PRACTICAL MANUAL

on

DISEASES OF VEGETABLE, ORNAMENTAL AND SPICE CROPS

Course No. HPP 326 Credit Hrs. 3(2+1)

For

B.Sc. (Horticulture) III-year (5th 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 symptoms, causal organisms and host parasitic relationship of important diseases, examination of cultures of important pathogens of vegetables, ornamental and spice crops in field as well as in protected cultivation.

Name of Student .....

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Batch .....

Session .....

Semester .....

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Date: Course Teacher

CONTENTS

Experiment No. 1

Objective: Identification and dry preservation of plant diseased specimens for herbarium

Activity: Collect ten disease samples from University research fields and prepare herbarium with all following details in it:

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. 2

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. 3

Objective: Identification of early blight of potato and tomato

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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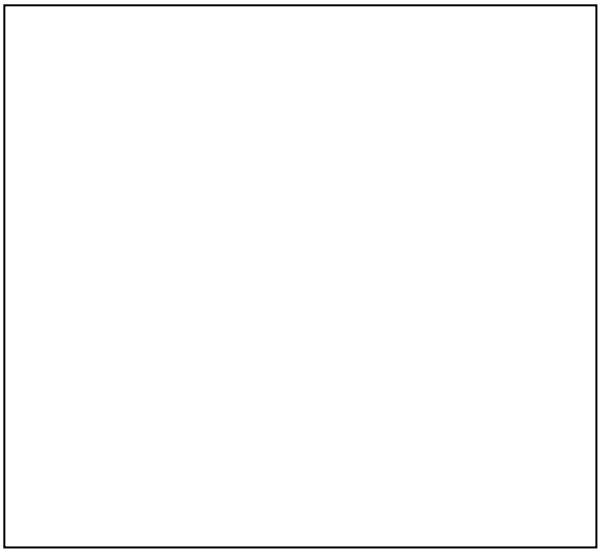

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Identification:……...……..……..

Experiment No. 4

Objective: Identification of late blight of potato and tomato

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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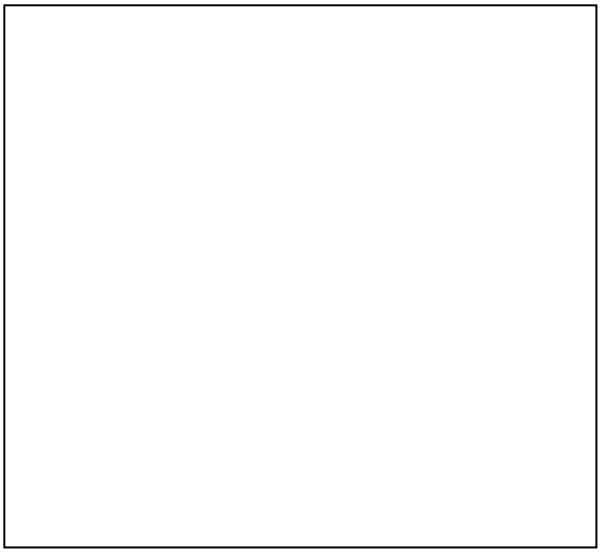

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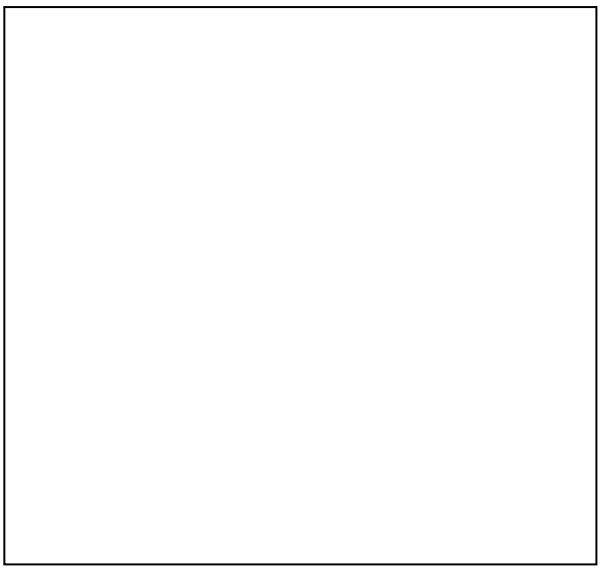

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Identification:……..……..……..

Experiment No. 5

Objective: Identification of leaf curl of tomato

Activity: Identify the pathogen from the disease sample provided to you. 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. 6

Objective: Identification of phomopsis blight and fruit rot of brinjal

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 anthracnose of chilli

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. 8

Objective: Identification of wilt of chilli

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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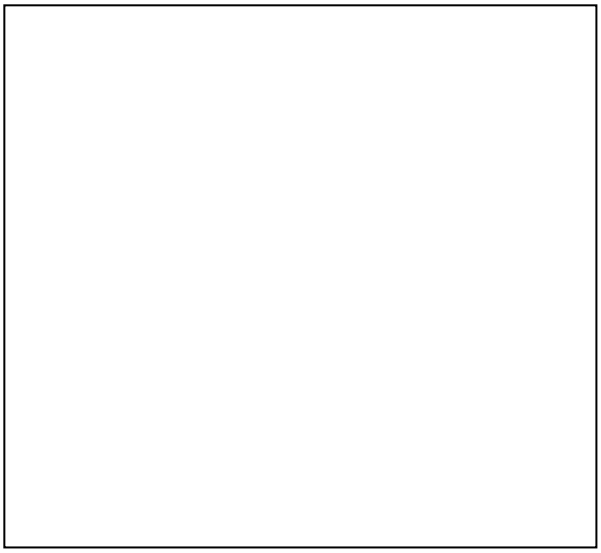

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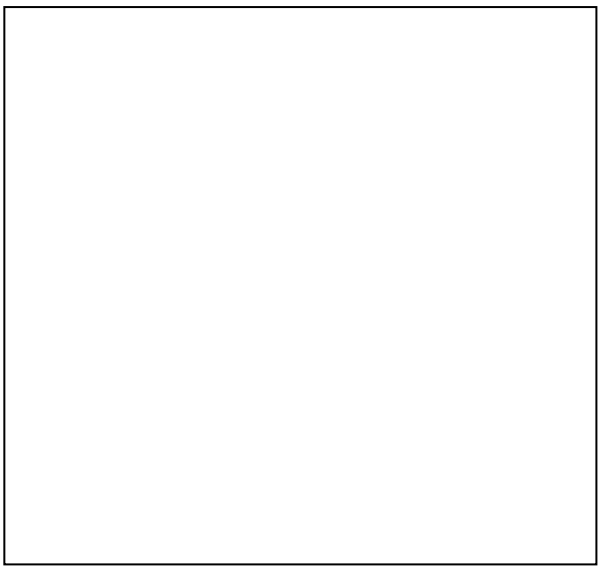

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Identification:…… ..……..……..

Experiment No. 9

Objective: Identification of damping-off of chilli

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 okra yellow vein mosaic

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. 11

Objective: Identification of anthracnose of bean

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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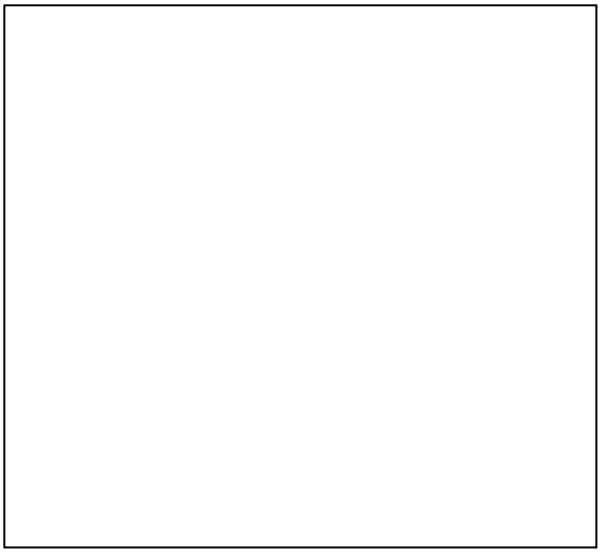

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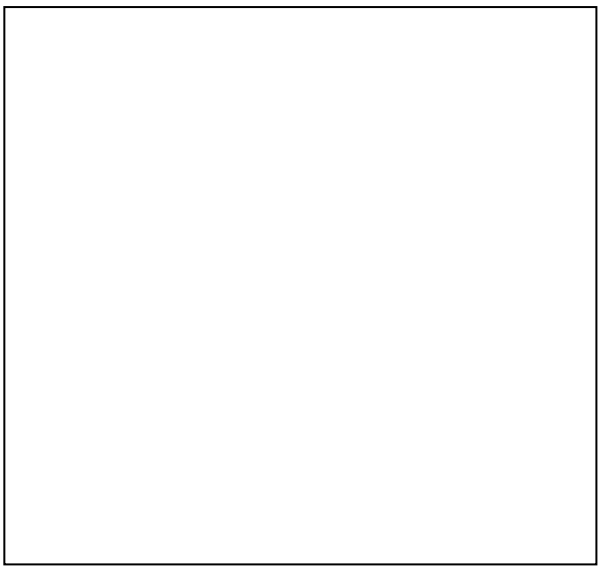

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Identification:…… ..……..……..

Experiment No. 12

Objective: Identification of bacterial blight of bean

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. 13

Objective: Identification of powdery mildew of pea

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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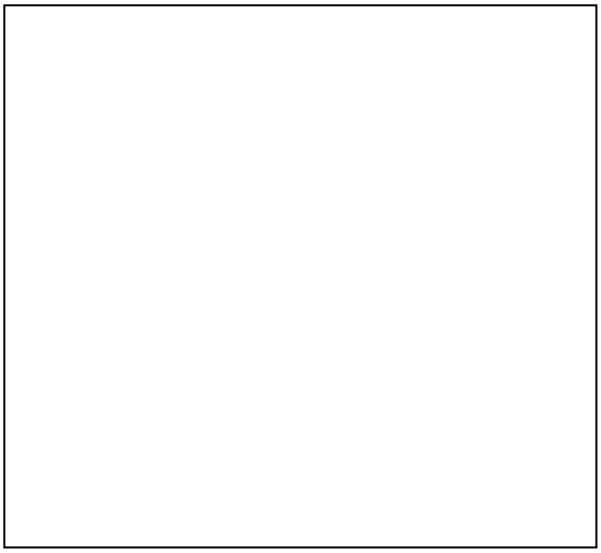

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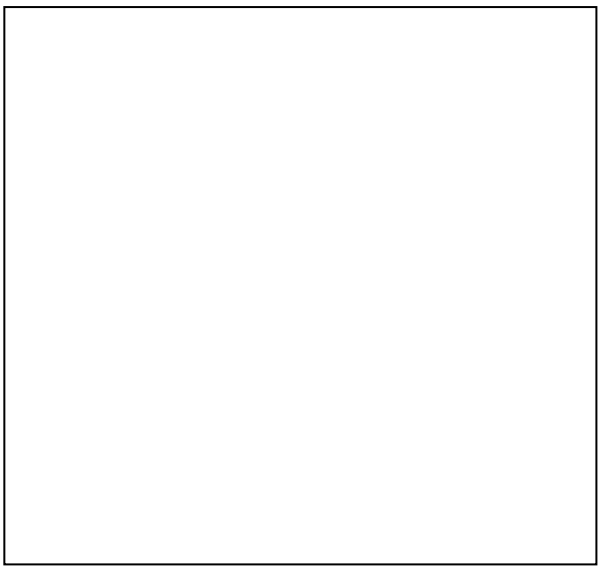

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Identification:…… ..……..……..

Experiment No. 14

Objective: Identification of soft rot of ginger

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. 15

Objective: Identification of bacterial blight of crucifers

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. 16

Objective: Identification of smudge of onion and garlic

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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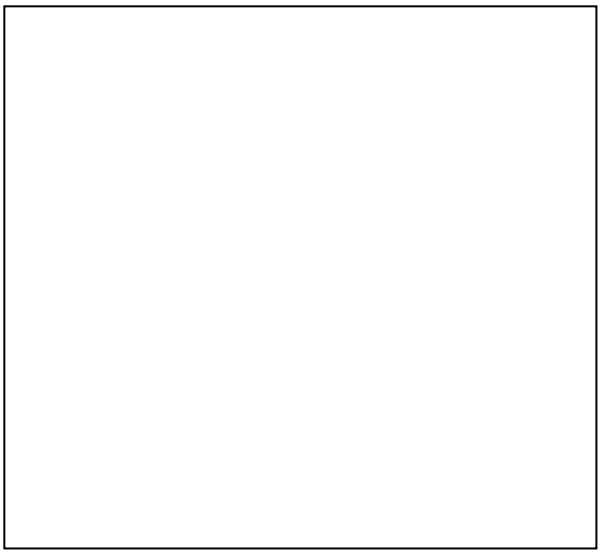

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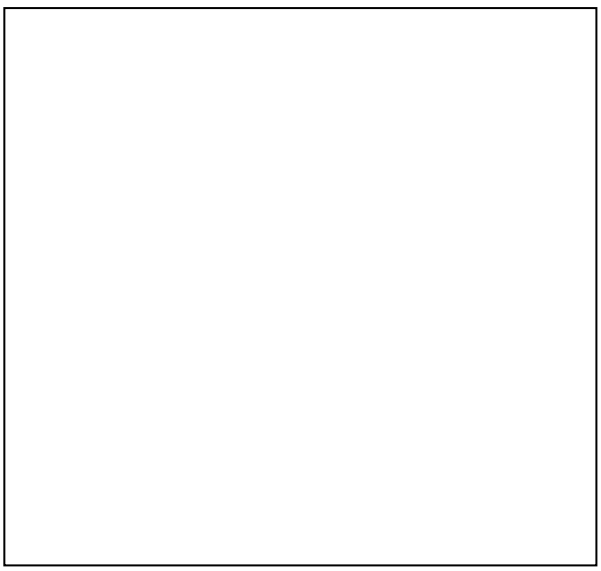

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Identification:…… ..……..……..

Experiment No. 17

Objective: Identification of leaf spot of turmeric

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. 18

Objective: Identification of black spot of rose

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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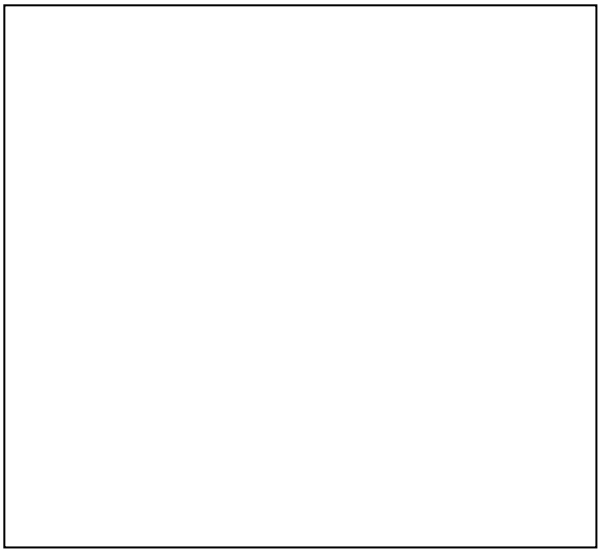

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Microscopic: ……..……

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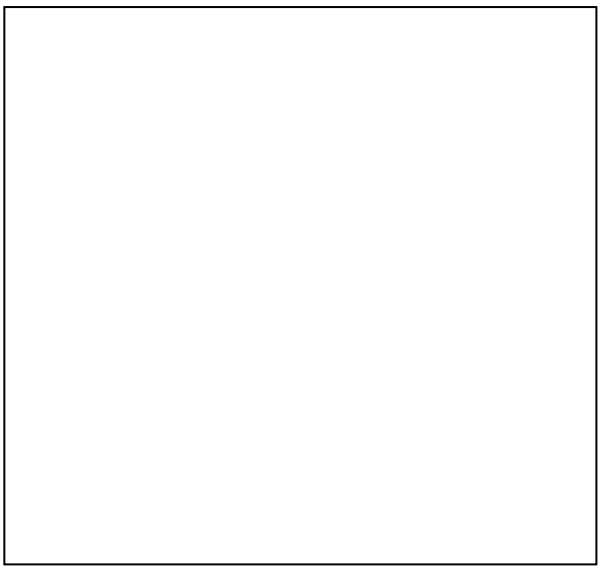

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Identification:…… ..……..……..

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

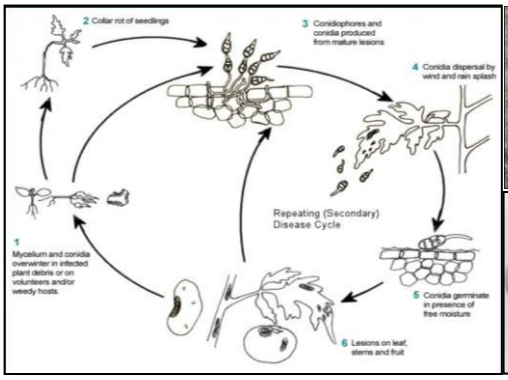
Preparation of Formalin Acetic Acid Alcohol (F.A.A.): It is a very good fixative and tissues could be left in it for a long period without any harm.
Composition: 50% Alcohol - 100 ml; 40% Formaldehyde - 6.5 ml; Glacial Acetic Acid - 2.5 ml

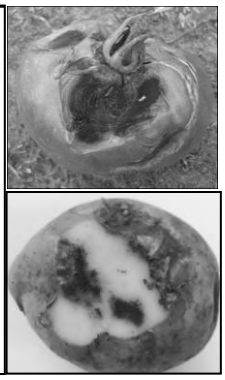
EARLY BLIGHT OF POTATO AND TOMATO (Pathogen: *Alternaria solani*)

Symptoms

1. Symptoms of early blight occur on fruit, stem and foliage of tomatoes and stem, foliage and tubers of potatoes.
2. First observed as small, black lesions mostly on the older foliage
3. Spots enlarge and by the time they are one-fourth inch in diameter or larger, concentric rings in a bull's eye pattern can be seen in the center of the diseased area
4. Tissue surrounding the spots may turn yellow
5. Symptoms on potato tubers are characterized by sunken, irregular lesions







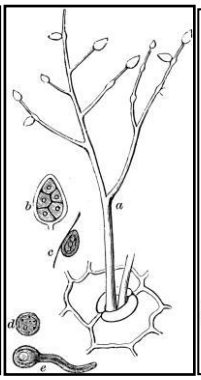
Microscopic:

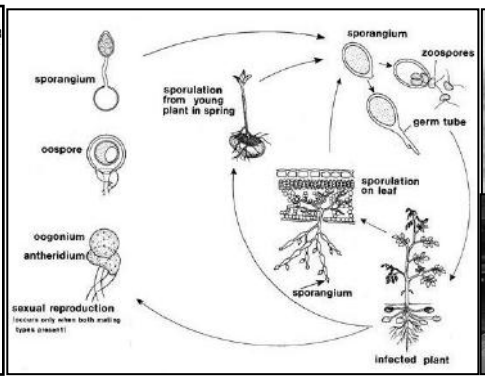
1. Mycelium - septate, branched, light brown which become darker with age
2. Conidia are borne singly or in a chain of two on distinct dark coloured conidiophores
3. Conidia - beaked, muriform, dark coloured and borne singly

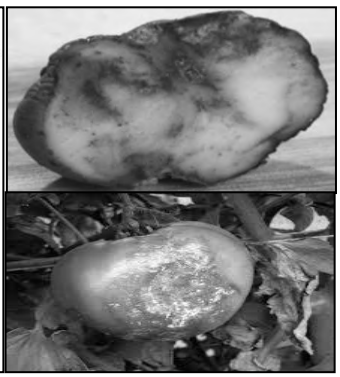
LATE BLIGHT OF POTATO AND TOMATO (Pathogen: *Phytophthora infestans*)

Symptoms

1. Water soaked spots appear on leaves, increase in size, turn purple brown and finally black in 2 or 3 days.
2. In humid conditions, *P. infestans* produces sporangia and sporangiophores on the surface of infected tissue and the resulting white sporulation on under surface of leaf.
3. The disease spreads to petioles, rachis and stems.
4. Stem infection frequently develops at nodes, stems break at these points and the plant topples over.
5. Dark brown spots developed on tomato fruits which spread to the entire fruit surface and whole fruit may rot.
6. Infected tuber tissues become copper brown, reddish or purplish in color.





Microscopic:

1. Mycelium - composed of tubular hyphae with few or no septa (cross-walls) in contrast to the septate hyphae of fungi
2. Sporangiophores – form at the margins of lesions, short and unbranched or sparingly branched
3. Sporangia - formed at the ends of sporangiophores, ovoid to ellipsoid with a distinct narrow apical plug (semi-papillate)
4. Zoospores- biflagellate (have two flagella), with one tinsel flagellum directed anteriorly and one whiplash flagellum directed posteriorly.

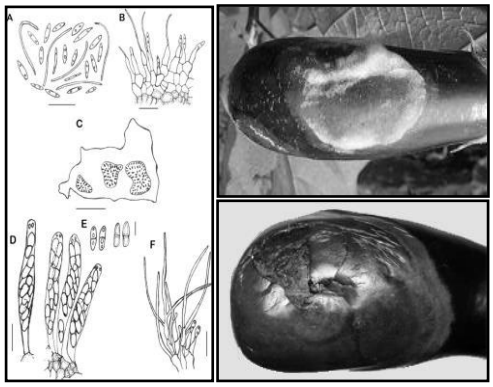
TOMATO YELLOW LEAF CURL (Pathogen: *Tomato yellow leaf curl virus* TYLCV) Infestation with whitefly (*Bemisia tabaci*)

Symptoms

1. Leaf curl disease is characterized by severe stunting of the plants with downward rolling and crinkling of the leaves.
2. The newly emerging leaves exhibit slight yellow colouration and later they also show curling symptoms.
3. Older leaves become leathery and brittle.
4. The nodes and internodes are significantly reduced in size. Infected plants looks pale and produce more lateral branches giving a bushy appearance.

PHOMPSIS BLIGHT AND FRUIT ROTOF BRINJAL (Pathogen: *Phomopsis vexans*)

Symptoms



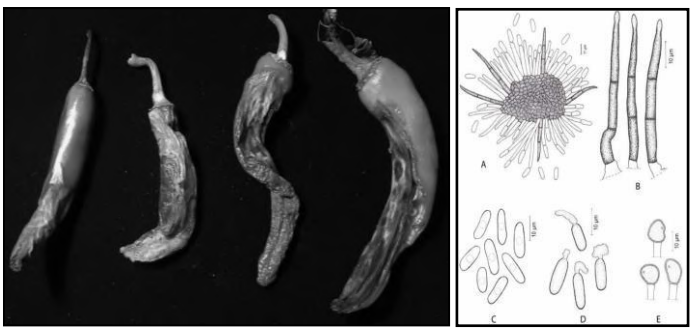
1. Spots generally appear first on seedling stems or leaves.
2. Girdle seedling stems and kill the seedlings
3. Leaf spots are clearly defined, circular, up to about 1 inch in diameter, and brown to gray with a narrow dark brown margin. Center of the spot becomes gray, and black pycnidia develop on it.
4. Fruit spots are much larger, affected fruit are first soft and watery, later on affected fruits may become black and mummified

Microscopic:

1. Pycnidia – with or without beak, found in the affected tissue, globose or irregularly shaped
2. Conidiophores – hyaline, simple or branched, found in the pycnidium
3. Conidia - hyaline, one celled and sub cylindrical
4. Ascospores - hyaline, narrowly ellipsoid to bluntly fusoid with one septum

CHILLI ANTHRACNOSE (Pathogen: *Colletotrichum capsici*)

Symptoms



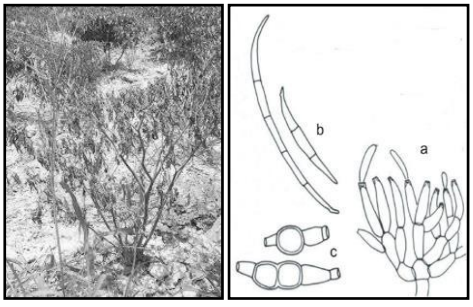
1. Symptoms are seen on leaf, stem and fruit.
2. Small, circular spots on the skin of the fruit and expanded in the direction of long axis of the fruit.
3. The fruits with many spots drop off prematurely resulting in heavy loss of yield.
4. Fungus may also attack the fruit stalk and spread along the stem causing dieback symptoms.

B. Microscopic:

1. Mycelium - septate and inter and intra cellular
2. Acervuli and stroma on the stem are hemispherical
3. Conidia - are barrel shaped, single celled, hyaline, small and elongated, in mass appear pinkish

CHILLI WILT (Pathogen: *Fusarium oxysporum* f.sp. *capsici*)

Symptoms



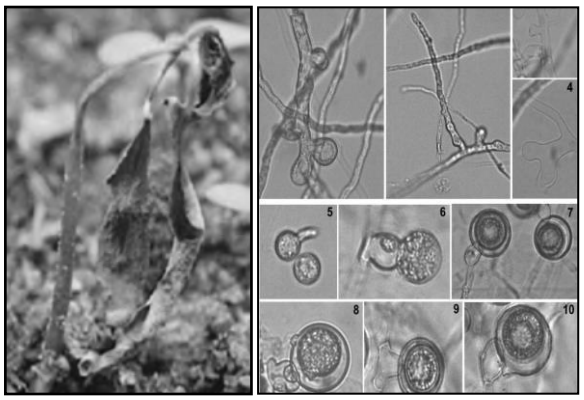
1. On the young seedlings initially, water-soaked areas developed at the collar region and a brown sunken lesion which soon appeared as girdled resulting in seedling collapse.
2. On adult plant initially, slight drooping of leaves which led to drying of leaves starting from lower ones extended from root to stem region and plants exhibited wilting symptom.

Microscopic:

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

CHILLI DAMPING-OFF (Pathogen: *Pythium aphanidermatum*)

Symptoms



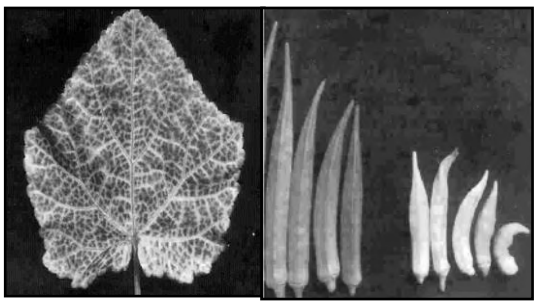
1. Seed may rot or the seedlings may be killed before they emerge from the soil.
2. Stem of young seedlings may also be attacked after emergence showing water soaking lesions and shriveling of stem which fall over and die.
3. In nursery the disease may appear in patches in 2-4 days, the entire lot of seedling may be destroyed.

Microscopic:

1. Sporangia produces terminal, inflated, lobate with hyphal swellings
2. Sporangia give rise to biflagellate zoospores, with tinsel and whiplash flagella.
3. Oogonia are terminal, globose, smooth, and 20 to 25 µm (av. 23 µm) in diameter.
4. Oospores are aplerotic, 18 to 22 µm (av. 20.2 µm) in diameter, with a 1 to 2 µm thick wall

OKRA YELLOW VEIN MOSAIC (Pathogen: *Okra yellow vein mosaic virus* (OYVMV) Whitefly infestation

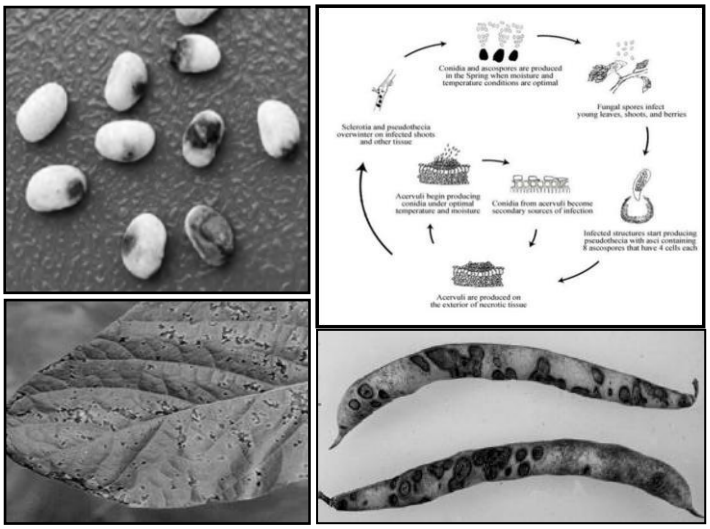
Symptoms



1. Yellowing of the entire network of veins in the leaf blade is the characteristic symptom.
2. In severe infections the younger leaves turn yellow, become reduced in size and the plant is highly stunted.
3. The veins of the leaves will be cleared by the virus and interveinal area becomes completely yellow or white.
4. In a field, most of the plants may be diseased and the infection may start at any stage of plant growth.
5. Infection restricts flowering and fruits, if formed, may be smaller and harder.
6. The affected plants produce fruits with yellow or white colour and they are not fit for marketing.

BEAN ANTHRACNOSE (pathogen: *Colletotrichum lindemuthianum*)

Symptoms



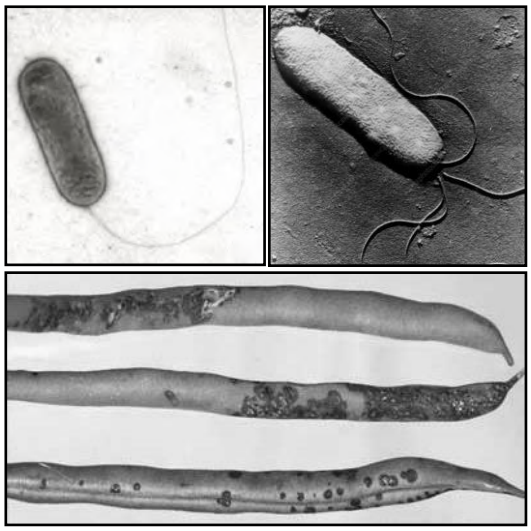
1. Black, sunken lesions about ½ inch in diameter or reddish-brown blotches develop on stems, pods and seedling leaves (cotyledons) but are most prominent on pods
2. Salmon coloured ooze on lesions and the veins on lower leaf surfaces turns black
3. On lima beans, symptoms are sooty-appearing spots on leaves and pods

Microscopic:

1. Acervuli – black colour, develop beneath the cuticle
2. Setae - few, brown and septate
3. Conidia - one celled, hyaline and cylindrical with rounded ends or one end slightly pointed, Conidiophores are short

BACTERIAL BLIGHT OF BEAN

There are two widespread bacterial blights that affect most types of beans,
Common blight (**Pathogen**: *Xanthomonas campestris* pv *phaseoli*)
Halo blight (**Pathogen**: *Pseudomonas syringae* pv. *phaseolicola*)



Symptoms

1. The stems, leaves and fruits of bean plants can be infected by either disease. Rain and damp weather favor disease development.
2. Halo blight occurs primarily when temperatures are cool.
3. Light greenish-yellow circles that look like halos form around a brown spot or lesion on the plant with age, the lesions may join together as the leaf turns yellow and slowly dies.
4. Stem lesions appear as long, reddish spots.
5. Leaves infected with common blight turn brown and drop quickly from the plant.

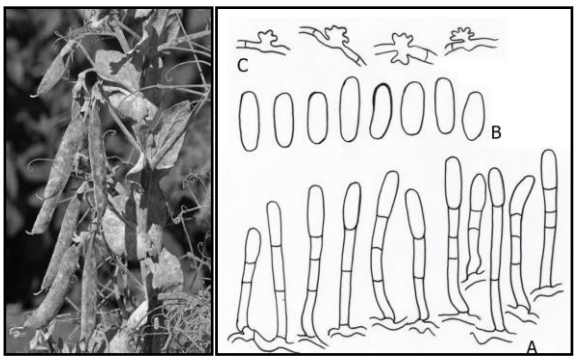
Microscopic:

1. The bacteria are rod-shaped, gram-negative, and has polar flagellum.
2. *Xanthomonas campestris* pv *phaseoli* colonies on laboratory media are usually yellow due to 'xanthomonadin' pigment production.

POWDERY MILDEW OF PEA (**Pathogen:** *Erysiphe polygoni*)

Symptoms

1. It is characterised by a white powdery growth on the leaves, stems and pods.
2. The initial symptoms consist of tiny slightly discoloured spots on the upper surface of leaves. Theses spots enlarge and become covered with powdery fungal growth.
3. The tissue beneath affected areas may turn purple and later brown.
4. If infection is severe, affected plants turn brown and die. Affected seeds become brown.



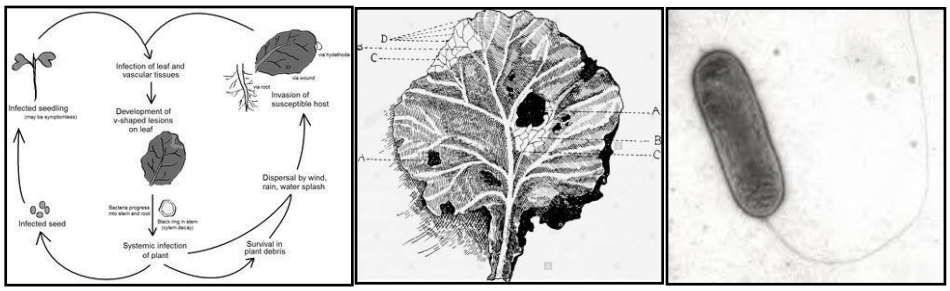
Microscopic:

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

BLACK ROT OF CRUCIFERS (**Pathogen**: *Xanthomonas campestris* pv. *campestris*)

Symptoms:

1. Early symptoms appear as wedge-shaped chlorotic (yellow) patches along the edge of the leaf that point toward the midrib.
2. Veins in the discolored area darken as the interveinal tissue dies and becomes brown and brittle.
3. The bacteria enter the vascular system of the plant eventually and the disease becomes systemic, producing leaf drop and head rot.



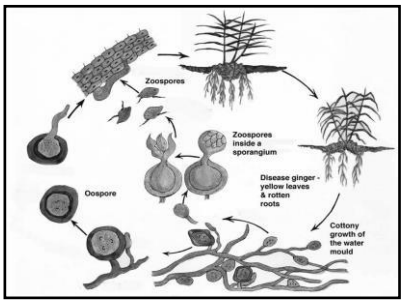
Microscopic

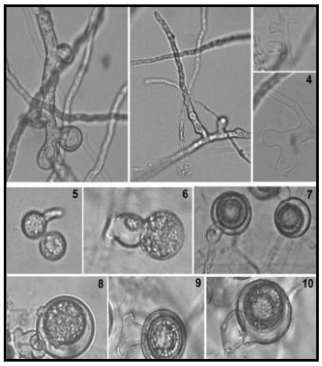
1. The bacterium is rod-shaped, gram-negative, and has a single polar flagellum.
2. Colonies on laboratory media are usually yellow due to 'xanthomonadin' pigment production.

GINGER SOFT ROT (Pathogens: *Pythium aphanidermatum. P. vexans and P. myriotylumare*)

Symptoms

1. Symptoms on rhizomes are of two types i.e. wet and dry rot.
2. Initial symptoms are noticed on leaves which turn slightly pale.
3. Yellowing of the leaves starts from the tip of the blade and spread downwards.
4. Infected leaves are killed which droop and hang down along the pseudostem.
5. Basal portion of the plant exhibits pale translucent colouration which becomes water soaked and soft.
6. Rotting extends from collar regions to rhizomes.
7. Later lesions decompose forming a watery mass of putrifying tissues enclosed by the tough rind of the rhizome and also the fibrovascular strands are not affected.
8. Roots arising from the affected regions of rhizome also show typical softening and rotting.

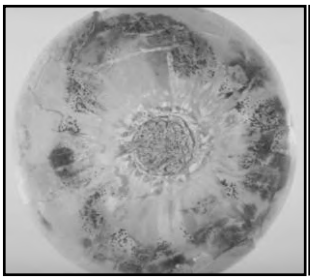


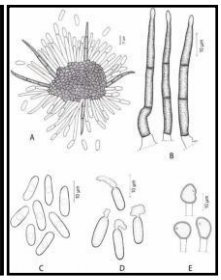


Microscopic:

1. Hyphae - hyaline, branched, non-septate
2. Sporangia - long tapering, formed by swelling of the hyphae
3. Zoospores – Kidney shaped, formed in vesicle, biflagellate and slightly depressed at the hilum end
4. Oospores - smooth walled, plerotic and spherical in shape

SMUDGE OF ONION AND GARLIC (**Pathogens:** *Colletotrichum circinans*)



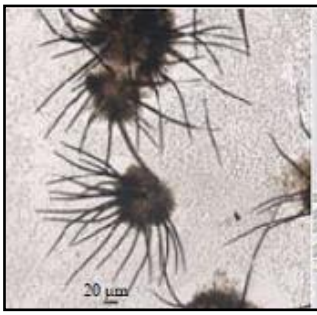


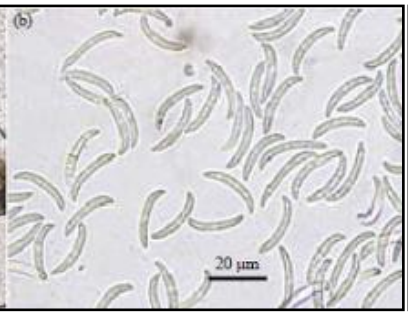
Symptoms

1. The disease occurs late in the season as the crop matures and continues to develop on bulbs in storage.
2. The fruiting bodies of the fungus turn from dark green to black as they mature, and form concentric rings around the neck and on the surface of dry outer bulb scales.
3. If the humidity is high, the disease may spread to the inner scales, causing small, yellow lesions.
4. If the disease continues to develop, the bulb may shrivel and sprout prematurely. Under warm, wet conditions, this fungus can cause damping-off and leaf spotting.

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

LEAF SPOT OF TURMERIC (Pathogen: ***Colletotrichum capsici***)





Symptoms

1. In affected leaves, elliptic or oblong spots with yellow halo are seen.
2. The centre of spots is greyish white and then with numerous black dots in centre
3. As the disease advances, the leaves dry up and give a scorched appearance.

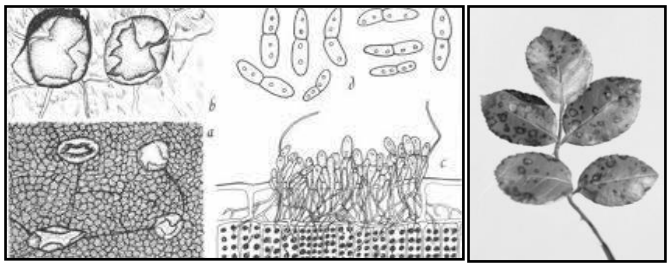
Macroscopic:

1. Mycelium - septate and inter and intra cellular
2. Acervuli and stroma on the stem are hemispherical
3. Conidia - are barrel shaped, single celled, hyaline, small and elongated, in mass appear pinkish

BLACK SPOT OF ROSE (Pathogens: *Diplocarpon rosae*)

Symptoms

1. Infected leaves develop black spots, especially on the upper leaf surface.
2. The circular or irregularly shaped spots can be up to 1/2 inch in diameter and typically have fringed borders.
3. As the disease progresses, the leaf margin begins to yellow, and then the entire leaf. The plant will then start to drop these leaves.



Microscopic: Black color acervulli are present in subcuticular layer, which produces hyaline, irregular, bi-celled conidia.