# PRACTICAL MANUAL

For

# DISEASES OF FIELD AND HORTICULTURAL CROPS & THEIR MANAGEMENT - II

Course No. PPA-321 Credit Hours 3(2+1)

For

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



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2024

# Department of Plant Pathology College of Agriculture

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Identification and histopathological studies of selected diseases of field and horticultural crops. Field visits are needed to diagnose field problems. Collection and preservation of plant-diseased specimens for herbarium. **Note:** Students should submit 50 pressed and well-mounted specimens.

| Name of Student                           |  |
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# **OBJECTIVE:** Prepare a temporary mount (slide) and stains.

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

| MATERIALS REQUIRED:  |       |
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# OBJECTIVE: Detection, collection and preservation of dry plant disease specimens for herbarium

ACTIVITY: Collect twenty disease samples from the university or nearby field and prepare a herbarium with the following details:

|                                | 1 1            | 2      | 3       | 4        | 5      |
|--------------------------------|----------------|--------|---------|----------|--------|
| a. Name of the plant           |                |        | 124     | -        | •      |
| b. Name of the disease         |                |        |         |          |        |
| c. Name of the causal organism | 5              |        |         | F        | 5      |
| d. Place of collection         | OLUE.          | REITY  | AOA     | The same | E)     |
| e. Date of collection          | 0              |        |         | 9        | 199    |
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| 1777 500 14                    | 6              | 7      | 8       | 9        | 10     |
| a. Name of the plant           | <i>y</i> -     |        | 1 1 1 N | A C      |        |
| b. Name of the disease         |                |        |         | 1 5      |        |
| c. Name of the causal organism | 35             | 74     | IJ      | 57       |        |
| d. Place of collection         | NY,            | 1      | _ 19    | 1 3      |        |
| e. Date of collection          |                |        | 100     | 000      | 1 5337 |
| MATERIALS REQUIRED:            |                |        |         |          |        |
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# OBJECTIVE: Detection, collection and preservation of plant diseased specimens for wet preservation

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

| MATERIALS REQUIRED:                      |  |
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# **OBJECTIVE:** Detection and identification of Leaf or brown rust of wheat

ACTIVITY: Preparation of a slide from the brown rust sample and observation under the microscope.

Draw the diagram of the symptoms and microscopic observation (uredospores and teliospores) as observed under the microscope.

| MATERIALS REQUIRED:     |  |
|-------------------------|--|
| OBSERVATION TO BE MADE: |  |
| A. Macroscopic          |  |
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| B. Microscopic          |  |
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# **OBJECTIVE:** Detection and identification of Loose smut of wheat.

| ACTIVITY: Identification of the pathogen from the giv<br>the symptom, and smut spores as observe | en sample. Prepare a slide, and draw the diagram o<br>ed under the microscope. |
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| MATERIALS REQUIRED:  |  |
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| OBSERVATION TO BE MADE:  |  |
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# **OBJECTIVE:** Detection and identification of Red-rot disease of sugarcane.

ACTIVITY: Cut the transverse section (T.S.) of the infected sugarcane stem. Write down the characteristic symptom and draw the diagram of symptom and spores as seen under the microscope MATERIALS REQUIRED: **OBSERVATION TO BE MADE:** A. Macroscopic ..... **B.** Microscopic .....

# **OBJECTIVE: Detection and identification of White rust of mustard**

ACTIVITY: Preparation of a slide from a leaf sample infected with white rust and observe under microscope and identify the pathogen. Draw the diagram of 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          | W W S W |
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# **OBJECTIVE: Detection and identification of Alternaria leaf spot of Mustard**

ACTIVITY: Preparation of a slide from infected leaf and identification of the pathogen. Draw the diagram of the symptoms and spores as 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: Detection and identification of Early blight of Potato**

ACTIVITY: Preparation of a slide from an infected potato leaf sample and observed under microscope for identification of the pathogen. Draw the diagrams of symptoms and spores seen under the microscope.

| MATERIALS REQUIRED:     |         |
|-------------------------|---------|
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| OBSERVATION TO BE MADE: |         |
| A. Macroscopic          |         |
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| B. Microscopic          | W W W W |
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# **OBJECTIVE: Detection and identification of Late Blight of Potato**

ACTIVITY: Preparation of a slide from infected potato leaf sample and identification of the pathogen.

Draw the diagrams of a 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: Detection and identification of wilt of chickpea**

ACTIVITY: Preparation of the slide from the cut section of the diseased root and observe under a microscope. Draw the diagram of a symptom and spores seen under the microscope.

| MATERIALS REQUIRED:     |  |
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| OBSERVATION TO BE MADE: | The same of the sa |
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# **OBJECTIVE:** Detection and identification of wilt cotton.

ACTIVITY: Prepare the slide of the fine section of the diseased root and prepare a temporary slide. Draw the diagram of symptoms seen under the microscope.

| MATERIALS REQUIRED:     |   |
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| OBSERVATION TO BE MADE: | • |
| A. Macroscopic          |   |
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| B. Microscopic          | Ü |
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# **OBJECTIVE: Detection and identification of Anthracnose of Mango**

| ACTIVITY: Prepare a slide from a cut section of a disernant the diagram of a symptom and spores s MATERIALS REQUIRED: | een under the microscope. |          |
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| OBSERVATION TO BE MADE:   |                           |          |
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# **OBJECTIVE: detection and identification of Powdery Mildew of Mango**

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

| MATERIALS REQUIRED:     |  |
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| OBSERVATION TO BE MADE: | The same of the sa |
| A. Macroscopic          |  |
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# **OBJECTIVE: Detection and identification of Mango Malformation**

| ACTIVITY: Prepare a slide from the diseased sample and Macroconidia and Microconidia as observed u | d observe under the microscope. Draw the diagrams of inder the microscope. |
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| MATERIALS REQUIRED:  |  |
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| OBSERVATION TO BE MADE:  |  |
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# **OBJECTIVE: Detection and identification of Citrus Canker** ACTIVITY: Draw the diagram of a symptom of the disease. MATERIALS REQUIRED: ..... **OBSERVATION TO BE MADE:** A. Symptom ..... **B. Microscopic** .....

# **OBJECTIVE: Detection and identification of Anthracnose and Fruit rot of chilli**

ACTIVITY: Prepare a slide from the infected given sample and observe under the microscope. Write characteristic symptoms as seen under a microscope.

| MATERIALS REQUIRED:     |  |
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| OBSERVATION TO BE MADE: |  |
| A. Macroscopic          |  |
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| B. Microscopic          |  |
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# **OBJECTIVE: Detection and identification of Black spot disease of rose**

| ACTIVITY: Preparation of a slide from infected samp diagrams of conidia and conidiophores as   | ele and observed under the microscope. Draw the seen under a microscope.   |
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| MATERIALS REQUIRED:  |  |
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| OBSERVATION TO BE MADE:  |  |
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#### **APPENDICES**

#### Temporary mounts (slides) and staining

Materials Required: Glass slide, coverslip, dropper, specimen, Lactophenol cotton blue, needle, watch glass

**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 take 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 placed on top. 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 coverslip into position. If this procedure is done correctly, the mount should be free from air bubbles.

#### **Preparation of Fungal Stain:**

**Lactophenol Cotton Blue:** It is used as a staining and mounting agent for the staining of 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 the 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 a layer of blotting sheets under sunlight or in a hot air oven for a few days.
- 2. Labelling and packaging: The material should be kept in good herbarium packets. This is attached to a chart paper sheet. The two sides of a packet are folded first, then the bottom flap and finally the top flap. The name of a pathogen, host, locality, date, and name of a 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 the 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 glacial acetic acid saturated with normal copper acetate crystals and 4 parts water till the green colour reappears and then preserved in 5 per cent formalin in the glass jars.
- 2. All mounted or preserved specimens must be labelled 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
- Name of the collector

**Preparation of Formalin Acetic Acid Alcohol (F.A.A.):** It is a very good fixative and tissues can 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

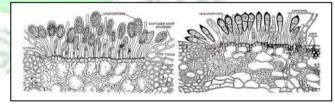
#### Identification of Leaf / brown rust of wheat

**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 than the uredia of yellow rust.
- 4. Teliospores are not seen.
- 5. Uredospores are brown and spherical.
- 6. Walls of the uredospores are minutely echinulate.



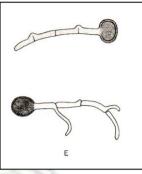
#### Identification of Loose smut of wheat

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 the affected ear.
- 3. Characteristic yellowing and chlorotic streaks terming into necrotic areas may be observed.
- **B. Microscopic:** Prepare a temporary slide of smut spores and observe under the microscope.
  - 1. Spores are olivaceous brown, lighter on one side and duster on the other.
  - 2. They are spherical or oval in morphology
  - 3. The wall of the spores is echinulated especially 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

- 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 examined. The pith is seen to be red along with scattered white mycelial growth of the fungus.
- **B. Microscopic:** Prepare a temporary slide of the fungus and observe under a 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, Coverslips, 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 give a zig-zag appearance.

#### **B.** Microscopic

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

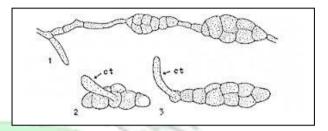
#### Identification of Alternaria leaf spot of Mustard

**Materials Required:** Diseased material, Slide, Coverslips, 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, 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, Coverslips, 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, and with concentric rings inside the spot giving a "target board" 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, and 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.
- Conidiophores— simple, straight or curved, 1-3 septate and dark coloured.
- 3. **Conidia** dictyospore, brown, obclavate with a beak, 3-8 tranversely 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, Coverslips, Microscope, Watch glass, Cotton blue, Razor,

Forceps, Needle

Pathogen: Phytophthora infestans

#### A. Symptoms:

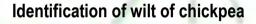
1. **Leaves**: Small faded green patches that turn into brown spots and downy growth of the pathogen is visible

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

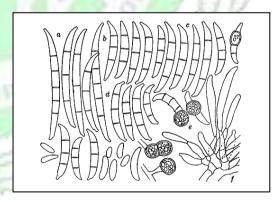


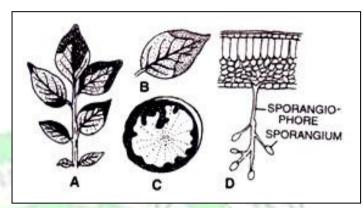
**Materials Required:** Diseased material, Slide, Coverslips, Microscope, Watch glass, Cotton blue, Razor, Forceps, Needle

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 upwards 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, Coverslips, Microscope, Watch glass, Cotton blue, Razor, Forceps, Needle

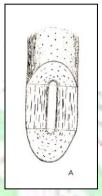
Pathogen: Fusarium oxysporum f. sp. vasinfectum

Observation: A. Symptoms:

- 1. Vein clearing on cotyledons and first leaf is observed.
- 2. Wilting of seedlings and adult plants as if they have suffered from water shortage even though there may be plenty of moisture in the soil.
- 3. Yellowing withering and drying of leaves and in some plants drying of the 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

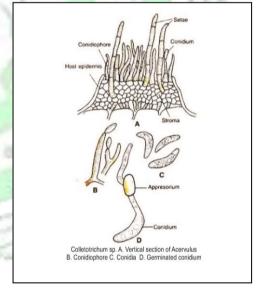
#### **Identification of Anthracnose of Mango**

**Materials Required:** Diseased material, Slide, Coverslips, 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 a '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.



#### **Identification of Powdery Mildew of Mango**

**Materials Required:** Diseased material, Slide, Coverslips, Microscope, Watch glass, Cotton blue, Razor, Forceps, Needle

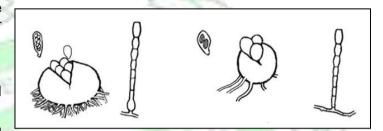
Pathogen: Oidium mangiferae.

#### A. Symptoms

- 1. White superficial powdery fungal growth on leaves, stalks of panicles, flowers and young fruits.
- 2. The affected flowers and fruits drop prematurely.
- 3. Young leaves are attacked on both 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.



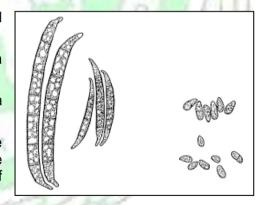
# **Identification of Mango Malformation**

**Materials Required:** Diseased material, Slide, Coverslips, Microscope, Watch glass, Cotton blue, Razor, Forceps, Needle

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

#### A. Symptoms

- 1. Three types of symptoms: bunchy top, floral malformation and vegetative malformation.
- 2. Bunchy top: Shoots remain short and stunted giving a bunchy top appearance.
- 3. Floral malformation: The malformed head dries up in a black mass and persists for a long time.
- 4. Vegetative malformation: Excessive vegetative branches of limited growth in seedlings. They are swollen with short internodes forming bunches of various sizes.



#### **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 that leads to yellow
- 2. halo.
- 3. Brownish corky outgrowth 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.



Material Required: Diseased material, Slide, Coverslips, Microscope, Watch glass, Cotton blue, Razor, Forceps,

Needle

Pathogen: Colletotrichum capsici

#### A. Symptoms

- 1. Shedding of flowers due to the infection at the pedicel and tips of branches.
- 2. A brown water soaked patch appears on the skin of the fruit.
- 3. Severe infection results in the shrivelling and drying of fruits. Such fruits become white and lose their pungency.
- 4. On the surface of the lesions minute black dots 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, Coverslips, Microscope, Watch glass, Cotton blue, Razor,

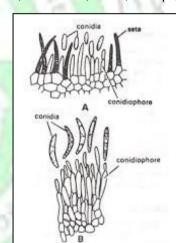
Forceps, Needle

Pathogen: Diplocarpon rosae

#### Symptoms:

- 1. Purplish or blackspots appear 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.



Overwintering stage matures

Qverwintering stage

