps, Needle

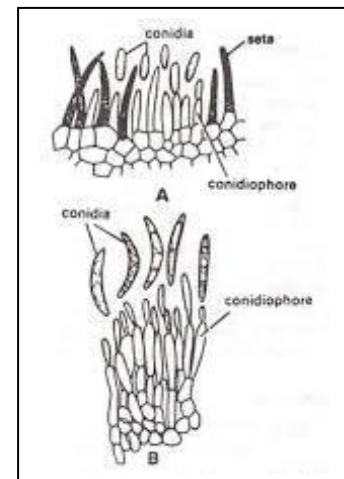
Pathogen: *Colletotrichum capsici*

A. Symptoms

1. Shedding of flowers due to the infection at pedicel and tips of branches.
2. Brown water soaked patch appears on the skin of the fruit.
3. Severe infection result in the shriveling and drying of fruits. Such fruits become white in colour and lose their pungency.
4. On the surface of the lesions minute black dot lie fruiting bodies called 'acervulli' occur.

B. Microscopic

1. Produces hyaline
2. Conidia are sickled-shaped.
3. Conidia are borne on small conidiophores in acervuli.



Identification of Black spot disease of rose

Materials Required: Diseased material, Slide, Cover slips, Microscope, Watch glass, Cotton blue, Razor, Forceps, Needle

Pathogen: *Diplocarpon rosae*

Symptoms:

1. Purplish or blackspots appears on the upper leaf surface, these expand rapidly into patches.
2. Within these spots diffuse and radiating strands of fungal hyphae are sometimes visible.
3. The leaf tissue may turn yellow around the spots and the leaf often drops early.

Microscopic observation: The fungus produced One or 2-celled conidia.

