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Research Paper

Study of phylloplane and phytopathogenic microbs

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Abstract
Airborne organisms are mostly trapped by the flattened surface of the leaf. They include fungi, bacteria and algae. Depending upon the major area of the spot and the anatomical study the disease can be identified. In our experiment we collected an infected curry leaves (Murrayakoenigii). The leaf showed white and brown coloured lesions, scattered throughout its surface area.

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I. Introduction

Physical Analysis of Infected leaf:

● Infected leaf is taken and the leaf area is calculated by using a graph paper.Total surface area percentage of leaf covered with lesion was calculated, with graphical interpretation. The total surface area of leaf was calculated, along with surface area of lesion. Nature and colour of the lesion was studied. To measure the area of the infected leaf, we first outlined the leaf on graph paper. The individual lesions were sectioned and surface area of lesion and the percentage area of the infection or the size of the spot are measured.

Microscopic Analysis of lesion:

● Transverse section of the leaf is cut off with the help of pith and the internal structure is studied under light microscope. A section of infected region of **curry leaves** was cut by using a sterile blade and viewed under a light microscope with objective lens of 45x magnification. Spores of the suspected pathogens were observed and morphology of the spore was studied. The dimension of the spores was measured by using ocular micrometer. The microscope was equipped with a scale (ocular lens 100 divisions) that is built into the eyepiece. The focus of the eyepiece was adjusted in order to view a proper sharp scale. Then the ocular scale was calibrated using a device called stage micrometer (2mm with divisions of 0.01mm). After calibration the length and the breadth of the spores were measured and recorded.

EXPERIMENT: STUDY OF PHYLLOPLANE AND PHYTOPATHOGENIC MICROBES

1.MORPHOLOGICAL AND ANATOMICAL DESCRIPTION :

1.1. Theory :

Airborne organisms are mostly trapped by the flattened surface of the leaf. They include fungi, bacteria and algae. Depending upon the major area of the spot and the anatomical study the disease can be identified. In our experiment we collected an infected curry leaves (*Murrayakoenigii*). The leaf showed white and brown coloured lesions, scattered throughout its surface area.

1.2. Procedure :

Physical Analysis of Infected leaf:

● Infected leaf is taken and the leaf area is calculated by using a graph paper.Total surface area percentage of leaf covered with lesion was calculated, with graphical interpretation. The total surface area of leaf was calculated, along with surface area of lesion. Nature and colour of the lesion was studied. To measure the area of the infected leaf, we first outlined the leaf on graph paper. The individual lesions were sectioned and surface area of lesion and the percentage area of the infection or the size of the spot are measured.

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1.3. Results :

The Host used for this experiment is ***Murrayakoenigii*(Curry leaves).**

Physical Analysis of Infected leaf:-

Fig:- Brown spots on curry leaves.

Lesions contained on the leaf surface was studied and its surface area was studied to be as follows:

Whole leaf area	1292 mm^2
Lesion area	17 mm^2
% of leaf infected	1.316 %

Microscopic Analysis of lesion:

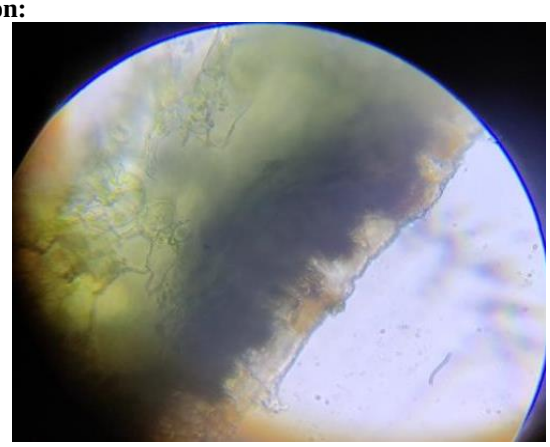

Fig:- Spores under the microscope

he spores were observed under a microscope using a micrometer. The stage micrometer and ocular micrometer were calibrated and the size of the spores was determined.

1 Stage division (SD):- 3 OD (Ocular Division)

1 SD = 10 micrometer

Size of the spore = 0.005 micrometer

1.4.Conclusion:-

Pathogen :*Phyllosticta sp*.

Diseasecaused :Formation of circular leaf spots that are light or dark brown in colour.

2. SPM CONTENT OF THE LEAF:

2.1.Theory :

The leaf surface also traps suspended particulate matter because of proximity to roads having heavy vehicles traffic. The dust particles and sediments can cause mechanical abrasions on the leaf surface, which aids the entry of various pathogens. This particulates are mostly SPM 10 /SPM 2.5. These mechanical abrasion of the leaf surface or sometimes form a thin layer rthe photosynthetic efficiency of the leaves and blocking transpiration. Sometimes they transport bacteria or fungal spores inside the leaves. The SPM measurement can be correlated with the effect of pollutants in disease progression or severity.

2.2.Procedure:

- The sample leaves were taken and weighed individually and noted then each sample leaf was washed in tap water to remove any particulates sticking to the surface.
- The leaves were then air-dried completely, and again weighed individually and noted. The subtracted weight between the pre-washed and post-washed leaf sample is the measurement of SPM of that particular sample and this was done for all the samples.

2.3.Result:

Weight of the sample leaves before washing	0.154g
Weight of the sample leaves after washing and air drying	0.151 g
SPM content as got by subtracting the post-washed plate from the pre-washed plate	0.003 g

3.MOTHER CULTURE AND PURE CULTURE PREPARATION:

3.1.Theory:

Pure culture, in microbiology, a laboratory culture containing a single species of organism. A pure culture is usually derived from a mixed culture (one containing many species) by transferring a small sample into new, sterile growth medium in such a manner as to disperse the individual cells across the medium surface or by thinning the sample many fold before inoculating the new medium.

3.2. Materials Required:

Host plant- Curry leaves, watch glass, solidified agar plates, blade, forceps, 0.1% -mercuric chloride, distilled water, antibiotic- ampicillin.

3.3. Procedure:

Mother Culture:

- A lesion was cut out from an infected Curry leavesusing sterile blade.
- The lesion was first washed in 0.1% HgCl2 solution for 30 seconds then was treated with three separate water washes for one minute each.
- Then the sample was inoculated on a PDA (Potato Dextrose Agar) Plate and incubated for 48 hours. 50 ml PDA plates were made by adding 1.2g of Potato Dextrose Broth (PDB) and 1gm in 50ml distilled water. It was poured in two petri dishes (25 ml in each).
- The colonies of microorganisms from the lesion were studied after incubation and the characters were noted. 50 ml PDA was made and poured in two petri dishes and allowed it to set.

Pure culture:

- The fungal colony obtained from the lesion were isolated in a sterile environment and pure culture of the respective organisms were made on the prepared PDA plates.
- Streaking method was performed for the isolation of pure culture.

Results:
Growth was observed after 3-4 days of incubation.

4. STAINING OF FUNGAL COLONIES:

4.1.Theory:

Lactophenol Cotton Blue:
The lactophenol cotton blue (LPCB) wet mount preparation is the most widely used method of staining and observing fungi and is simple to prepare. The preparation has three components: phenol, which will kill any live organisms; lactic acid which preserves fungal structures, and cotton blue which stains the chitin in the fungal cell walls.

4.2. Materials Required:
Staining of fungal culture: Glass slides, needle, lactophenol cotton blue, spirit lamp, cover slips, oil immersion light microscope.

4.3. Procedure:
For the fungal organism obtained Lactophenol Cotton Blue wet mount preparation was performed and viewed under microscope and characters were noted.

1. Place a drop of 70% alcohol on a microscope slide.
2. Immerse the specimen/material in the drop of alcohol.
3. Add one, or at most two drops of the lactophenol/cotton blue mountant/stain before the alcohol dries out.
4. Holding the coverslip between forefinger and thumb, touch one edge of the drop of mountant with the coverslip edge, and lower gently, avoiding air bubbles. The preparation is now ready for examination.

4.4.Observations:
The spores of the pathogen were observed.

5.COMPLETION OF KOCH'S POSTULATES

5.1. Theory:
According to molecular Koch's Postulates:
1) Specific microorganism must be found in abundance in all diseased organism and not in healthy organisms
2) Microorganisms must be isolated from diseased organisms and grown in pure culture.
3) Cultured microorganisms should cause disease when introduced into healthy organism
4) Microorganisms must be re-isolated from the inoculated diseased host and identified as being identical to the original specific causative agent.
Pure Culture is a culture containing single species of organism and is usually derived from a mixed culture.

5.2. Materials Required:
Petri plate, cotton, needle, spirit lamp, centrifuge tube, inoculation loop, pure culture plates, distilled water, vortex.

5.3. Procedure:
- The inoculation sample of the pathogen were first prepared by taking a loopful of fungal in a centrifugation tube and adding 1ml of distilled water.
- The centrifugation tube is introduced to vortex for 2-3 minutes, for homogenization of the solution.
- Two healthy Curry leaveswere then inoculated by the pure culture of fungal organism separately and incubated for 5-7 days.
- After incubation it was observed that whether the inoculated leaves developed similar lesions as compared to the initial infected leaf taken.
- A control was also made where the leaf was incubated only with distilled water.

5.4. Observation:
No infection was observed in a healthy leaf inoculated with the pure culture of the pathogen.

5.5.Results:
Results revealed the successful completion of Koch's Postulates.