PRACTICAL MANUAL

on

DISEASES OF FRUIT, PLANTATION, MEDICINAL AND AROMATIC CROPS

Course No. PPH 214 Credit Hrs. 3(2+1)

For

B.Sc. (Horticulture)

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2024

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College of Agriculture

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Syllabus: Diseases of Fruit, Plantation, Medicinal and Aromatic Crops

Practical: Observations of disease symptoms, identification of casual organisms and host parasite relationship of important diseases.

Examination of scrapings and cultures of important pathogens of fruits, plantation, medicinal and aromatic crops.

Name of Student .....

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Batch .....

Session .....

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Date: Course Teacher

CONTENTS

Experiment No. 1

Objective: To prepare and observe temporary slides of symptomatic plant tissue

Activity:

1. Discuss the use and the composition of lactophenol cotton blue.
2. Prepare a slide using the provided sample and stain it using lactophenol cotton blue.

Materials Required: ……

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Procedure:

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Preparation of lactophenol cotton blue:

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Use of Stain:

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Precautionary Measures:

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Experiment No. 2

Objective: Identify and preserve plant disease specimens for herbarium preparation

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

a. Host (name of the diseased plant)

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

c. Place where collected

d. Date of collection

e. Name of the collector

Materials Required: ……

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Procedure for Dry Preservation:

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Preparation of Specimen:

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Experiment No. 3

Objective: Identification and wet preservation of diseased plant specimens

Activity:

1. Prepare FAA solution for the preservation of plant disease samples
2. Collect the disease samples and preserve them in glass bottles following the wet preservation protocol.

Materials Required:……

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Procedure for Wet Preservation:

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Preparation of Formalin Acetic Acid Alcohol (F.A.A)

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Experiment No. 4

Objective: Identification of mango powdery mildew pathogen

Activity: Identify the pathogen from the disease sample provided to you by preparing slide. Draw neat diagrams of characteristic symptoms and spores observed under the microscope.

Materials Required: …….

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Observations:

Symptoms: ……

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Microscopic: ……..……

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Identification:…… ..……..……..

Experiment No. 5

Objective: Identification of mango anthracnose pathogen

Activity: Identify the pathogen from the disease sample provided to you by preparing slide. Draw neat diagrams of characteristic symptoms and spores observed under the microscope.

Materials Required: …….

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Observations:

Symptoms: ……..……

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Microscopic: ……..……

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Identification:…… ..……..……..

Experiment No. 6

Objective: Identification of mango malformation pathogen

Activity: Identify the pathogen from the disease sample provided to you by preparing slide. Draw neat diagrams of characteristic symptoms and spores observed under the microscope.

Materials Required: …….

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Observations:

Symptoms: ……..……

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Microscopic: ……

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Identification:…… ..……..……..

Experiment No. 7

Objective: Identification of citrus canker disease

Activity: Identify the pathogen from the disease sample and draw neat diagrams of characteristic symptoms observed.

Materials Required: …….

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Observations:

Symptoms: ……

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Microscopic: ……..……

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Identification:…… ..……..……..

Experiment No. 8

Objective: Identification of citrus scab pathogen

Activity: Identify the pathogen from the disease sample provided to you by preparing slide. Draw neat diagrams of characteristic symptoms and spores observed under the microscope.

Materials Required: …….

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Observations:

Symptoms: ……

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Microscopic: ……..……

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Identification:…… ..……..……..

Experiment No. 9

Objective: Identification of banana panama wilt pathogen

Activity: Identify the pathogen from the disease sample provided to you by preparing slide. Draw neat diagrams of characteristic symptoms and spores observed under the microscope.

Materials Required: ……

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Observations:

Symptoms: ……

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Microscopic: ……

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Identification:…… ……

Experiment No. 10

Objective: Identification of banana sigatoka pathogen

Activity: Identify the pathogen from the disease sample provided to you by preparing slide. Draw neat diagrams of characteristic symptoms and spores observed under the microscope.

Materials Required: …….

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Observations:

Symptoms: ……..……

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Microscopic: ……..……

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Identification:…… ..……..……..

Experiment No. 11

Objective: Identification of banana bunchy top disease

Activity: Identify the pathogen from the disease sample provided to you and draw neat diagrams of characteristic symptoms observed.

Materials Required: …….

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Observations:

Symptoms: ……

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Microscopic: ……

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Identification:…… ..……..……..

Experiment No. 12

Objective: Identification of guava wilt pathogen

Activity: Identify the pathogen from the disease sample provided to you by preparing slide. Draw neat diagrams of characteristic symptoms and spores observed under the microscope.

Materials Required: …….

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Observations:

Symptoms: ……

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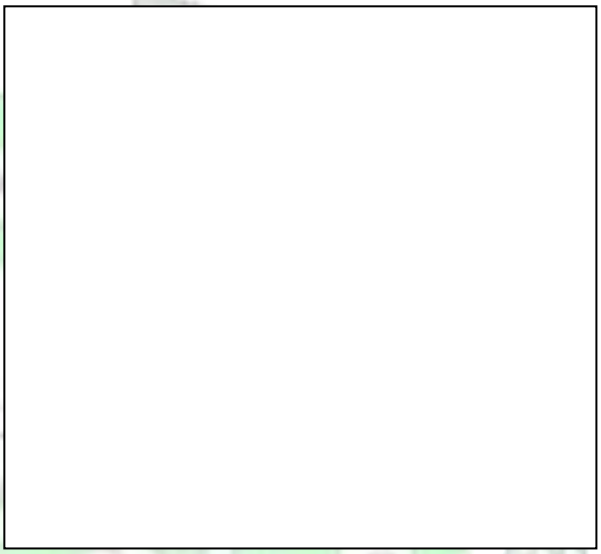

Microscopic: ……

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Identification:…… ……..……..

Experiment No. 13

Objective: Identification of papaya ring spot disease

Activity: Identify the pathogen from the disease sample provided to you and draw neat diagrams of characteristic symptoms observed.

Materials Required: …….

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Observations:

Symptoms: ……..……

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Microscopic: ……..……

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Identification:…… ……..……..

Experiment No. 14

Objective: Identification papaya leaf curl disease

Activity: Identify the pathogen from the disease sample provided to you and draw neat diagrams of characteristic symptoms observed.

Materials Required: …….

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Observations:

Symptoms: ……..……

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Microscopic: ……

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Identification:…… ..……..……..

Experiment No. 15

Objective: Identification of pomegranate bacterial blight disease

Activity: Identify the pathogen from the disease sample provided to you and draw neat diagrams of characteristic symptoms observed.

Materials Required: …….

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Observations:

Symptoms: ……

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Microscopic: ……..……

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Identification……

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Experiment No. 16

Objective: Identification of pomegranate anthracnose pathogen

Activity: Identify the pathogen from the disease sample provided to you by preparing a slide. Draw neat diagrams of characteristic symptoms and spores observed under the microscope.

Materials Required: …….

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Observations:

Symptoms: ……..……

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Microscopic: ……..……

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Identification:……..……..……

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Experiment No. 17

Objective: Identification of ber powdery mildew pathogen

Activity: Identify the pathogen from the disease sample provided to you by preparing slide. Draw neat diagrams of characteristic symptoms and spores observed under the microscope.

Materials Required: ……..

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Observations:

Symptoms: ……

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Microscopic: ……

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Identification:……..……..……

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PREPARATION OF TEMPORARY MOUNTS AND STAIN

Procedure:

Microscope Slide:

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

Fungal Stain:

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

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

Mounting Agent:

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

Purpose of the Stain:

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

Precautionary Measures:

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

DRY PRESERVATION

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

Specimen: A herbarium specimen can include a single sporocarp or a portion of it, a dried culture, a slide, or material attached to its host or substrate (e.g., leaf, stem, bark, rock, soil, paper, cloth). There are two primary preservation methods for diseased plant specimens: Dry Preservation and Wet Preservation.

Procedure for Dry Preservation:

1. **Collection and Drying:**
    - Ensure the sample displays distinct symptoms of disease.
    - Dry the specimen between layers of blotting sheets under sunlight or in a hot air oven for a few days.
2. **Labeling and Packaging:**
    - Place the dried material in herbarium packets, attaching these to chart paper sheets.
    - Fold the two sides of the packet first, followed by the bottom flap, and finally the top flap.
    - Label the packet with the pathogen's name, host, locality, date, and the name of the scientist who identified the specimen.
3. **Disinfection and Storage:**
    - Fumigate the specimen folders with methyl bromide vapors in a fumigation chamber for 24-48 hours before storage.

Preparation of Specimen:

- The ideal specimen should be 25–40 cm long and up to 26 cm wide, allowing it to fit on a standard herbarium mounting sheet (42 x 27 cm).
- For large plant parts, such as a palm or cycad frond, cut them into sections and press each section onto separate sheets.
- Long, narrow specimens (e.g., grasses and sedges) can be folded once, twice, or even three times during pressing. This allows plants up to 1.6 meters tall to fit on a single sheet.
- For very small plants, multiple individuals can be placed on each sheet.

WET PRESERVATION

Preservative:
A preservative is a chemical used to fix plant and animal tissues, preventing decomposition and maintaining their original shape, form, size, and structure. It hardens tissues and prevents decay by rapidly penetrating the tissue.

Procedure:

1. **Preparation and Preservation:**
   Fresh, diseased specimens should be washed and then placed in a boiling mixture of one part

glacial acetic acid saturated with normal copper acetate crystals and four parts water until the green color reappears. Afterward, store the specimens in 5% formalin within glass jars.

2. **Labeling:**
   All mounted or preserved specimens should be labeled with the following information, where possible:
   - Host (name of the diseased plant)
   - Disease Parasite (name of the organism causing the disease)
   - Place of collection (nearest town and state)
   - Date of collection
   - Name of the collector

Preparation of Formalin Acetic Acid Alcohol (F.A.A.):
F.A.A. is an excellent fixative that allows tissues to be preserved for an extended period without degradation.

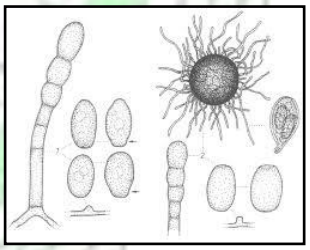
Composition:

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

POWDERY MILDEW OF MANGO (Pathogen: *Oidium mangiferae*)

Symptoms

1. A white, superficial powdery fungal growth appears on the leaves, panicle stalks, flowers, and young fruits.
2. Affected flowers and fruits tend to drop prematurely.
3. Young leaves are attacked on both surfaces, with the lower surface showing more prominent symptoms.



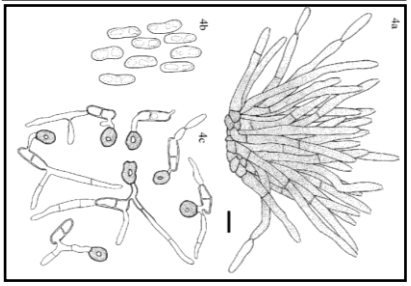
Microscopic Examination**:** To examine microscopically, prepare a temporary slide using cotton blue dye and observe under a microscope.

1. **Conidiophores:** Short and hyaline (transparent).
2. **Conidia:** Single-celled, barrel-shaped, and produced in chains.

MANGO ANTHRACNOSE (pathogen: *Colletotrichum gloeosporioides*)

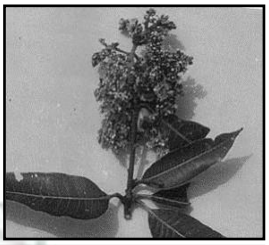
Symptoms

1. Leaves develop oval or irregular, greyish-brown spots that may merge, covering a large area of the leaf.
2. Infected leaves often display a 'shot hole' appearance due to tissue damage.
3. Ripening fruits exhibit black spots on the skin that gradually become sunken and may merge together.



Microscopic Examination: To examine under a microscope, cut a section of the diseased tissue and prepare a temporary slide. Conidia are barrel-shaped, single-celled, hyaline (transparent), small, and elongated.

MANGO MALFORMATION



Pathogens: Different species of Fusarium (*F. mangiferae* var. *subglutinans, F. sterilihyphosum, F. mexicanum*)

Symptoms

1. **Types of Symptoms:** The disease manifests in three ways: bunchy top, floral malformation, and vegetative malformation.
2. **Bunchy Top:** The shoots remain short and stunted, resulting in a "bunchy top" appearance.
3. **Floral Malformation:** The malformed flower heads dry up, turning into black masses that persist for an extended period.
4. **Vegetative Malformation:** Seedlings show excessive vegetative branching with limited growth. The branches are swollen, have short internodes, and form clusters of various sizes.

Microscopic Examination

To conduct a microscopic examination, prepare a temporary slide from the diseased material and observe under the microscope.

1. **Microconidia:** These are one or two-celled, oval to fusiform in shape, and are produced from polyphialides.
2. **Macroconidia:** These are two to three-celled and falcate (curved or sickle-shaped).

CITRUS CANKER (**Pathogen**: *Xanthomonas axonopodis* pv. *citri*)

Symptoms:

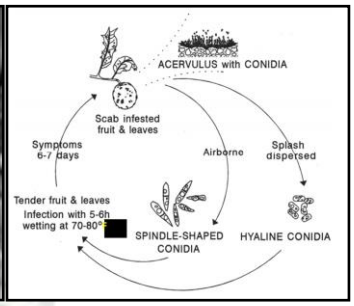
1. Initially, water-soaked patches appear on the affected areas, which gradually turn brown and develop corky, raised spots surrounded by yellow halos.
2. During warm, rainy seasons, brownish corky outgrowths with cracks appear, from which bacteria may ooze out.

Microscopic Examination:

1. **Bacterial Morphology:** The bacterium is rod-shaped, gram-negative, and possesses a single polar flagellum.
2. **Colony Appearance:** On laboratory media, the colonies are typically yellow due to the production of the pigment 'xanthomonadin.'

CITRUS SCAB (Pathogen: *Elsinoe fawcetti*)

Symptoms



1. Initially small, semi-translucent dots like lesion develops on leaves which become sharply defined pustular elevations.
2. Affected leaves become stunted, malformed, wrinkled or puckered, with irregular torn margins.
3. On the fruit, lesions consist of corky projections are observed.

Microscopic Examination:

Prepare a temporary slide of spores and examine under the microscope.

1. Conidia are hyaline, oblong or spindle shaped and 2-4 x 4-8 µm in size.
2. Asci are ovoid, ascospore are 1- 3 septate oblong to elliptical.
3. The acervulus are typically saucer-shaped

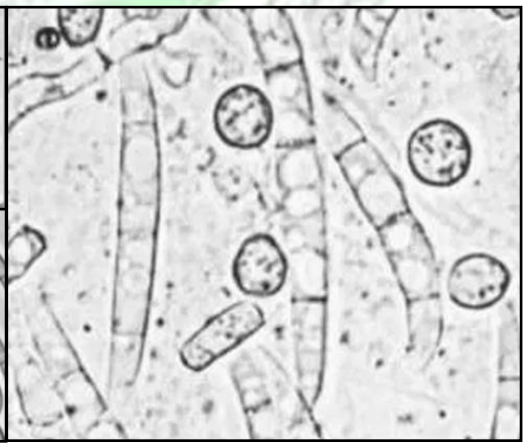
BUNCHY TOP OF BANANA (Pathogen: *Banana bunchy top virus*)

Vectored by aphid.

Symptoms

1. Initially, dark green streaks appear in the veins of lower portion of the leaf midrib and the leaf stem.
2. The leaves appear to be “bunched” at the top of the plant, the symptom for which this disease is named.
3. Severely infected banana plants usually will not fruit, but if fruit is produced small banana which are likely to be distorted and twisted.

PANAMA WILT OF BANANA (Pathogen: *Fusarium oxysporum* f. sp *cubense*)



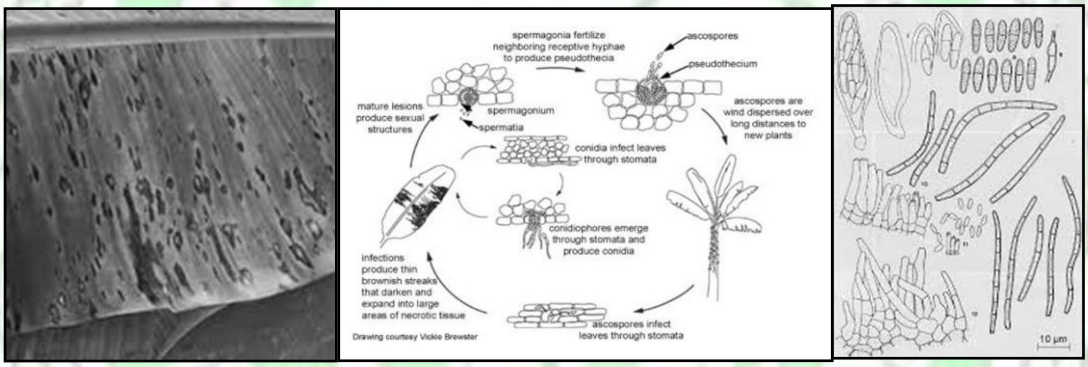
Symptoms

1. The lowermost leaves begin to yellow, starting from the margins and moving towards the midrib.
2. The yellowing gradually extends upwards to the other leaves.
3. Affected leaves break near their base and hang down around the pseudostem.
4. There is longitudinal splitting of the pseudostem.
5. The vascular vessels show discoloration, appearing as red or brown streaks.

Microscopic Examination: To perform a microscopic examination, cut a section of the diseased root, prepare a temporary slide, and observe under a microscope.

1. **Microconidia:** These are single-celled, septate, hyaline, and elliptical or oval in shape.
2. **Macroconidia:** These are sickle-shaped, hyaline, have 3-5 septa, and taper at both ends.
3. **Chlamydospores:** These are thick-walled, spherical to oval, and range in color from hyaline to slightly yellowish.

SIGATOKA DISEASE OF BANANA (Pathogens: *Mycosphaerella musicola and Mycosphaerella fijiensis*)



Symptoms

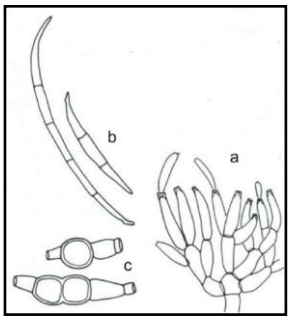
1. A slight discoloration appears between the leaf's secondary veins.
2. These discolored areas gradually develop into pale yellow streaks, brown streaks, and elliptic necrotic spots arranged parallel to the secondary veins.
3. The spots have a depressed grey center surrounded by a yellow halo.
4. As the disease progresses, the lesions merge and cover a larger area of the leaf.

Microscopic Examination: To examine microscopically, prepare a temporary slide of the fungus and observe under a microscope.

1. **Conidia:** These are hyaline, cylindrical or curved, and usually have 3-5 (or more) septa.
2. **Ascospores:** These are septate, hyaline, ellipsoidal, with the upper cell slightly broader than the lower, measuring 14.5-18.0 x 3-4 µm.

GUAVA WILT (Pathogen: *Fusaium oxysporum* f. sp. *Psidii*)

Symptoms



1. Browning and wilting of leaves from tip.
2. The leaves shed of premature, fruit size remain smaller, bare twig fail to develop new leaves and flower dry up.
3. Discolouration of stem and death of branches and then the whole tree dry up.

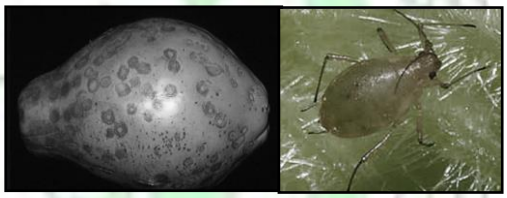
Microscopic Examination: To perform a microscopic examination, cut a section of the diseased root, prepare a temporary slide, and observe under a microscope.

1. **Microconidia:** These are oval to ellipsoid, cylindrical, straight to curved, and measure 7 to 10 x 2 to 3 µm. They are borne on simple phialides that arise laterally on the hyphae.
2. **Macroconidia:** These are 3 to 4 septate, fusoid to subulate, pointed at both ends, and measure 32 to 50 x 3 to 7 µm.

PAPAYA RING SPOT (Pathogen: *Papaya Ring Spot virus* (PRSV)

Vectored by aphid

Symptoms

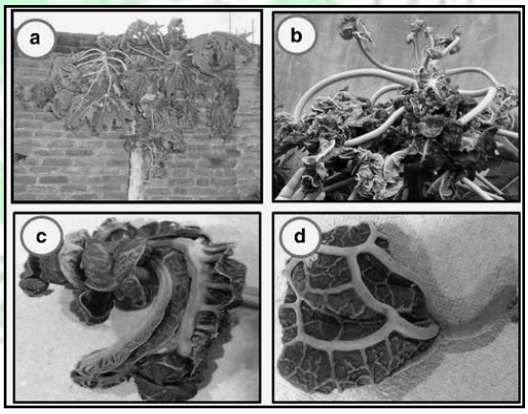


1. The earliest symptoms on papaya are a yellowing and vein-clearing of the young leaves.
2. Dark-green streaks and concentric rings appear in the leafstalks and stems. The rings are darker green than the background-green fruit colour.
3. Malformation and reduction of the lamina which may become extremely filliform.

PAPAYA LEAF CURL (Pathogen: *Papaya leaf curl virus* (PaLCuV)

Vectored by Whitefly

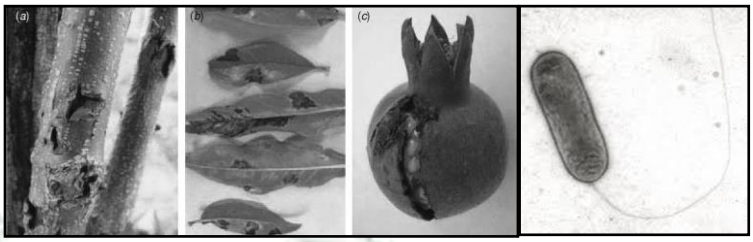
Symptoms



1. Curling, crinkling and distortion of leaves, reduction of leaf lamina, rolling of leaf margins inward and downward, thickening of veins.
2. Leaves become leathery, brittle and distorted. Plants stunted. Affected plants do not produce flowers and fruits.

BACTERIAL BLIGHT OF POMEGRANATE (Pathogen: *Xanthomonas axonopodis pv. punicae*)

Symptoms

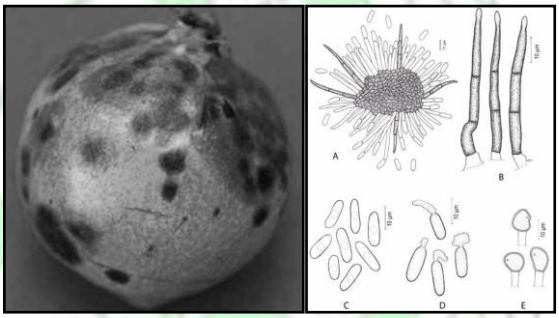


1. Appearance of one to several small water soaked, dark colored irregular spots on leaves resulting in premature defoliation under severe cases.
2. Spots on fruits were dark brown irregular slightly raised with oily appearance, which split open with L-shaped cracks under severe cases.

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.

POMEGRANATE ANTHRACNOSE (Pathogen: *Colletotrichum gloeosporioides*)



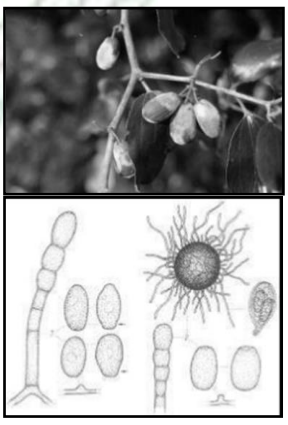
Symptoms: Small, regular to irregular black spots appear on the leaves, calyx region, and fruits. These spots later turn into dark brown, depressed lesions. Infected leaves turn yellow and eventually drop off.

Microscopic Examination: To examine microscopically, cut a section of the diseased tissue, prepare a temporary slide, **and observe under a microscope.**

1. **Conidia:** The conidia are barrel-shaped, single-celled, hyaline (transparent), small, and elongated.

POWDERY MILDEW OF BER (Pathogen: *Oidium erysiphoides* f.sp. *zizyphi*)

Symptoms



1. Small, white powdery growth appear on the young fruits which later enlarge and coalesce and final turn brown to dark brown.
2. Affected young fruits drop off prematurely or become corky, cracked, mis-shapen and underdeveloped.

Microscopic:

Prepare a temporary slide by using cotton blue and examine under the microscope.

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