

Practical manual
on
Diseases of Field and Horticultural Crops and Their
Management-I

Course No. APP-338, Credit Hrs. 3(2+1)

For
B.Sc. (Agri.) III Year (V Semester)



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Syllabus: Diseases of Field and Horticultural Crops and their Management-I APP 338 3(2+1)

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 field and horticultural crops.

Name of Student

Roll No.

Batch

Session

Semester

Course Name :

Course No. :

Credit

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CERTIFICATE

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

Date:

Course Teacher

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Experiment No. 2

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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Identification:.....

Experiment No. 7

Objective: Identification of sorghum 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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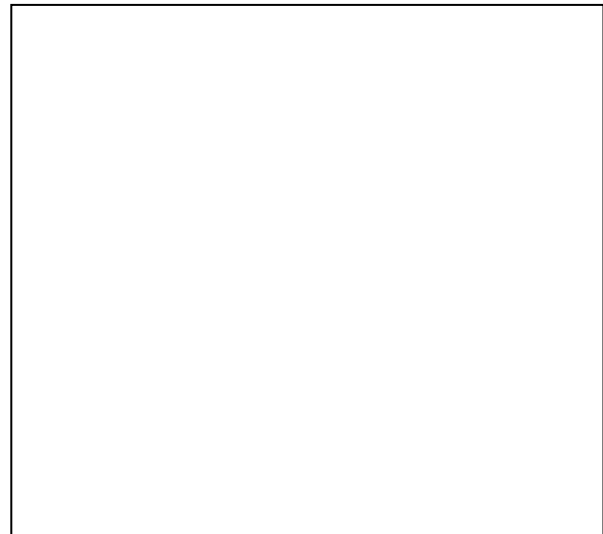
Observations:

Symptoms:

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Microscopic:

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Identification:.....

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Identification:.....

Identification:.....

Experiment No. 12

Objective: Identification of guava anthracnose pathogen

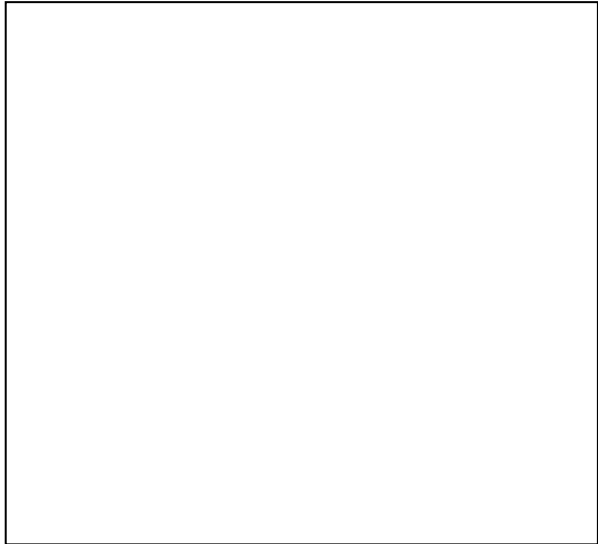
Activity: Identify the pathogen from the disease sample provided to you by preparing slide. Draw neat diagrams of characteristic symptoms and spores observed under the microscope.

Materials Required:

Observations:

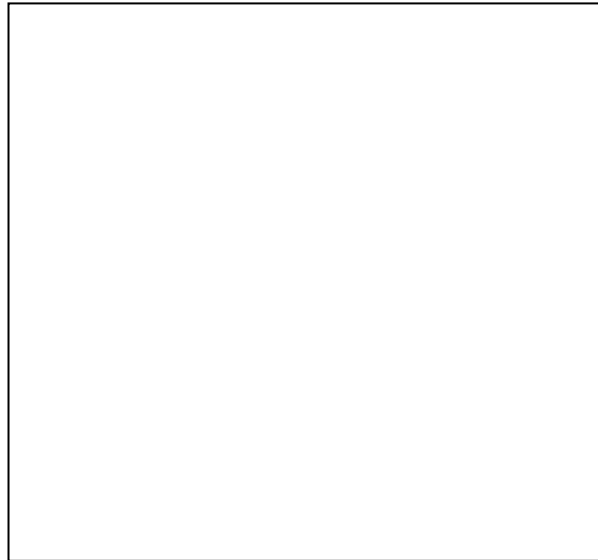
Symptoms:

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Microscopic:

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Identification:.....

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 or 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:** Bundles of specimens (15-20) packed in double layered polythene bags are tight sealed and exposed to -20°C for 48-72 hours at 6-8 months interval.
- 4. 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:

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

BLAST OF PADDY (*Oryza sativa*)

Symptoms

Leaf blast:

- Symptoms appear on leaves, nodes, rachis and glumes
- Common on leaves and the neck of the panicle
- On leaves, the lesions originate as small specks, which enlarges from few millimetres to several centimetres
- Characteristic symptom is spindle shaped spots with grey centre and brown margin

Nodal blast:

- Spots also appear on sheath, culm, culm nodes and glumes
- Spots coalesce and covering large areas of the leaves
- Black lesions appear on nodes girdling them.
- Affected nodes may break up and all the plant parts above the infected nodes may die
- Severely infected nursery and field appear as burnt

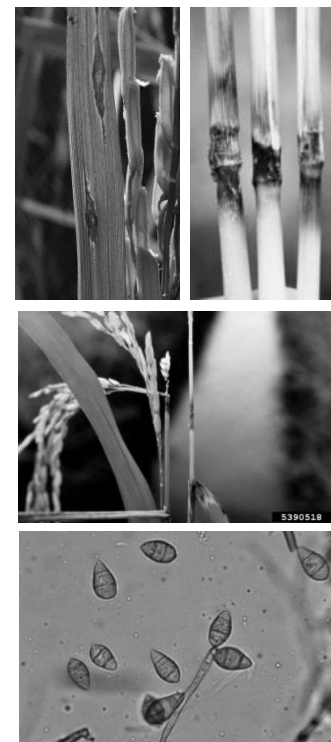
Neck blast (rotten neck / neck rot / panicle blast):

- Affects at flower emergence
- Attacks the peduncle and the lesion turns to brownish-black

Microscopic

Pathogen- *Pyricularia oryzae* (Syn: *P. grisea*) (Sexual stage: *Magnaporthe oryzae*, *M. grisea*)

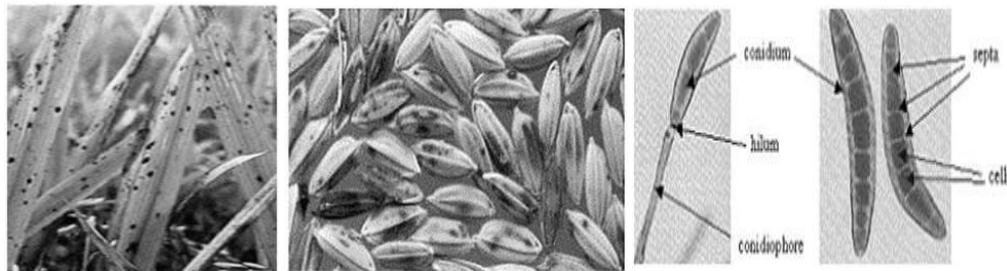
- Hyaline to olivaceous and septate mycelium
- Conidia are pyriform to ellipsoid, attached at the broader base by a hilum and are hyaline to pale olive green, usually 3 celled
- Conidia are produced sympodially in clusters on long, septate and olivaceous conidiophores



BROWN SPOT OF PADDY (*Oryza sativa*)

Symptoms

- Minute spots on the coleoptile, leaf blade, leaf sheath, and glume
- Spots most prominent on the leaf blade and glumes
- The spots become cylindrical or oval, dark brown with yellow halo later becoming circular
- Several spots coalesce and the leaf dries up
- The seedlings die and affected nurseries can be often recognised from a distance by scorched appearance
- Dark brown or black spots also appear on glumes leading to grain discoloration
- It causes failure of seed germination, seedling mortality and reduces the grain quality and weight



Microscopic

Pathogen- *Helminthosporium oryzae* (Syn: *Drechslera oryzae*, *Bipolaris oryzae* (Perfect stage: *Cochliobolus miyabeanus*))

- Mycelium - brown septate
- Conidiophores - single or in small groups, geniculate, brown in colour
- Conidia - pale to golden brown in colour, usually curved with a bulged centre and tapered ends, 6-14 septate

MAYDIS AND TURCICUM LEAF SPOTS OF MAIZE (*Zea mays*)

Symptoms (Maydis)

- The fungus affects the crop at young stage
- Early symptoms are oval, water-soaked spots on leaves
- Mature symptoms are characteristic cigar shaped lesions that are 3 to 15cm long
- Lesions are elliptical and tan in colour, developing distinct dark areas
- Lesions typically first appear on lower leaves, spreading to upper leaves and the ear sheaths as the crop matures
- Under severe infection, lesions may coalesce, blighting the entire leaf



Symptoms (Turcicum)

- Affects the crop at young stage
- Small yellowish round to oval spots are seen on the leaves
- Spots gradually increase into bigger elliptical spots and are straw to grayish brown in the centre with dark brown margins
- The spots coalesce giving blighted appearance
- The surface is covered with olive green velvety masses of conidia and conidiophores



Microscopic

Pathogen - *Helminthosporium maydis* (Syn: *H. turcicum*)

- Conidia are distinctly curved, fusiform, pale to mid dark golden brown with 5-11 septa
- Conidiophores are in group, geniculate, mid dark brown, pale near the apex and smooth

Pathogen – Pathogen – *Exserohilum turcicum*

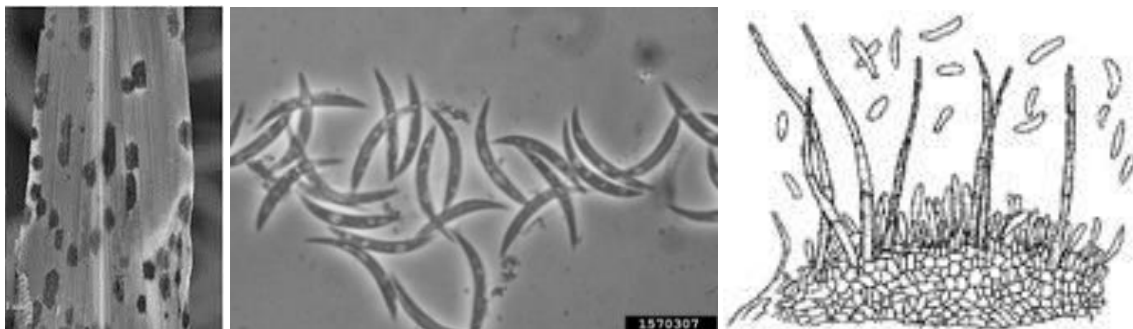
- The conidia are 18-23 u wide and 73-137 u long with 4-9 septa and born singly at the tips of the conidiophores.
- The conidial shape is fusoid, obclavate, straight, or curved; the hilum is strongly protruding.
- Conidia germinate commonly from one or both polar cells, rarely from intermediate cells.
- The conidiophores (7-11 x 165-283 u) are brown, irregularly cylindrical and 3-7 septate.
- They emerge in groups of two to six or more through stomata, or less frequently directly through the epidermis.



ANTHRACNOSE OF SORGHUM (*Sorghum bicolor*)

Symptoms

- The fungus causes both leaf spot (anthracnose) and stalk rot (red rot)
- The disease appears as small red coloured spots on both surfaces of the leaf
- The centre of the spot is white in colour encircled by red, purple or brown margin
- Numerous small black dots like acervuli are seen on the white surface of the lesions
- Red rot can be characterized externally by the development of circular cankers, particularly in the inflorescence
- Infected stem when split open shows discoloration, which may be continuous over a large area or more generally discontinuous giving the stem a marbled appearance



Microscopic

Pathogen - *Colletotrichum graminicolum*

- Fungus mycelium is localised in the spot
- Acervuli with setae arise through epidermis
- Conidia are hyaline, single celled, vacuolate and falcate in shape

BLAST OF FINGER MILLET (*Eleusine coracana*)

Symptoms

- Symptoms observed on the seedling, leaf, peduncle and finger
- Elliptical or diamond shaped lesions on leaves with grey centers, water-soaked and chlorotic halo surrounding the lesions are characteristic symptoms
- Neck blast symptoms develop as elongated black colour lesion mostly one-two inches below the ear
- Finger blast symptom starts at the tip and proceeds toward the base of the finger, which becomes brown
- Neck infection is the most serious phase of the disease that causes major loss in grain number, grain weight and increase in spikelet sterility





Microscopic

Pathogen: *Pyricularia grisea* (Perfect state: *Magnaporthe grisea*)

- Hypha is hyaline and septate, becomes brown with age
- The hyphal cell size ranges from 1.5–6.0µm in length
- Under high humidity, large number of conidiophores and conidia are produced giving a dirty brown colour to the lesion
- The growth of the pathogen is relatively more on the upper surface, making the spot darker
- Conidiophores are simple, septate, basal portion being relatively darker, may emerge either through stomatal opening or directly from the epidermal layer
- Conidia produced acrogenously, are hyaline and obpyriform in shape
- Conidia are three celled, the middle cell being wider and darker, and measure 19-31µm x 10-15µm
- The end cells germinate giving out germ tubes
- Formation of terminal or intercalary chlamydospores is common are globose, thick-walled, olive brown measuring 4-10µm in diameter

LEAF SPOTS OF GROUNDNUT (*Arachis hypogaea*)

Symptoms: Tikka disease/Early leaf spot and Late leaf spot

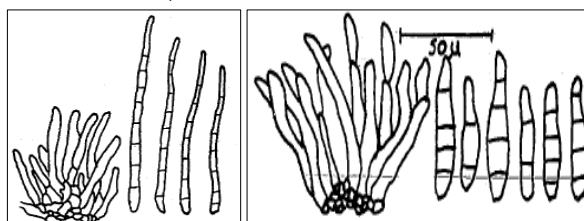
Early leaf spot	Late leaf spot
Appears within a month after sowing	Appears towards the maturity of the crop
Lesions are brown when seen on the under surface of the leaf	Carbon black
Spots encircled by a bright yellow halo	Yellow halo is absent
Spots are circular to irregular 1-10mm in dia., larger than late leaf spot	Spots are smaller 1-6 mm in dia. and more circular than early leaf spot
	

Microscopic

Early leaf spot

Pathogen - *Cercospora arachidicola* (Sexual Stage: *Mycosphaerella arachidis*)

- The fungus produces abundant sporulation on the upper surface of the leaves
- Conidiophores are olivaceous brown or yellowish brown in colour, short, 1 or 2 septate, unbranched and geniculate and arise in clusters
- Conidia are sub hyaline or pale yellow, obclavate, often curved 3-12 septate, 35- 110 x 2.5 - 5.4 μm in size with rounded to distinctly truncate base and sub-acute tip



Late leaf spot

Pathogen - *Phaeoisariopsis personata* (Sexual stage: *Mycosphaerella berkeleyii*)

- The conidiophores are long, continuous, 1-2 septate, geniculate, arise in clusters on lower surface of leaves and are olive brown in colour.
- The conidia are cylindrical or obclavate, short, measure 18-60 x 6-10 μm , hyaline to olive brown, usually straight or curved slightly with 1-9 septa, but mostly 3-4 septate.

PHYTOPHTHORA BLIGHT AND STERILITY MOSAIC OF PIGEONPEA (*Cajanus cajan*)

Symptoms: (Phytophthora blight)

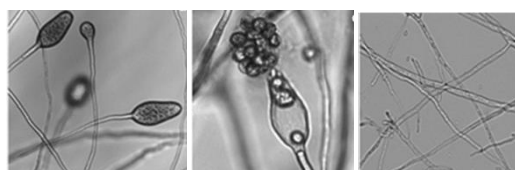
- Affected plants show as water-soaked brown to dark lesions on the leaves later becomes necrotic
- The lesions on stem and petiole are somewhat brown and sunken
- The lesions enlarge in size and girdle the stem resulting drying of branches and foliage
- The seedlings die suddenly due to infection
- No symptoms are found on root system
- Branches and petioles lead to desiccation
- In severe cases, the whole foliage becomes blighted
- Infected stem can easily break by the wind
- In advanced stages, the stem is commonly swollen into cankerous structures near the lesions
- The seedlings are highly prone to this infection and dry plants are common during rainy season
- The disease is serious when continuous rains occur or there is water logging in the field



Microscopic

Pathogen - *Phytophthora drechsleri* f. sp. *cajani*

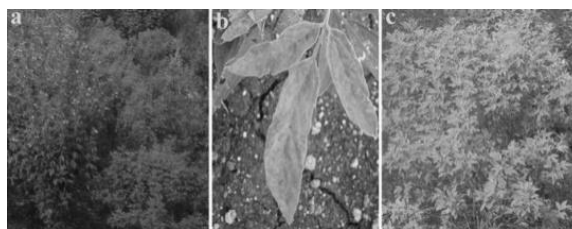
- Mycelium - hyaline, coenocytic
- Sporangiohores - hyaline bearing ovate or pyriform, non-papillate sporangia
- Each sporangium produces 8-20 zoospores
- Oospores - globose, light brown, smooth and thick walled



STERILITY MOSAIC

Symptoms

- The plants infected remain stunted
- The leaves show mosaic symptoms
- The symptoms may develop on all the leaves of infected plants
- The flowering is partially or completely stopped and a few flowers which develop are sterile



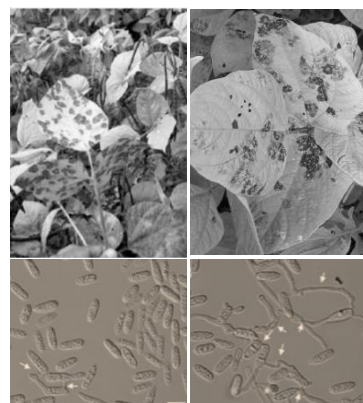
Pathogen - Pigeonpea sterility mosaic (PPSMV)

- Shows properties similar to viruses in the genus Tenuivirus
- Asymmetric morphology measuring 3-8 nm in diameter with 32 kDa coat protein
- Genetic material RNA 4-5 segmented size 0.8-3.5 kilo-base
- The virus particles are highly flexuous and of branched filamentous
- Ultrastructural studies of PPSMV infected tissues showed 100 – 150 nm quasi-spherical membrane bound bodies (MBBs) and fibrous inclusions
- The sterility mosaic causal agent is transmitted by the arthropod mite vector – *Aceria cajani* an eriophyid mite

ANTHRACNOSE OF BLACK GRAM (*Vigna mungo*) AND GREEN GRAM (*Vigna radiata*)

Symptoms

- Observed in all aerial parts of the plants and at any stage of crop growth
- Dark brown to black sunken lesions appears on the hypocotyl area and cause death of the seedlings
- Small angular brown lesions appear on leaves, mostly adjacent to veins, which later become greyish white centre with dark brown or reddish margin
- Lesions may be seen on the petioles and stem
- The prominent symptom is seen on the pods
- Minute water-soaked lesion appears on the pods initially and becomes brown and enlarges to form circular, depressed spot with dark centre with bright red or yellow margin
- Several spots join to cause necrotic areas with acervuli
- The infected pods have discoloured seeds



Microscopic

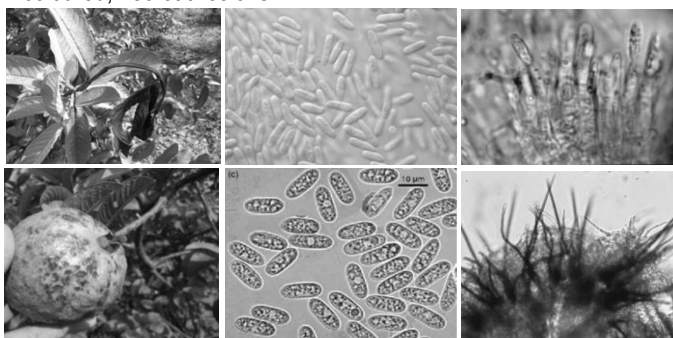
Pathogen: *Colletotrichum lindemuthianum* (Sexual stage: *Glomerella lindemuthianum*)

- Mycelium is branched, septate, hyaline becomes dark coloured with age
- Conidiophores on short, erect, hyaline, unbranched which measure 40-60 µm long
- Conidia are formed singly at the ends of free hyphae or on stromatoid masses in acervuli.
- On the host the conidial stage develops on stomata beneath the cuticle, later rupturing it and becoming erumpent
- Conidia are 1-celled, hyaline, oblong, cylindrical with rounded ends or with one end slightly pointed, measure 13-22(11-20) x 2.5-55 µm borne in acervuli acrogenously.

ANTHRACNOSE OF GUAVA (*Psidium guajava*)

Symptoms

- Symptoms are observed on mature fruits on the tree
- The characteristic symptoms consist of sunken, dark coloured, necrotic lesions
- Under humid conditions, the necrotic lesions become covered with pinkish spore masses
- The disease progresses, the small sunken lesions coalesce to form large necrotic patches affecting the flesh of the fruit



Microscopic

Pathogen: *Colletotrichum gloeosporioides*

- Conidia - hyaline, aseptate, oval to elliptical
- Conidiophore is cylindrical
- Acervuli - dark brown to black

LEAF CURL OF PAPAYA (*Carica papaya*)

Symptoms

- Curling, crinkling and distortion of leaves
- Reduction of leaf lamina
- Rolling of leaf margins inward and downward
- Thickening of veins
- Leaves become leathery, brittle and distorted
- Plants become stunted
- Affected plants does not produce flowers and fruits

Pathogen: Papaya leaf curl virus (*PaLCuV*)

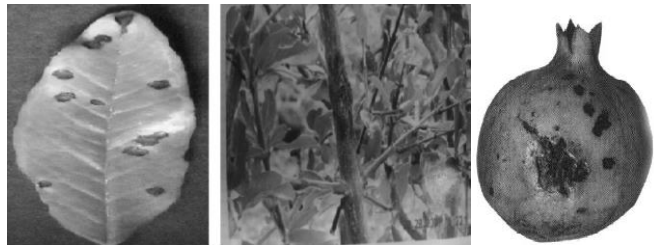
- The virus belongs to the begomovirus genus of Geminiviridae
- It is a single stranded DNA virus
- It is transmitted in nature by the whitefly (*Bemisia tabaci*)
- This virus is not transmitted through seed and mechanically



BACTERIAL BLIGHT OF POMEGRANATE (*Punica granatum*)

Symptoms

- Dark red spots appear on leaves
- Affected leaves gets distorted and malformed
- Severely affected young leaves fall off
- Brown to black spots around the nodes of the stem
- Cracking of nodes in infected plants due to girdling of the stem
- Fruits brown to black spots developed on the pericarp of fruit with L or Y-shaped cracks
- Raised dark brown lesions with indefinite margins are seen on the fruit surface



Microscopic

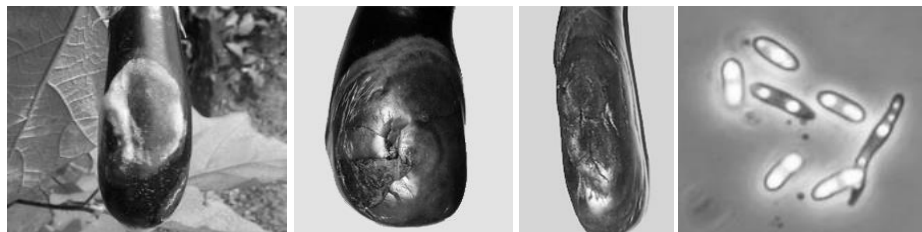
Pathogen: *Xanthomonas axonopodis* pv. *punicae*

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

PHOMOPSIS BLIGHT AND FRUIT ROT OF BRINJAL (*Solanum melongena*)

Symptoms

- Affects all above the ground plant parts
- Spots generally appear first on seedling stems or leaves
- Girdle seedling stems and kill the seedlings
- Leaf spots are clearly defined, circular, up to about 1 inch in diameter, brown to grey with a narrow dark brown margin
- Fruit spots are much larger, affected fruit are first soft and watery
- Later on affected fruits may become black and mummified
- Centre of the spot becomes grey and black pycnidia develop on it



Microscopic

Pathogen: *Phomopsis vexans*

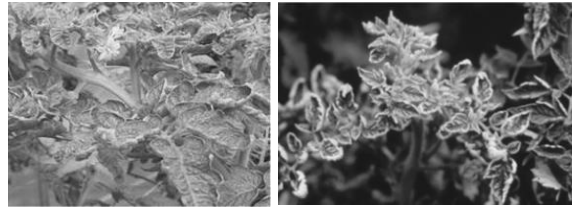
- Pycnidia –
 - With or without beak
 - Found in the affected tissue
 - Globose or irregularly shaped
- Conidiophores – found in the pycnidium are hyaline, simple or branched
- Conidia - hyaline, one celled and sub cylindrical

- Ascospores - hyaline, narrowly ellipsoid to bluntly fusoid with one septum

LEAF CURL OF TOMATO (*Solanum lycopersicum* / *Lycopersicon esculentum*)

Symptoms

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



Pathogen: Tomato leaf curl virus (ToLCV)

- The genus *Begomovirus* is a circular single-stranded DNA virus
- Virus exclusively transmitted by whitefly (*Bemisia tabaci*)
- Two species namely *Tomato leaf curl New Delhi virus* and *Tomato leaf curl Palampur virus* predominantly distributed in Northern India
- One species namely *Tomato leaf curl Bangalore virus* is dominant in Southern India
- The virus particles are 80nm in diameter

EARLY BLIGHT OF TOMATO (*Solanum lycopersicum* / *Lycopersicon esculentum*)

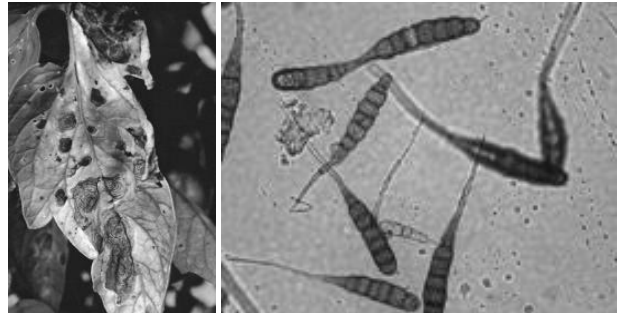
Symptoms

- Affects the foliage at any stage of the growth
- Early blight first observed on the plants as small, black lesions mostly on the older foliage
- Spots enlarge and become one-fourth of an inch in diameter or larger and concentric rings in a bull's eye pattern can be seen in the center of the diseased area
- Tissue surrounding the spots may turn yellow

Microscopic

Pathogen: *Alternaria solani*

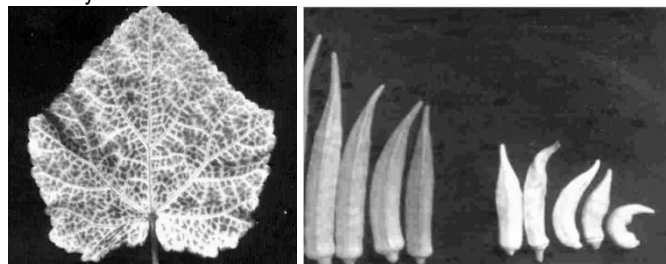
- Mycelium septate, branched, light brown becomes darker with age
- Conidiophores are dark coloured
- Conidia are beaked, muriform, dark colored and borne singly



YELLOW VEIN MOSAIC OF OKRA (*Abelmoschus esculentus*)

Symptoms

- Infection may start at any stage of plant growth
- Characteristic symptom is the yellowing of the entire network of veins in the leaf blade
- Veins of the leaves will be cleared due to the virus
- Interveinal area may become completely yellow or white
- Severe infections - younger leaves turn yellow, reduced in size and the plant is highly stunted
- Infection restricts flowering and fruits, if formed, may be smaller and harder
- Affected plants produce fruits with yellow or white colour



Pathogen- Bendi yellow vein mosaic virus (MYVMV) / okra yellow vein mosaic (OYVMV)

- Viral disease caused by monopartite Begomovirus
- Transmitted by white fly (*Bemisia tabaci*)
- The virus particles are 16 – 18nm in diameter