THE NEOTIA UNIVERSITY

ज्ञानम् आत्म प्रदीपाय

Diseases of Field and Horticultural Crops and their Management- I

Practical manual

Course Code – CC-AGL 538

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Index

Preface

This course will create awareness on diseases of Field and horticultural crops not only reduce the productive capability but also adversely affect the quality. With the changing climate, Agricultural & horticultural crops have become vulnerable to hazards of diseases, playing a major role in causing threat of food security.

This course will be very helpful to clear concept of the students about the detection and diagnosis of important diseases of field and horticultural crops by their symptoms. This Practical manual will also provide practical knowledge on methods for microscopic slide preparation (Teasing method) and Lactophenol Cotton Blue (LPCB) staining, free hand section method for histopathology study, methods for preparation of Herbarium specimens of plant disease sample, identification and histopathological studies of different field and horticulture plant diseases viz. Brown spot disease of Rice, Sheath blight disease of Rice, leaf spot/ blight disease of maize, Cercospora / Anthracnose leaf spot of gram, Tikka Disease of Groundnut, Anthracnose disease of Guava, Alternaria leaf spot diseases of Cruciferous Vegetables, Early Blight/Late Blight diseases of Tomato, Anthracnose diseases of Bean and Panama wilt of Banana. This course will have a field visit. As a result, students will be better aware of the Plants diseases.

Practical (P) 1: Methods for microscopic slide preparation (Teasing method) and Lactophenol Cotton Blue (LPCB) Staining

Objectives: In plant disease sample the pathogen remain in the embedded state. So, it is necessary to tease the disease sample to identify the pathogen and to study the cell and spore structure of the pathogen. Lactophenol Cotton Blue (LPCB) Staining is a simple histological staining method used for the microscopic examination and identification of fungi.

Principle:

Lactophenol Cotton Blue (LPCB) staining method works on the principle of aiding the identification of the fungal cell walls.

- Fungi are eukaryotic organisms with both macroscopic and microscopic characteristics.
- The fungal spore cell wall is made up of chitin of which the components of the Lactophenol Cotton Blue solution stains for identification.
- The lactophenol cotton blue solution acts as a mounting solution as well as a staining agent.
- The solution is clear and blue in colour and it is made up of a combination of three main reagents:
    - **Phenol**: It acts as a disinfectant by killing any living organisms
    - **Lactic acid**: To preserve the fungal structures
    - **Cotton blue**: To stain or give colour to the chitin on the fungal cell wall and other fungal structures
- The stain will give the fungi a blue-coloured appearance of the fungal spores and structures, such as hyphae.

Materials required: Infected disease sample, Slide, Cover Slip, Blotting Paper, Fine pointed needle, Lactophenol, Cotton blue, new blade and Microscope.

Procedure of Lactophenol Cotton Blue (LPCB) Staining and teasing methods:

Method-1

1. Place a drop of cotton blue on the slide.
2. Cut a small portion of the infected samples (lesion) and put it on that cotton blue fluid.
3. Wait for one minute for getting stain the sample and after that wash the cotton blue with the help of Lactophenol.
4. Scrap the disease portion from the infected leaf.
5. Place the cover slip slowly at an angle with support of the needle on the drop of the mounting fluid.
6. Dry excess of lactophenol with a small piece of blotting paper.
7. Expel air bubbles, if any under the cover slip.

8. Examine the sample at low power (10x) then at high power (40x) of the microscope.

Method-2

1. On a clean microscopic glass slide, add a drop of 70% ethanol
2. Add the fungal specimen to the drop of alcohol using a sterile mounter such as an inoculation loop (from solid medium), / diseased specimen with the help of forceps or needle, depending on the sample of use.
3. Tease the fungal/ diseased sample of the alcohol, using a needle mounter, to ensure the sample mixes well with the alcohol.
4. Using a dropper or pipette, add one or two drops of Lactophenol Cotton Blue Solution (prepared above) before the ethanol dries off.
5. Remove the excess stain carefully and cover the specimen with a clean coverslip without making air bubbles to the stain.
6. Examine the stain microscopically at 10X/ 40X, to observe for fungal spores and other fungal structures.

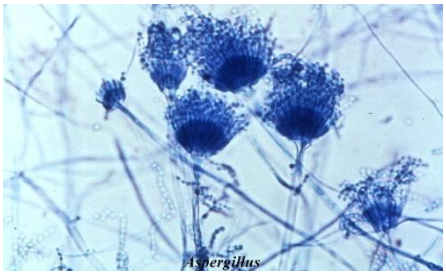
Applications:

- Used in the identification of suspected fungal samples.
- General identification of fungi and its structures.

Limitations:

- It can only be used as a presumptive identification method of fungi which should be followed up with other diagnostic tools such as biochemical and cultural examination.
- The components of the solution should be used before expiry, including the use of the solution before it expires.
- The solution may disrupt the original morphology of the fungi.
- The stain can only be used to identify mature fungi and its structures and not the young vegetative forms of fungi.
- The stain can't be stored for a long period of time.

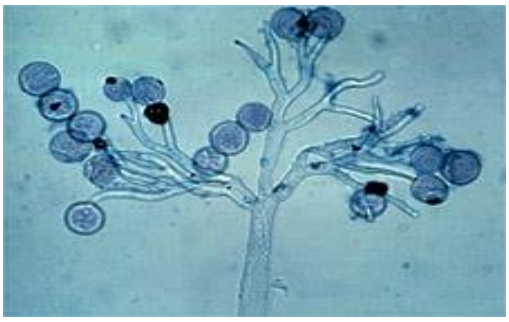
***Fusarium* sp. stained with**



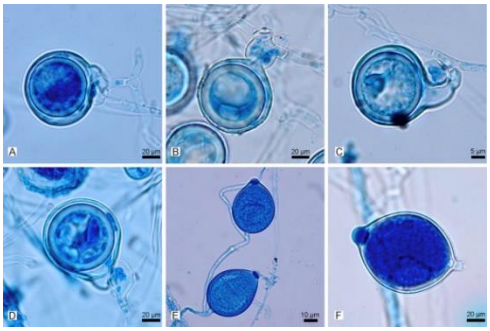
***Aspergiullus* sp. stained with LPCB**

***Penicillium* sp. stained with LPCB**

***Colletotrichum* sp. stained with LPCB**



***Peronospora* sp. stained with LPCB**



***Phytophthora* sp. stained with LPCB**

Useful internet links: https://youtu.be/zRMD8rmd3l8

Practical (P) 2: Free hand section method for histopathology study

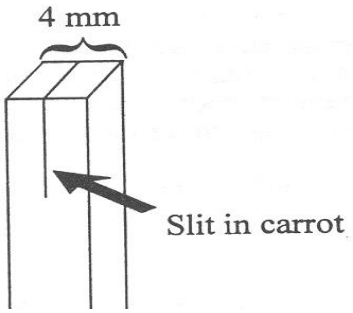
Objectives: In plant disease sample the pathogen remain in the embedded state with epidermis layer of the host tissue or specialized structures formed by pathogens. So, it is necessary for sectioning the fungal fruit bodies or structures attached with host (viz. acervuli, pycnidia, rust sori, tumerous outgrowth). Free hand section method is also used to identify the pathogen and to study the cell and spore structures or arrangement of the pathogen inside host tissue.

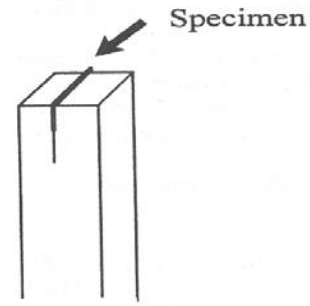
Materials required: Infected disease sample, Slide, Cover slip, Blotting paper, Razor blade, dropper, Fine pointed needle, Forceps, Brush, Watch glass, Carrot disc, Cotton blue, Lactophenol, Sterile water, Microscope

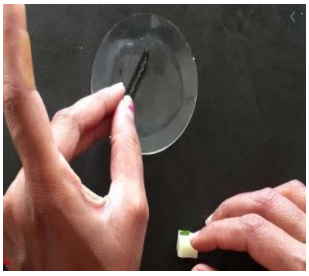
Methods:

1. Obtain a new double edge razor blade. To minimize the risk of cutting oneself, cover one edge of the razor blade with masking tape.
2. Insert the thin diseased leaves, small and soft specimens such as roots, tissue pieces into a small piece of pith such as a carrot disc.
3. Hold piece of carrot firmly. The material should be held against the side of the first finger of the left hand (or right hand) by means of the thumb.
4. Take the razor blade in the right hand (or left hand) and place it on the first finger of the left hand (or right hand), more or less at a right angle to the specimen. Draw the razor across the top of the material in such a way as to give the material a drawing cut (about 45° in the horizontal direction).

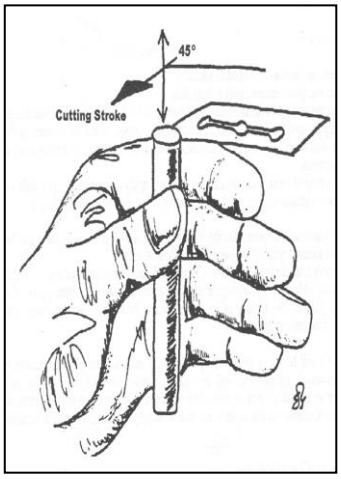
5. Cut several sections at a time. Sections will certainly vary in thickness.
6. Transfer sections to water, always using a brush, not a forceps or needle.
7. Select and transfer the thinnest section and stain with LPCB.

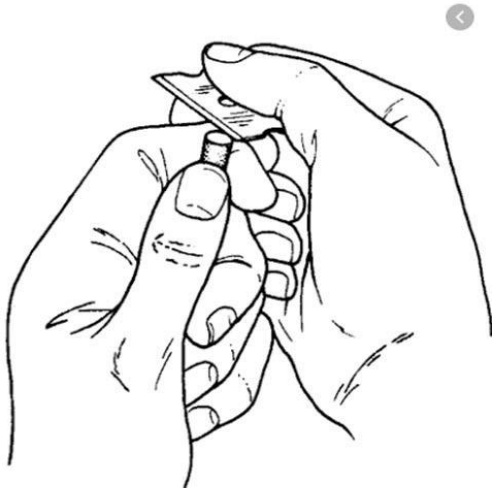






Staining and visualization under microscope





Method of holding a specimen and sectioning for free hand section

Useful internet links: **https://youtu.be/-bLBHiqlmOU**

Practical (P) 3: Identification and histopathological studies of Brown spot disease of Rice.

Objectives: To identify the causal organism of the disease and to study etiology.

Symptoms: Circular, brown lesion on seedling; distorted primary and/or secondary leaves on seedlings; black discoloration on roots are occur due to this disease. Circular or oval lesions with grey centre and reddish-brown margin that surrounding those lesions are arise on an older leaves. This is the characteristic symptom of brown spot of Rice. Grains are also affected produce brown or black spots on grain, reduced number of grains per Kernel and also reduced the kernel weight.

Causal organism:

Anamorphic stage: *Helminthosporium oryzae* (Syn: *Bipolaris oryzae*); *Drechslera oryzae* .

Teleomorphic stage: *Cochliobolus miyabeanus*.

(**Division:** Ascomycota, **Class:** Dothideomycetes, **Order:** Pleosporales, **Family:** Pleosporaceae)

Mycelium is septate, greyish-brown to dark brown in colour. The hyphae are dark brown or olivaceous. Conidiophores are straight, sometime geniculate, pale to brown colour found single or in groups. The conidia measure 35-170 x 11-17 µm. typical conidia are slightly curved, widest at the middle and tapering toward end, occasionally straight pale olive green to golden brown and septate. Mature conidia are brownish with a moderately thin peripheral wall. Sexually they produce Perithecia, which is globose and black in colour. Asci are cylindrical to fusiform, hyaline or pale olive green in colour, slightly curved. Ascospores are filiform.

Materials required: Infected disease sample, Slide, Cover slip, Blotting paper, Fine pointed needle, Scissor, Cotton blue, Lactophenol, Microscope

Methods: Teasing process **[See Practical (P) 1]**

Observation:

Result and conclusion:

Useful internet links: https://www.youtube.com/watch?v=FT7aEzqOQJw

Practical (P) 4: Identification and histopathological studies of Sheath blight disease of Rice.

Objectives: To identify the causal organism of the disease and to study etiology.

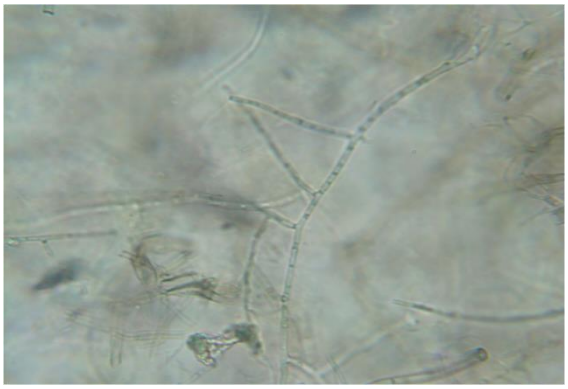
Symptoms: The fungus affects the crop from tillering to heading stage. Initial symptoms are noticed on leaf sheaths near water level. On the leaf sheath oval or elliptical or irregular greenish grey spots are formed. As the spots enlarge, the centre becomes greyish white with an irregular blackish brown or purple brown border. Lesions on the upper parts of plants extend rapidly coalescing with each other to cover entire tillers from the water line to the flag leaf. The presence of several large lesions on a leaf sheath usually causes death of the whole leaf, and in severe cases all the leaves of a plant may be blighted.

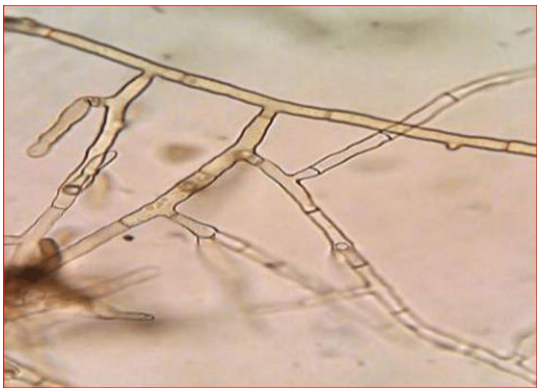
Causal organism: Anamorphic stage: *Rhizoctonia solani*

Teleomorphic stage: *Thanetophorus cucumeris*

(**Division:** Basidiomycota, **Class:** Agaricomycetes, **Order:** Cantharellales, **Family:** Ceratobasidiaceae)

Mycelium produces long multinucleate cells that grow approximately at right angles to the main hypha with a slight constriction at the junction of main hypha and branches. Hyphal cells are separated by septum containing a pore which allows movement of mitochondria, nuclei, and cytoplasm from cell to cell.





Materials required: Infected disease sample, Slide, Cover slip, Blotting paper, Fine pointed needle, Scissor, Cotton blue, Lactophenol, Microscope

Methods: Teasing process **[See Practical (P) 1]**

Observation:

Result and conclusion:

Useful internet links: https://www.youtube.com/watch?v=HPlthxaStKw

Practical (P) 5: Identification and histopathological studies of leaf spot disease of Maize. `

Objectives: To identify the causal organism of the disease and to study etiology.

Northern corn leaf blight:

Symptom: 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 as they mature that are associated with fungal sporulation. 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.

Causal organism: Anamorphic: *Exserohilum turcicum*

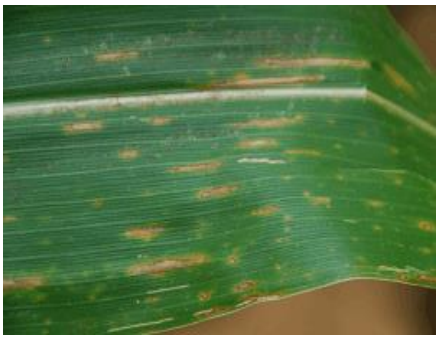
Telomorphic: *Setosphaeria turcica*

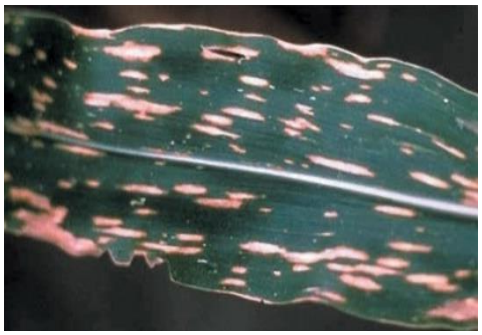
(**Division:** Ascomycota, **Class:** Dothideomycetes, **Order:** Pleosporales, **Family:** Pleosporaceae)

Colonies of *Exserohilum* is grey to blackish-brown colour. The conidia from which the hilum extends, are either straight, curved, slightly bent or ellipsoidal to fusiform.

Southern corn leaf blight:

Symptom: Small yellowish round or oval spots appear on the leaves. These spots enlarge, become elliptical and the centre becomes straw coloured with a reddish-brown margin. Conidia and conidiophores are formed in the centre.

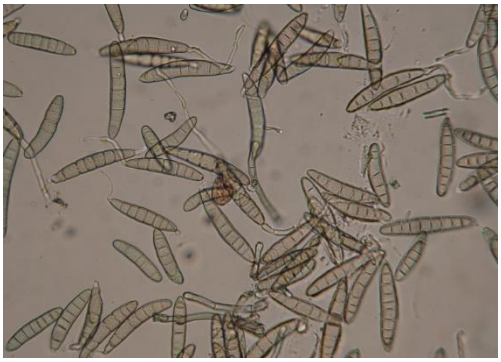


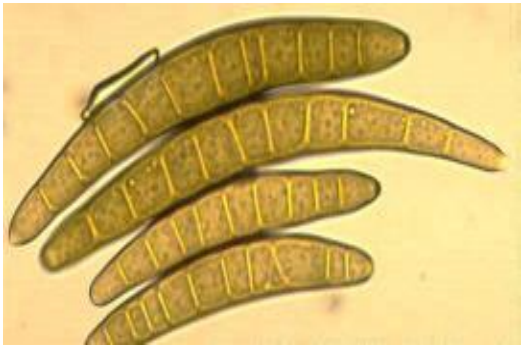


Causal organism: Anamorphic: *Bipolaris maydis* (*Helminthosporium maydis*)
Teleomorphic: *Cochliobolus heterostrophus*

(**Division:** Ascomycota, **Class:** Dothideomycetes, **Order:** Pleosporales, **Family:** Pleosporaceae)

The pathogen produces brown colour hyphae, conidiophores and conidia. Conidia are brown in colour, straight some time curved, fusiform and having several numbers of septa.





Gray leaf spot:

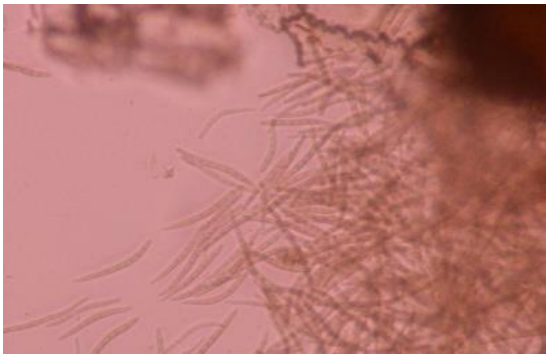
Symptom: Gray leaf spot lesions begin as small necrotic pinpoints with chlorotic halos. Coloration of initial lesions can range from tan to brown before sporulation begins. Early lesions are ambiguous, so they are easily confused with other foliar diseases such

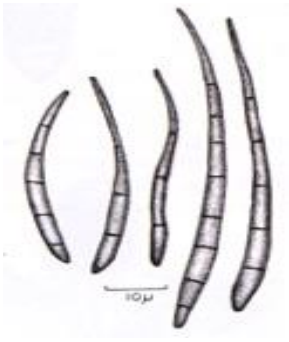
as anthracnose leaf blight, eyespot, or common rust. At letter stage as infection progresses, lesions begin to take on a more distinct shape. Lesion expansion is limited by parallel leaf veins, resulting in the blocky shaped “spots”. As sporulation commences, the lesions take on a more gray coloration. Entire leaves can be killed when weather conditions are favourable, and rapid disease progression causes lesions to merge.

Causal organism: *Cercospora zeae-maydis*

(**Division:** Ascomycota, **Class:** Dothideomycetes, **Order:** Capnodiales, **Family:** Mycosphaerellaceae)

Pathogen produce mycelium, conidia. Conidia are olivaceous in color, thin hair like structure and pointed at the end.





Materials required: Infected disease sample, Slide, Cover slip, Blotting paper, Fine pointed needle, Scissor, Cotton blue, Lactophenol, Microscope

Methods: Teasing process **[See Practical (P) 1]**

Observation:

Result and conclusion:

Useful internet links: https://www.youtube.com/watch?v=UcKKEjKkZJU,
https://www.youtube.com/watch?v=oAI6o__Ccsk,
https://www.youtube.com/watch?v=107Xz8HslXM

Practical (P) 6: Identification and histopathological studies of Cercospora / Anthracnose leaf spot of gram.

Objectives: To identify the causal organism of the disease and to study etiology.

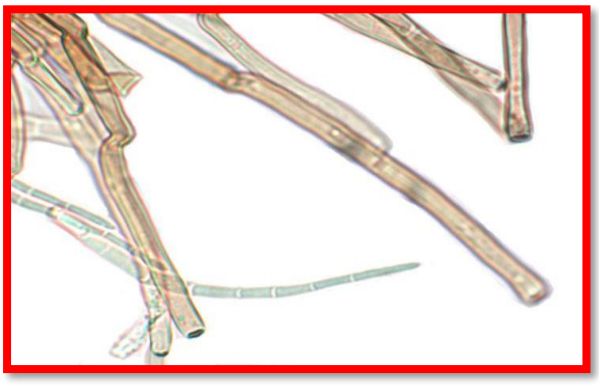
Cercospora Leaf Spot:

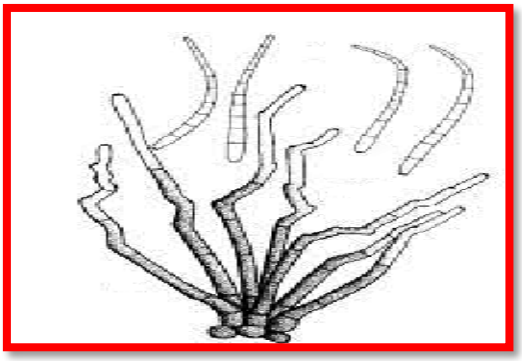
Symptom: The symptoms generally characterized by the appearance of leaf spot that are circular and / or irregular in shape with greyish- white centres and reddish brown to dark brown margins are visible on the leaves. Sometimes powdery growths are there, due to the sporulation of the fungus at the centre of the spots. The spots may increase in numbers and size. In some cases, the affected tissue is killed and the dead portion drops out leaving shot-hole symptoms on the leaves.

Causal organism: *Cercospora canescens*

(**Division:** Ascomycota, **Class:** Dothideomycetes, **Order:** Capnodiales, **Family:** Mycosphaerellaceae)

The fungus produces clusters of dark brown septate conidiophores. The conidia are linear, hyaline and whip-like, thin walled and 5-10 septate and are borne singly at the tip of the conidiophores.





Anthracnose Leaf Spot:

Symptom: water soaked lesions appear on leaves and pods, later becoming brown and enlarging to form circular spots of varying size. The spots are usually depressed with dark centers and bright red, yellow and orange margins. When the infections are severe on the leaf petiole and stem, the affected parts wither off. Often the seedlings are blighted due to infection soon after the seed germinate.

Causal organism: Anamorphic: *Colletotrichum lindemuthiana*

Teleomorphic: *Glomerella lindemuthiana*

(**Division:** Ascomycota, **Class:** Sordariomycetes, **Order:** Glomerellales, **Family:** Glomerellaceae)

The mycelium is localized in the lesions and forms stroma or cushion like structure just beneath the epidermis. Short conidiophore and pointed, septate and dark colored setae are arise from the stroma. On the tip of the hyaline conidiophore, oblong, thin walled, single celled, hyaline conidia are produce. Sexually the pathogen produce Perithecia. Perithecia are ostiolate having limited number of asci, which contain typically eight spores, which are one or two celled.

Materials required: Infected disease sample, Slide, Cover slip, Blotting paper, Fine pointed needle, Scissor, Cotton blue, Lactophenol, Microscope

Methods: Teasing process **[See Practical (P) 1]**

Observation:

Result and conclusion:

Useful internet links: https://www.youtube.com/watch?v=wr83FIHa5Kw

Practical (P) 7: Identification and histopathological studies of Tikka Disease of Groundnut.

Objectives: To identify the causal organism of the disease and to study etiology.

Tikka Leaf Spots:

Early leaf spot:

Symptom: At first small chlorotic spots appear on leaflets, with time they enlarge and turn brown to black and assume sub circular shape on upper leaf surface. On lower surface of leaves light brown colouration is seen. Lesions also appear on petioles, stems, stipules. In severe cases several lesions coalesce and result in premature senescence.

Causal organism: Anamorphic: *Cercopora arachidicola*

Teleomorphic: *Mycosphaerella arachidis*

(**Division:** Ascomycota, **Class:** Dothideomycetes, **Order:** Capnodiales, **Family:** Mycosphaerellaceae)

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 arise in clusters. Conidia are sub hyaline or pale yellow, obclavate, often curved 3-12 septate with rounded to distinctly truncate base and sub-acute tip. The perfect stage of fungi has been found on the dead host tissue. The perfect stage of the fungus produces Pseudothecium. Asci are cylindrical to clavate and contain 8 ascospores. Ascospores are hyaline, slightly curved and two celled.

Late leaf spot:

Symptom: Black & nearly circular spots appear on the lower surface of the leaflets. Lesions are rough in appearance. In extreme cases many lesions coalesce resulting in premature senescence and shedding of the leaflets.

Causal organism: Anamorphic: *Phaeoisariopsis personata* (Syn :*Cercospora personata*)
Teleomorphic : *Mycosphaerella berkeleyii)*

(**Division:** Ascomycota, **Class:** Dothideomycetes, **Order:** Capnodiales, **Family:** Mycosphaerellaceae)

The pathogen produce conidiophore. They are long, continuous, 1-2 septate, arise in clusters and olive brown in colour. The conidia are cylindrical or obclavate, short, hyaline to olive brown, usually straight or curved slightly with 1-9 septa, mostly 3-4 septate. The fungus in its perfect stage produces Pseudothecium which are globose. Asci are cylindrical to ovate, contain 8 ascospores. Ascospores are 2 celled and constricted at septum and hyaline.

Materials required: Infected disease sample, Slide, Cover slip, Blotting paper, Fine pointed needle, Scissor, Cotton blue, Lactophenol, Microscope

Methods: Teasing process **[See Practical (P) 1]**

Observation:

Result and conclusion:

Useful internet links: https://www.youtube.com/watch?v=OO3HBrDNKG8

Practical (P) 8: Identification and histopathological studies of Anthracnose disease of Guava.

Objectives: To identify the causal organism of the disease and to study etiology.

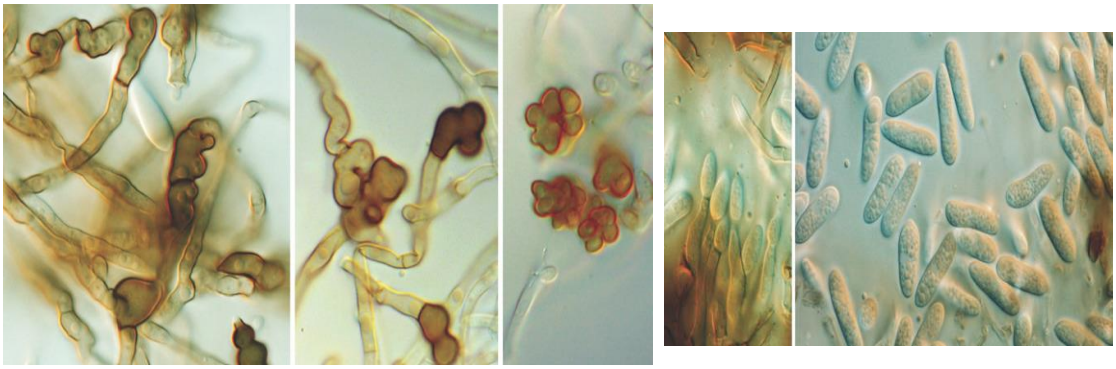
Symptom: The most characteristic symptoms appear during the rainy season as small pin-head sized spots on the unripe fruits.They gradually enlarge to form sunken and circular, dark brown to black spots.The infected area of the unripe fruits becomes harder and corky. Infections also occur on leaves and twig portions. In moist weather, acervuli appear as black dots on twigs or fruits, which later produce pinkish spore mass.

Causal organism: Anamorphic**:** *Gloeosporium psidii* (sym. *Colletotrichum gloeosporioides*)

Teleomorphic: *Glomerella cingulate*

(**Division:** Ascomycota, **Class:** Sordariomycetes, **Order:** Glomerellales, **Family:** Glomerellaceae)

Pathogen produces brown to dark brown coloured acervuli on the affected parts of the plant. Setae, conidiophores and conidia are formed in the acervuli. Mycelium is intercellular, branched and light brown in colour. Conidiophores are hyaline and small. Conidia are formed at the tip of the conidiophores and are cylindrical shaped, unicellular, hyaline. They germinate by germ tube. Setae are long, tapering at the end, dark brown to black in colour.



Materials required: Infected disease sample, Slide, Cover slip, Blotting paper, Fine pointed needle, Scissor, Cotton blue, Lactophenol, Microscope

Methods: Teasing process **[See Practical (P) 1]**

Observation:

Result and conclusion:

Useful internet links: https://www.youtube.com/watch?v=p0n0WRJryrU

Practical (P) 9: Identification and histopathological studies of Alternaria leaf spot diseases of Cruciferous Vegetables.

Objectives: To identify the causal organism of the disease and to study etiology

Symptom: The first symptoms of the disease are minute yellow specks on the oldest leaves and stems. The spots darken and enlarge into circular, tan to dark brown spots. Alternating light and dark concentric rings give the spots the appearance of a target; a yellow halo may surround the lesion. Older spots may be black, brown, or tan in colour, papery in texture and may fall off, giving the appearance of a shot-hole. Infection of seedling stems may result in damping-off or stunted plants. As the disease progresses, it spreads to all aerial parts of the plant. If humid, lesions can produce spores.

Causal organism: *Alternaria brassicicola*

(**Division:** Ascomycota, **Class:** Dothideomycetes, **Order:** Pleosporales, **Family:** Pleosporaceae)

The conidia are dark brown and smooth-walled, up to 60 x 14µm. The conidia are cylindrical to oblong in shape and are muriform and produced in chains of 8-10 spores. They are firmly attached to conidiophores that are olive-brown, septate. Although conidia can be spread by rain, the most common means of spread is through the air.

Colonies of *A. brassicicola* tend to be dark brown or black in colour.

Materials required: Infected disease sample, Slide, Cover slip, Blotting paper, Fine pointed needle, Scissor, Cotton blue, Lactophenol, Microscope

Methods: Teasing process **[See Practical (P) 1]**

Observation:

Result and conclusion:

Useful internet links: https://www.youtube.com/watch?v=V2YqYgoELP

Practical (P) 10: Identification and histopathological studies of Early Blight/Late Blight diseases of Tomato.

Objectives: To identify the causal organism of the disease and to study etiology

Early Blight of Tomato:

Symptoms: Early blight is first observed on the plants as small, black lesions mostly on the older foliage. 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 centre of the diseased area. Tissue surrounding the spots may turn yellow. Lesions on the stems are similar to those on leaves, sometimes girdling the plant if they occur near the soil line. The fungus also infects the fruit, generally through the calyx or stem attachment. Lesions attain considerable size, usually involving nearly the entire fruit; concentric rings are also present on the fruit.

Causal organism: *Alternaria solani.*

(**Division:** Ascomycota, **Class:** Dothideomycetes, **Order:** Pleosporales, **Family:** Pleosporaceae)

Mycelium is septate, branched, light brown which become darker with age. Conidiophores are dark coloured. Conidia are beaked, muri form, dark coloured.

Late Blight of Tomato:

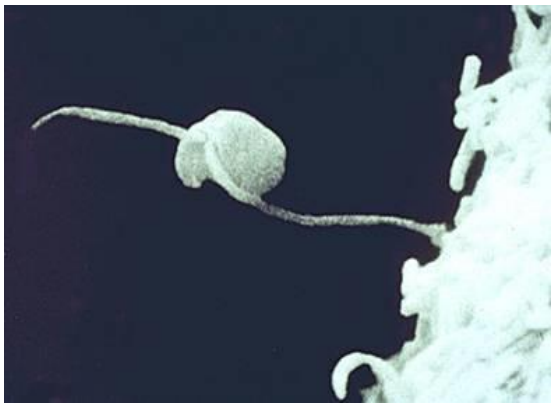
Symptoms: The first symptoms of late blight on tomato leaves are irregularly shaped, water-soaked lesions, often with a lighter halo or ring around them; these lesions are typically found on the younger, more succulent leaves in the top portion of the plant canopy. As the disease progresses, lesions enlarge causing leaves to brown, shrivel and die. Late blight can also attack tomato fruit in all stages of development. Rotted fruit are typically firm with that eventually become leathery and chocolate brown in colour, these spots can enlarge to the point of encompassing the entire fruit.

Causal Organism: *Phytophthora infestans*

(Phylum: Oomycota, **Order:** Peronosporales, **Family:** Peronosporaceae)

The mycelium is hyaline and coenocytic (few septa). *Phytophthora infestans* produces sporangia or sac-like structures on sporangiophores. In cool, wet conditions, zoospores will form and emerge from the sporangia and in warmer conditions, sporangia may function as a single spore and germinate directly. Zoospores are biflagellate (have two

flagella), with one tinsel flagellum directed anteriorly and one whiplash flagellum directed posteriorly. Sexually they produce Antheridium and Oogonium.



Materials required: Infected disease sample, Slide, Cover slip, Blotting paper, Fine pointed needle, Scissor, Cotton blue, Lactophenol, Microscope

Methods: Teasing process **[See Practical (P) 1]**

Observation:

Result and conclusion:

Useful internet links: https://www.youtube.com/watch?v=5gWsmYFQE7w

Practical (P) 11: Identification and histopathological studies of Anthracnose diseases of Bean.

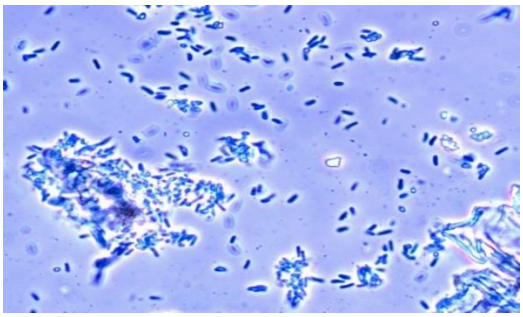
Objectives: To identify the causal organism of the disease and to study etiology
Symptoms: Leaf lesions typically appear first on lower leaf surfaces near the veins and, as the disease progresses, appear on upper leaf surfaces. The lesions are elongate, angular, and brick red to purple. They turn brown to black with age. Lesions may also form on petioles. Circular to irregular, sunken lesions on bean pods are the most noticeable symptom of anthracnose. Pod lesions are up to 1/2-inch in diameter and are tan to rust-coloured with a brown or purple border. Inside the pod, the seed coat may have brown to black lesions. During severe outbreaks of anthracnose, pods may dry and fail to fill.

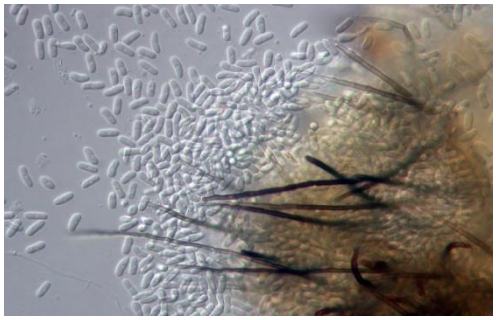
Causal Organism: Asexual stage: *Colletotrichum lindemuthianum*

Sexual stage: *Glomerella cingulate*

(**Division:** Ascomycota, **Class:** Sordariomycetes, **Order:** Glomerellales, **Family:** Glomerellaceae)

It produced hyaline, single celled, oblong, sometimes with one end slightly pointed spores or conidia on conidiophore along with setae on Acervulus. The pathogen sexually reproduces to form ascospores inside of asci, and subsequently packed into Perithecia.





Materials required: Infected disease sample, Slide, Cover slip, Blotting paper, Fine pointed needle, Scissor, Cotton blue, Lactophenol, Microscope

Methods: Teasing process **[See Practical (P) 1]**

Observation:

Result and conclusion:

Useful internet links: https://www.youtube.com/watch?v=Dn3iblzb4ws

Practical (P) 12: Identification and histopathological studies of Panama wilt of Banana.

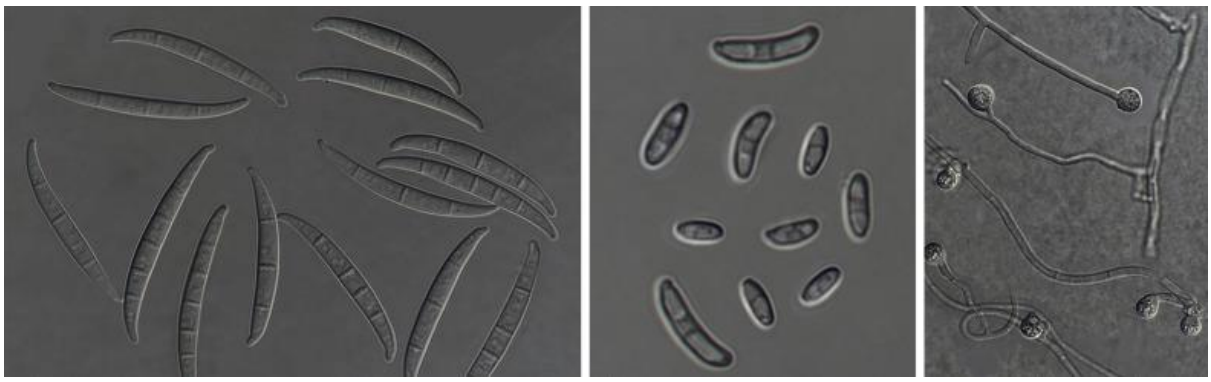
Objectives: To identify the causal organism of the disease and to study etiology.

Symptoms: Yellowing of the lower most leaves starting from margin to midrib of the leaves. Yellowing extends upwards and finally heart leaf alone remains green for some time and it is also affected. The leaves break near the base and hang down around pseudo stem. Longitudinal splitting of pseudo stem. Discolouration of vascular vessels as red or brown streaks are the characteristic symptoms. The fungus spreads through use of infected rhizomes. Continuous cultivation results in build-up of inoculum.

Causal organism: *Fusarium oxysporum* f. sp. *cubense*

(**Division:** Ascomycota, **Class:** Sordariomycetes, **Order:** Hypocreales, **Family:** Nectriaceae)

Mycelium is septate, hyaline and branched. Fungus produces micro, macro conidia and also chlamydospores. Micro conidia - Single celled or rarely one septate hyaline elliptical or oval. Macro conidia - Sickle shaped hyaline, 3-5 septate and tapering at both ends. Chalamydospores - Thick walled, spherical to oval, hyaline to slightly yellowish in colour.



Materials required: Infected disease sample, Petriplate, PDA Slant test tube, inoculating needle, Slide, Cover slip, Blotting paper, Fine pointed needle, Scissor, Cotton blue, Lactophenol, Microscope

Methods:

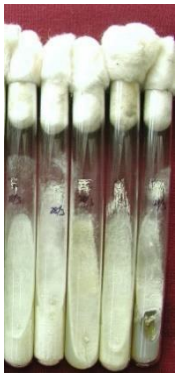
Isolation of fungus from rhizome infected banana corm from different location:

The following technique was followed for isolation of fungus from crop plants:

1. Selection of infected banana corm is done from rhizome infecting banana plant.
2. 2-2.5 cm cubical blocks are being taken out from collected banana corms with the help of steel knife.
3. Firstly, cubical corms pieces were washed in running tap water properly. And then surface sterilized in 70% ethyl alcohol/0.1% mercuric chloride under laminar air flow to avoid surface contamination.
4. The blocks are then washing properly with the sterilized distilled water up to 5-6 times to wash in the surface sterilizing agents.
5. Then the blocks are aseptically transferred to sterilized Petri plates with sterilized blotting paper inside the plates. Then the blotting paper is soaked with sterilized distilled water in aseptic condition.
6. After 5-7 days a white cottony growth will come out from the surface of the blocks.

7. The cottony mycelial growth of the pathogen was transferred to PDA slant with the help of inoculating needle.
8. After that teasing process was done of fungal mycelium for histological studies of pathogen.



Observation:

Result and conclusion:

Useful internet links: https://www.youtube.com/watch?v=J5oLHmCLBbo

Practical (P) 13: Field visit for detection and diagnosis of Diseases of field crops

Listed the diseases of Field Crops during field visit–

Diseases of Field Crop			
Sl. No.	**Name of the Disease**	**Causal Organism**	**Symptoms**

Diseases of Field Crop			
Sl. No.	**Name of the Disease**	**Causal Organism**	**Symptoms**

Useful internet links: https://www.youtube.com/watch?v=h6-mAoY-B6k

Practical (P) 14: Field visit for detection and diagnosis of Diseases of Horticultural crops.

Listed the diseases of Horticultural Crops during field visit–

Diseases of Horticultural Crop			
Sl. No.	**Name of the Disease**	**Causal Organism**	**Symptoms**

Diseases of Horticultural Crop			
Sl. No.	**Name of the Disease**	**Causal Organism**	**Symptoms**

Practical (P) 15: Methods for preparation of Herbarium specimens of plant disease sample

A Herbarium is defined as a collection of plants that usually have been dried, pressed, preserved on sheets and arranged according to any accepted system of **classification** for future reference and study.

In fact, it is a great fileting system in Plant Pathology for information about plant disease, symptoms, both primary in the form of actual specimens of the plants, and secondary in the form of published information, pictures and recorded notes.

Materials required: Knife, Scissor, Plant disease sample, Old newspaper or blotting paper, Adhesive glue or cello-tape, Label, Weight for proper pressing

Methods for preparation of a herbarium:

1. **Collection of plant disease sample:** Disease sample should be collected from fields or gardens and packed in polypropylene bag.
2. **Pressing and drying:** The specimens are spread out between the folds of old newspapers or blotting sheets avoiding overlapping of parts. The blotting sheets with plant disease specimen should be placed in the plant press for drying. After 24 to 48 hrs the press is opened. The process should be repeated for proper drying.
3. **Mounting:** The dried specimens are mounted on herbarium sheets of standard size (41 x 29 cm). Mounting is done with die help of glue, adhesive or cello-tape.
4. **Labelling:** A label is pasted or printed on the lower right hand corner. The label should indicate the information about the name of disease, host (common and scientific name), causal organism, date of collection, place of collection and name of collector.

Citrus canker herbarium specimen

Potato late blight herbarium specimen

Rust disease herbarium specimen

Useful internet links: https://youtu.be/xj7KQR6ck5g , https://youtu.be/6MDfQjAOxcs

Practical (16): Collection and preservation of plant disease specimen for herbarium.

Enlist the number of Herbarium of plant disease specimen submitted by the student –