

PRACTICAL MANUAL
on
**DISEASES OF FRUIT, PLANTATION,
MEDICINAL AND AROMATIC CROPS**

Course No. HPP 227 Credit Hrs. 3(2+1)

For

B.Sc. (Horticulture) I-year (2nd Semester)



By

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2019

Department of Plant Pathology
College of Agriculture
Rani Lakshmi Bai Central Agricultural University
Jhansi-284003

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

Roll No.

Batch

Session

Semester

Course Name :

Course No. :

Credit

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in the year.....in the respective lab/field of College.

Date:

Course Teacher

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Experiment No. 3

Objective: Identification and wet preservation of plant diseased specimens

Activity:

1. Prepare FAA solution for preservation of plant disease sample
2. 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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Preparation of Formalin Acetic Acid Alcohol (F.A.A.).....

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Experiment No. 4

Objective: Identification of mango powdery mildew pathogen

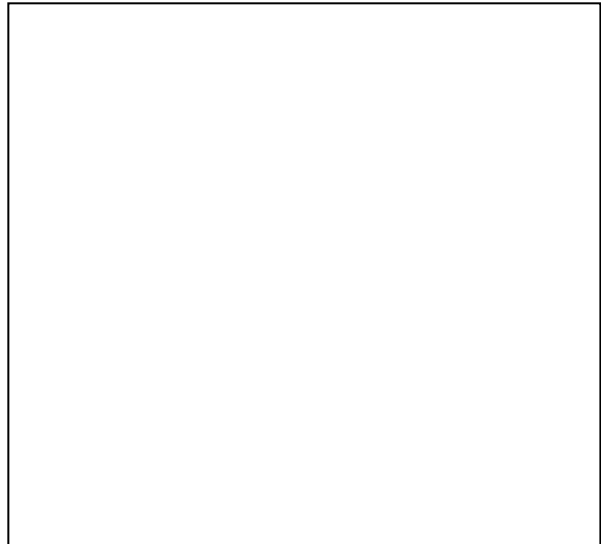
Activity: Identify the pathogen from the disease sample provided to you by preparing slide. Draw neat diagrams of characteristic symptoms and spores observed under the microscope.

Materials Required:

Observations:

Symptoms:

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Microscopic:

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Identification:

Experiment No. 6

Objective: Identification of mango malformation pathogen

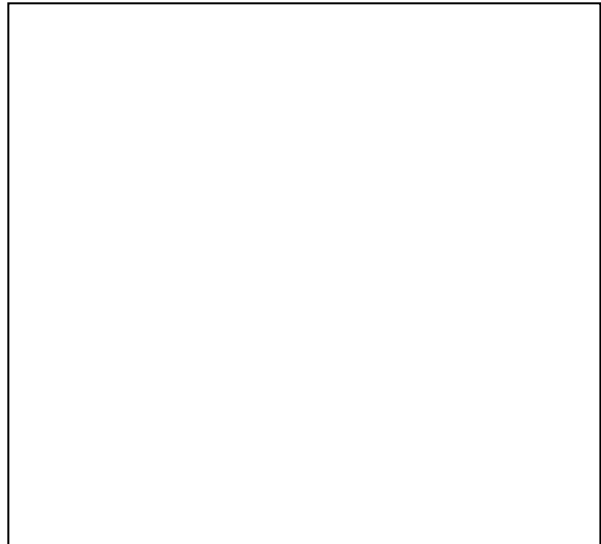
Activity: Identify the pathogen from the disease sample provided to you by preparing slide. Draw neat diagrams of characteristic symptoms and spores observed under the microscope.

Materials Required:

Observations:

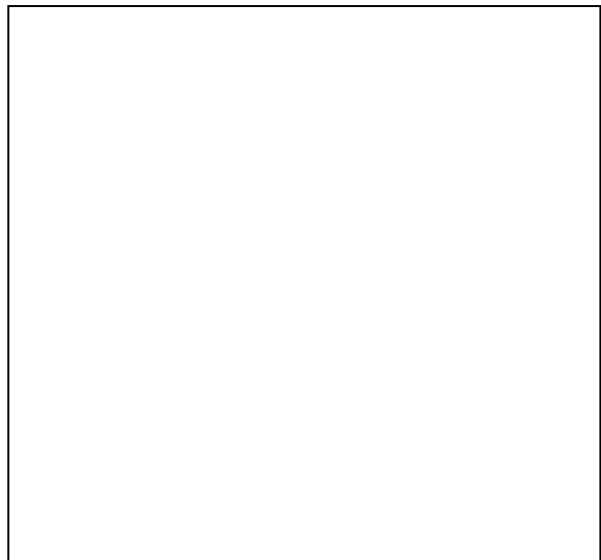
Symptoms:

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Microscopic:

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Identification:

Experiment No. 9

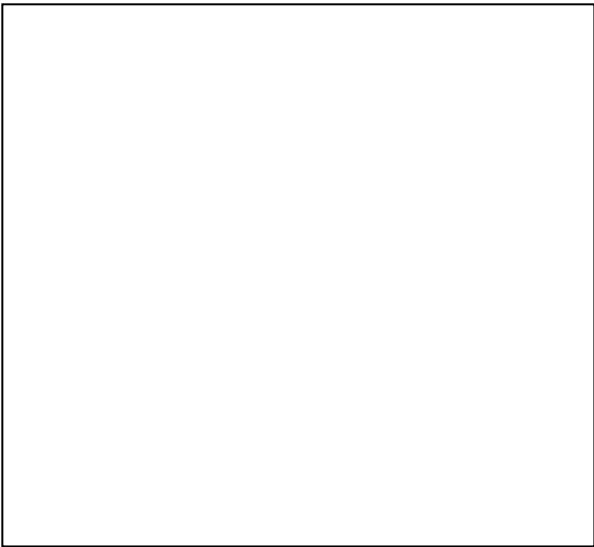
Objective: Identification of banana panama wilt pathogen

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:

Observations:

Symptoms:



Microscopic:

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Identification:

Experiment No. 10

Objective: Identification of banana sigatoka pathogen

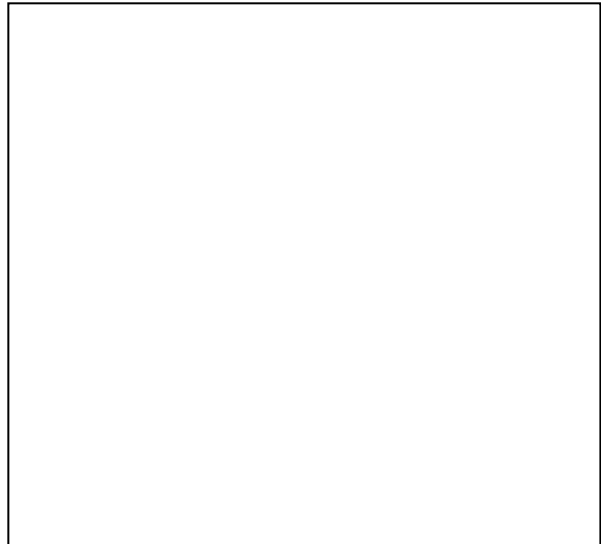
Activity: Identify the pathogen from the disease sample provided to you by preparing slide. Draw neat diagrams of characteristic symptoms and spores observed under the microscope.

Materials Required:

Observations:

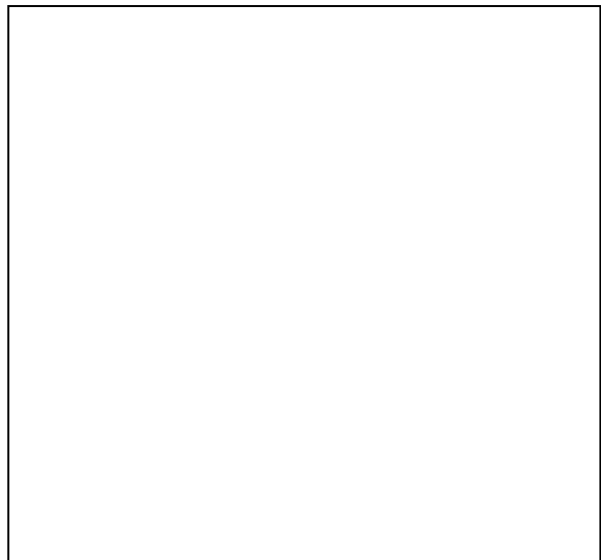
Symptoms:

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Microscopic:

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Identification:

Experiment No. 12

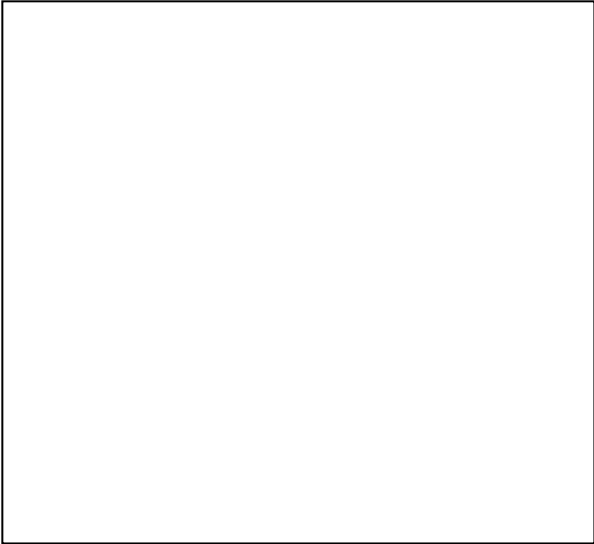
Objective: Identification of guava wilt pathogen

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:

Observations:

Symptoms:



Microscopic:

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Identification:

Experiment No. 13

Objective: Identification of guava anthracnose pathogen

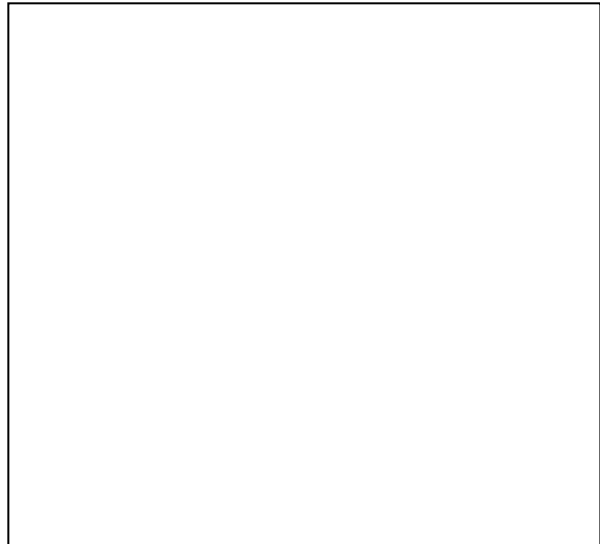
Activity: Identify the pathogen from the disease sample provided to you by preparing slide. Draw neat diagrams of characteristic symptoms and spores observed under the microscope.

Materials Required:

Observations:

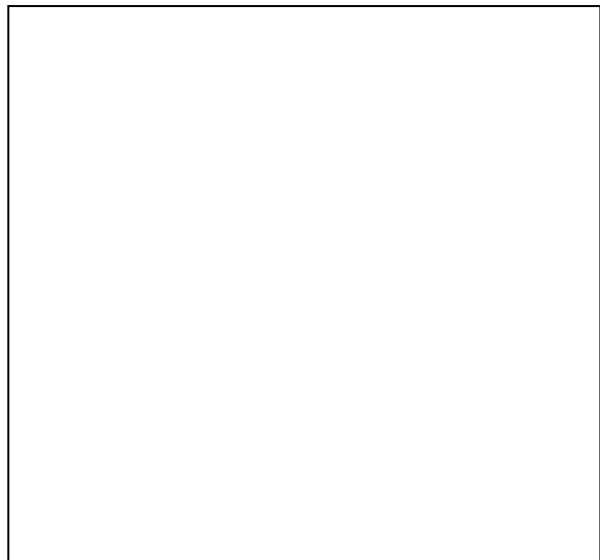
Symptoms:

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Microscopic:

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Identification:

Experiment No. 14

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:

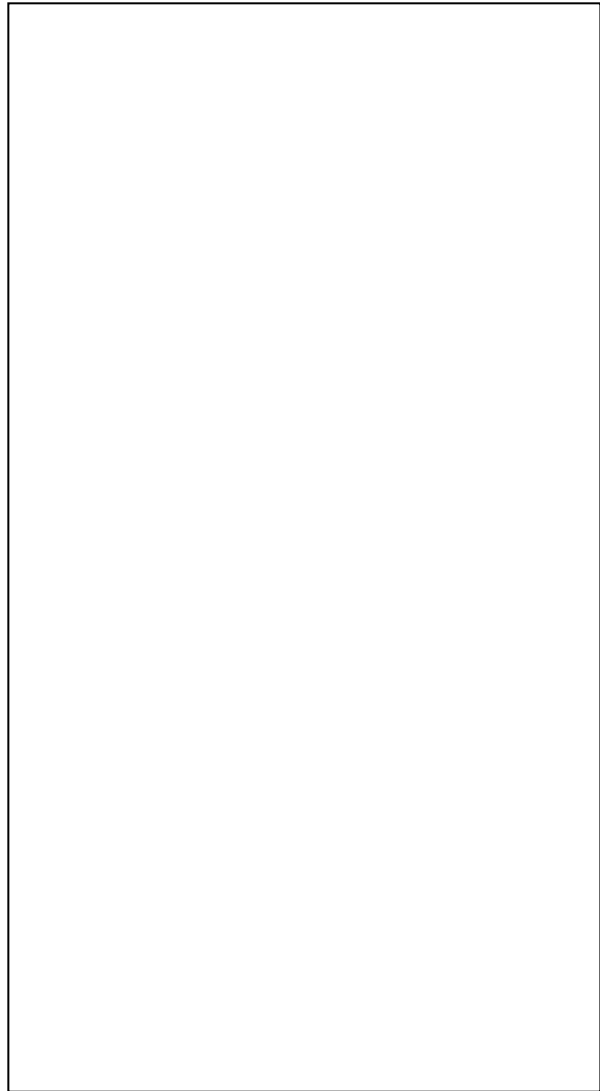
Observations:

Symptoms:

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Microscopic:

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Identification:.....

Experiment No. 15

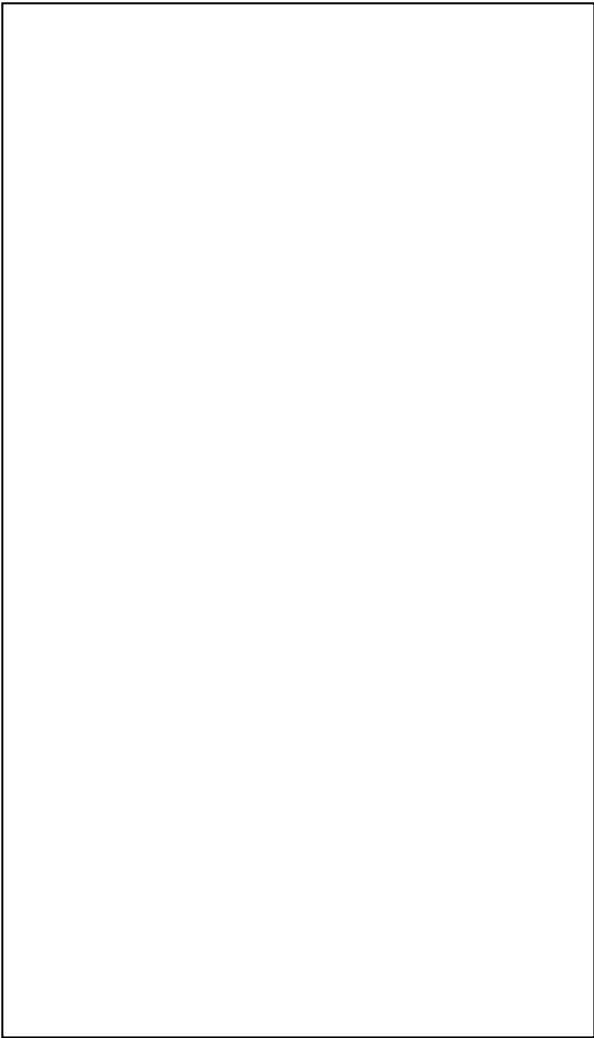
Objective: Identification papaya leaf curl disease

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

Materials Required:

Observations:

Symptoms:



Microscopic:

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Identification:.....

Experiment No. 16

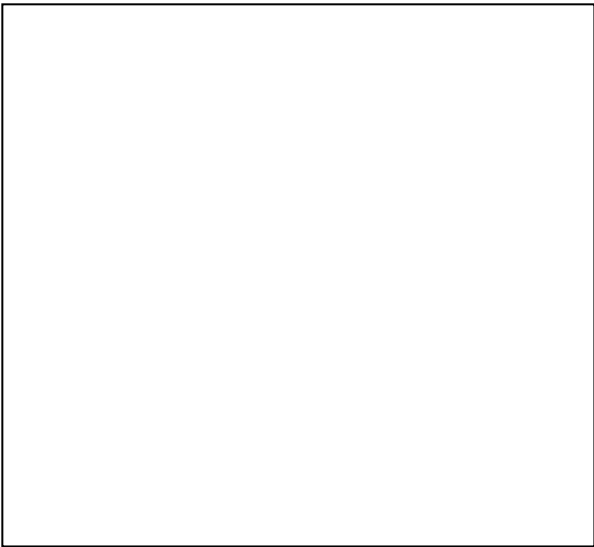
Objective: Identification of ber powdery mildew pathogen

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:

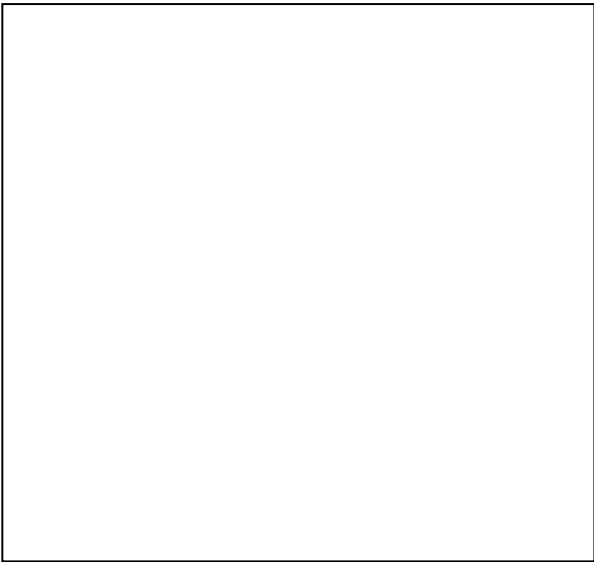
Observations:

Symptoms:



Microscopic:

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Identification:

Experiment No. 17

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:

Observations:

Symptoms:

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Microscopic:

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Identification:.....

Experiment No. 18

Objective: Identification of pomegranate anthracnose pathogen

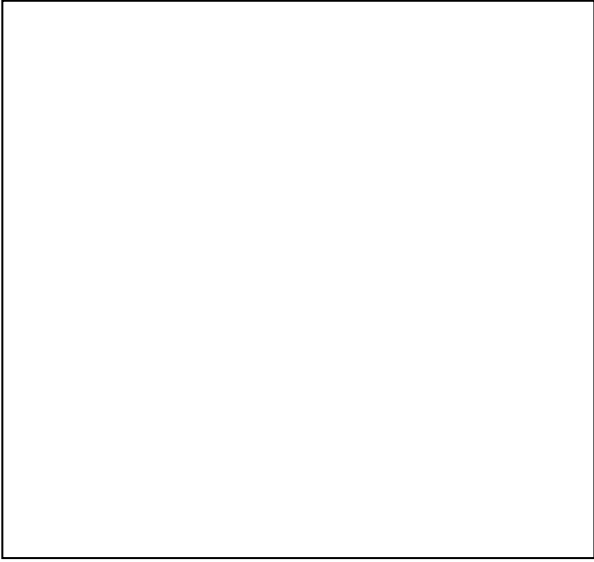
Activity: Identify the pathogen from the disease sample provided to you by preparing slide. Draw neat diagrams of characteristic symptoms and spores observed under the microscope.

Materials Required:

Observations:

Symptoms:

Microscopic:



Identification:

TEMPORARY MOUNTS AND STAIN

Procedure:

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.

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:

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

Use of Stain:

- (i) It helps in proper and correct study of the micro-organisms under the microscope.
- (ii) It differentiates between the host tissue and the micro-organism.
- (iii) 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.

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 may be a single sporocarp or a portion of it, dried culture, slide or the material on its host or substrate (e.g. leaf, stem, bark, rock, soil, paper, cloth). Two types of preservation methods are used for diseased plant specimen: Dry preservation and Wet preservation.

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 h 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.

WET PRESERVATION

Preservative is a chemical which is used to fix (to maintain) the tissues of plants and animals for a long time so that decomposition does not take place. Chemicals are used to kill, preserve and fix plant/animal tissues and specimens in such a way that they retain their original shape, form size and structure. These make the tissues hard and prevent them from decaying. A fixative must penetrate rapidly the tissue removed from the body.

Procedure:

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)
 - d. Date collected
 - e. 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

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

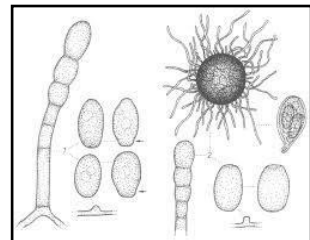
Symptoms

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.



Microscopic: Prepare a temporary slide by using cotton blue microscope.

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



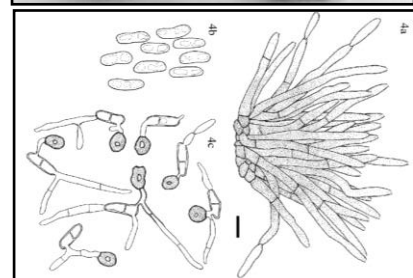
MANGO ANTHRACNOSE (pathogen: *Colletotrichum gloeosporium*)

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.



Microscopic: Cut the section of diseased part. Prepared a temporary slide and examine under the microscope. Conidia are barrel shaped, single celled, hyaline, small and elongated.



MANGO MALFORMATION

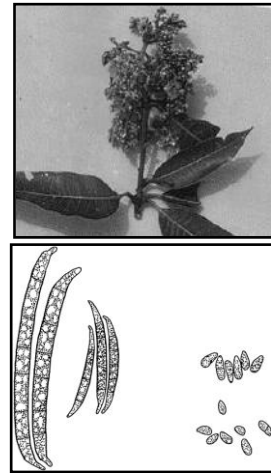
Pathogens: Different species of Fusarium (*F. mangiferae* var. *subglutinans*, *F. sterilihyphosum*, *F. mexicanum*)

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: 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.

Microscopic: Prepare a temporary slide from the diseased material and examine under the microscope.

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



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

Symptoms:

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



Microscopic

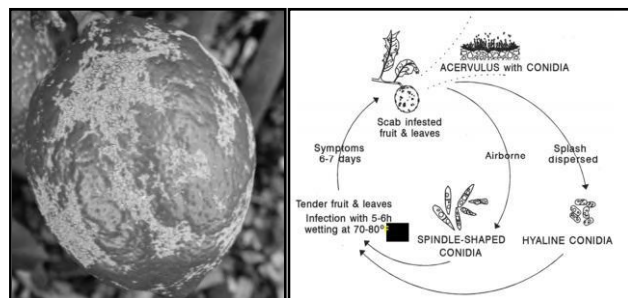
1. The bacterium is rod-shaped, gram-negative, and has a single polar flagellum.
2. Colonies on laboratory media are usually yellow due to 'xanthomonadin' pigment production.

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: 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*)

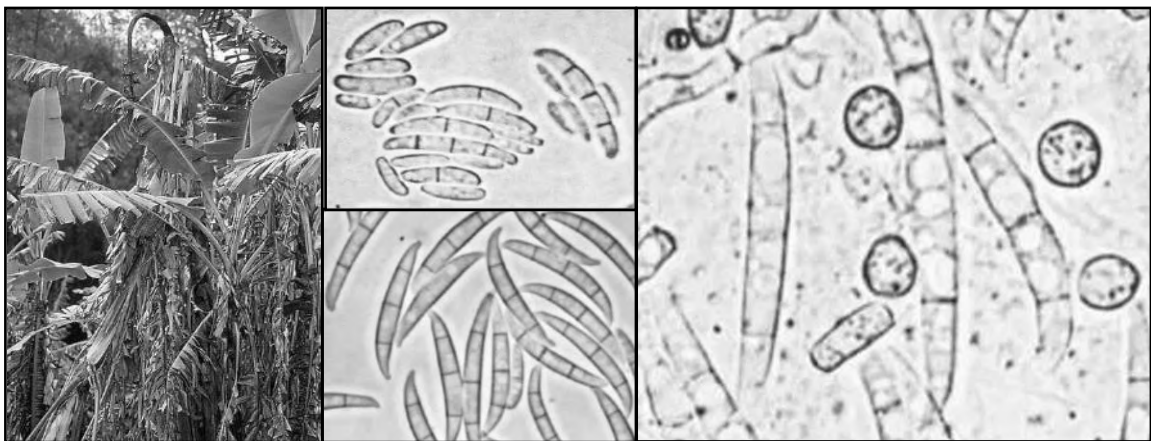
Infestation by aphid.

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. *cabense*)



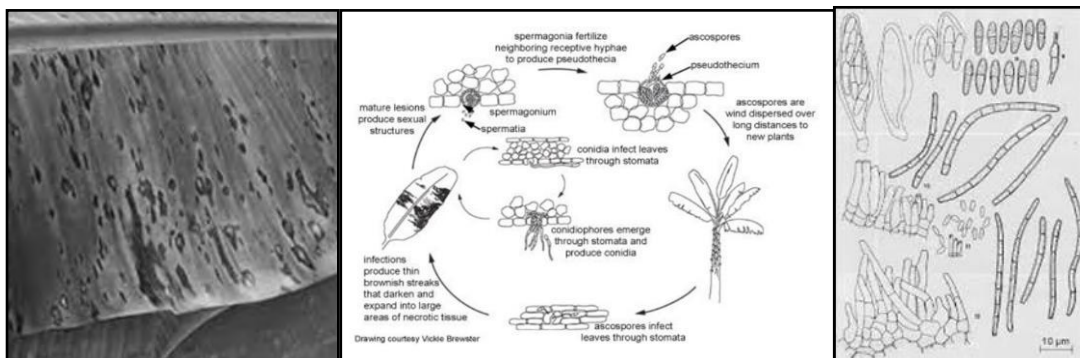
Symptoms

1. Yellowing of the lower most leaves starting from margin to midrib of the leaves.
2. Yellowing extends upwards.
3. The leaves break near the base and hang down around pseudostem.
4. Longitudinal splitting of pseudostem.
5. Discolouration of vascular vessels as red or brown streaks.

Macroscopic: Cut section of diseased root and prepare a temporary slide and observe under microscope.

1. Micro conidia are single celled, septate hyaline elliptical or oval.
2. Macro conidia are sickle shaped hyaline, 3-5 septate and tapering at both ends.
3. Chlamydospores are thick walled, spherical to oval, hyaline to slightly yellowish in colour.

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



Symptoms

1. A slight discoloration between the leaf's secondary veins.
2. Over time, these points develop into pale yellow streaks, brown streaks and elliptic necrotic spots arranged parallel to the secondary veins.
3. The spot have depressed grey center is surrounded by a yellow halo.
4. As the disease progresses, the lesions coalesce and cover a large area of the leaf.

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

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

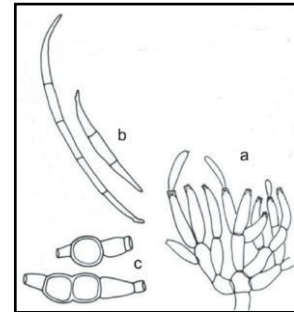
GUAVA WILT (Pathogen: *Fusarium oxysporum* f. sp. *Psidii*)

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: Cut section of diseased root and prepare a temporary slide and observe under microscope.

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



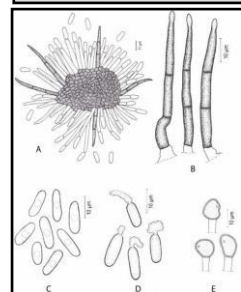
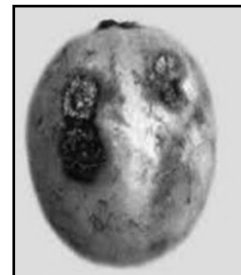
GUAVA ANTHRACNOSE (Pathogen: *Gloeosporium psidii*)

Symptoms

1. Pin-head spots are first seen on unripe fruits, which gradually enlarge.
2. Spots are dark brown in colour, sunken, circular and have minute black stromata in the center of the lesion, which produce creamy spore masses in moist weather.
3. The infected area on unripe fruits become corky and hardy, and often develops cracks in case of severe infection.
4. The plant begins to die backwards form the top of a branch.

B. Microscopic: Cut section of diseased part and prepare a temporary slide and observe under microscope.

1. Conidia are formed at the tip of conidiophore, sickle shaped, unicellular and hyaline.
2. Conidiophores are small, hyaline; setae are long, tapering at the end and dark brown in colour.
3. Acervulli are brown to black in colour.

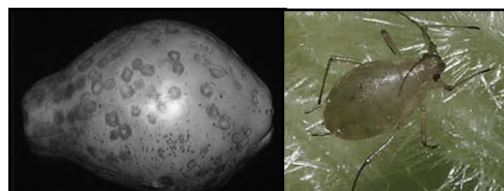


PAPAYA RING SPOT (Pathogen: *Papaya Ring Spot virus* (PRSV))

Aphid infestation

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.

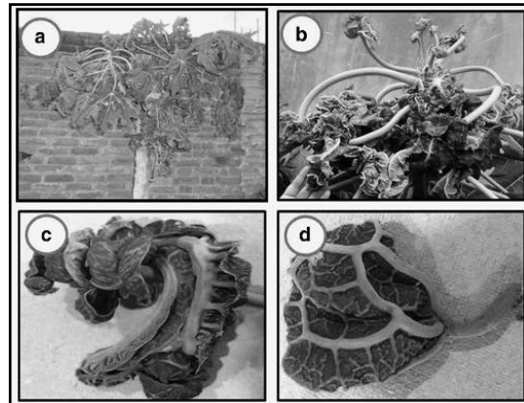


PAPAYA LEAF CURL (Pathogen: *Papaya leaf curl virus* (PaLCuV))

Whitefly infestation

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.



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

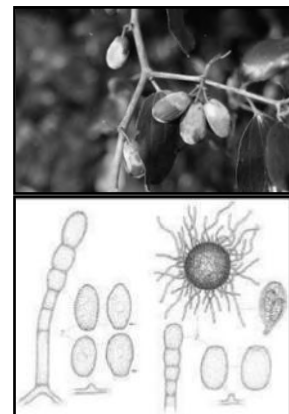
Symptoms

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 examined under the microscope.

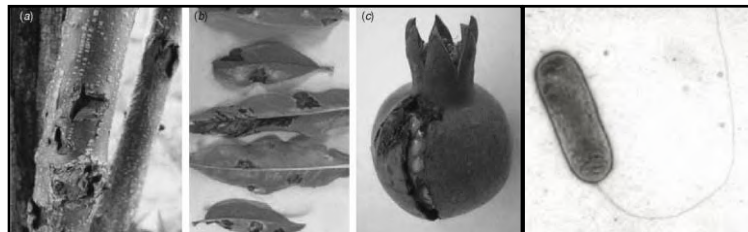
1. Conidiophores are short and hyaline.
2. Conidia are single celled, barrel shaped produced in chain.



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 on leaves, calyx region and fruits which turn later on as dark brown depressed spots. Infected leaves turn yellow and drop off.

Microscopic: Cut the section of diseased part. Prepared a temporary slide and examine under the microscope. Conidia are barrel shaped, single celled, hyaline, small and elongated.

