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Practical Manual

B.Sc. (Hons) Agriculture (V Semester)

PRINCIPLES OF INTEGRATED PEST AND DISEASE MANAGEMENT

Ent- 312 3(2+1)

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2022

Syllabus: Principles of Integrated pest and Disease Management (Ent-312)
Methods of diagnosis and detection of various insect pests, and plant diseases. Methods of insect pests and plant disease measurement. Assessment of crop yield losses. Calculations based on economics of IPM. Identification of biocontrol agents, different predators and natural enemies. Mass multiplication of *Trichoderma*, *Pseudomona*s, *Trichogramma*, NPV, etc. Identification and nature of damage of important insect pests and diseases and their management. Crop (agro-ecosystem) dynamics of a selected insect pest and diseases. Plan & assess preventive strategies (IPM module) and decision making crop monitoring attacked by insect pest and diseases. Awareness campaign at farmer's fields.
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CONTENTS

Experiment No. 1

Objective: To study the methods of diagnosis and detection of various insect pests

- Observe the insect damaging symptoms in the field

Defoliators……

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Leaf folder…..……..……

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Leaf roller……..……

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Leaf miners (Burrowers) ……

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Gall making insects……..……

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The Wood/Phloem borers..…….

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Root borers……..……...……

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Pod borers ……..……

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Stem borers..……..…….**.....**

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Sap feeders..……..…….**..** ……..

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Pollen feeders.……..…… ……

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Experiment No. 2

Objective: To study about the methods of Insect Pest Management

Principles: ……..…….....
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Components of IPM:

Monitoring: ……..…….
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Cultural control: ……..……
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Physical control: ……..……..……..
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Mechanical control: ……..……
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Biological control: ……..……

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Legislative control: ……..…….

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Chemical control: ……..……

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Host Plant Resistance: ……..……

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Botanicals: ……..……..……

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History of IPM……..……

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Write the Advantages of Integrated Pest Management

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Experiment No. 3

Objective: To learn the methods of assessment of crop yield increase and losses

Procedure:

- Step 1: Observe the yield of treated and untreated plot kg/quadrate from each quadrate.
- Step 2: Convert the yield obtained from each quadrate in hectare and perform statistical analysis. The percent increase in yield over control was calculated by using following formula:

Yield increase over control = T - C /C X 100
Where, T = Yield of protected plot (kg/ha) C = Yield of unprotected plot (kg/ha)
The percentage avoidable loss in yield due to sucking pests was calculated as per the formula given by (Khosla, 1977).
Percentage avoidable loss = T - C /T X 100
Where, T = Yield in the protected plot (kg/ha) C = Yield in the unprotected plot (kg/ha)

Experiment No. 4

Objective: To learn the calculations based on economics of IPM

Economic Injury Level (EIL):……..…

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Economic threshold Level (ETL):……

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EIL = C/VIDK, where
C = cost of control
V = value of the commodity
I = injury (e.g. defoliation or damaged fruit) based on a given density of insects
D = economic damage (e.g. bushels lost or quality discount) caused by a given level of injury
K = proportion of reduction in injury resulting from a control measure (usually the proportion of the insect population that is killed, or "percent control")

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Experiment No. 5

Objective: To identify the biocontrol agents, predators and natural enemies

Required Materials:.....

Write down the important characters of identification

Insect Predators

Lady bird beetle:…….....……...

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Green Lacewing:…..……..…….…

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Praying mantis:……..……..

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Syrphid fly:…..……..…….……

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Predatory Bug:……..……

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Insect Parasitoids; Order: Diptera

Family: Tachinidae:..……..…….

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Family: Sarcophagidae:……..……

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Order: Hymenoptera;

Family: Trichogrammatidae: ……..……

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Family: Braconidae: ...……..……..

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Family: Chalcididae: ……..……

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Family: Ichneumonidae: ……..……..

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Family: Bethylidae:..……..……

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Family: Eulophidae:……..……

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Experiment No. 6

Objective: To study the crop dynamics of *Helicoverpa armigera* on chickpea

Observations:

- Select 10 plants randomly in each plot in the field and tag them
- Record the number of insect population on each selected plant at weekly interval after incidence.

Sl. No.	Ist week	IInd week	IIIrd week	IVth week	Mean number of insect population
1.					
2.					
3.					
4.					
5.					
6.					
7.					
8.					
9.					
10.					

Experiment No. 7

Objective: To assess the preventive strategies and decision-making crop monitoring attacked by insect pest and diseases.

The **8 recommended steps:** Identify the Pest(s), Determine the Pest, Beneficial and Crop Dynamics, Plan Preventative Strategies, Monitor the Pests, Decision on Need to Control, Select Optimal Pest Control Tactics, Implement and Evaluate

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Experiment No. 8

Objective: To learn about the mass multiplication of *Trichogramma* sp.

Material Required:

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Procedure:……..……

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Experiment No. 9

Objective: To learn about the mass multiplication of NPV

Material Required:

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Procedure:

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Experiment No. 10

Objective: To study the identification and dry preservation of plant diseased specimens

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

a. Host plant (name of the diseased plant)
b. Name of the pathogen (organism causing the disease)
c. Place where collected
d. Place of collection
e. Date of collection
f. 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. 11

Objective: To study the 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. 12

Objective: To study the method of detection and diagnosis of plant diseases

Step 1: To study different types of disease symptoms produced by pathogen

Blights: ……

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Leaf spots:……

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Chlorosis:……

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Canker:……

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Anthracnose:……

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Damping off:……

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Downy mildew:……..……..…….

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Powdery mildew:……..……..…….

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Wilt:……..…….

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Leaf curl:……..

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Leaf distortion:……..……..

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Phyllody:……..……..

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Mottle:……..……..

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Yellows:……...……...

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Step 2: Preparation of temporary mounts (slides).

Materials Required:

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Procedure: ……..……..……..

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Preparation of Fungal Stain:

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Use of Stain: .....……

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Precautionary Measures:

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Experiment No. 13

Objective: To learn the different techniques for the isolation of plant pathogens

Step 1: Preparation of Potato Dextrose Agar media for growth of pathogens

Materials Required:

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Procedure:……

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Precautionary Measures:

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Step 2: Isolation of plant pathogens from rizosphere zoneof soil, seed and affected plant parts

Materials Required:

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Procedure for isolation of fungi from diseased leaves:…….

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Procedure for isolation of bacteria from infected leaves:……

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Procedure for isolation of pathogen from stems, fruits, seeds, and other aerial plant parts:……

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Procedure for isolation of pathogen from soil:……

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Precautionary measures:……

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Step 3: Koch's postulates for estimating pathogenicity

Material Required:

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Steps for Koch Postulates:……..…..

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Precautionary Measures: ……

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Experiment No. 14

Objective: To learn the preparation of fungicide formulation

Exercise 1: Preparation of 1% Bordeaux Paste and Bordeaux Mixture

Materials Required:

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Procedure:……

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Precautionary Measures: ……

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Exercise 2: Cheshunt compound Preparation.

Materials Required:

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Procedure:……

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Precautionary Measures: ……

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Exercise 3: Preparation of Chaubattia Paste.

Materials Required:

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Procedure:……

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Precautionary Measures: ……

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Exercise 3: Calculate the amount of Indofil M-45 (50WP) @0.2% is required to spray 1 hectare area.

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Results:……..…

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Exercise 3: Calculate amount of Thiram @0.2% is required for the seed treatment of 8 kg seeds.

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Results:……..…

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Calculations: ……

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Experiment No. 15

Objective: To learn the assessment of disease and yield loss

Exercise 1: Calculate disease incidence and disease severity of Alternaria blight of mustard.

Disease incidence:

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Number of infected plants: ……

Total number of plants: ……..

Results: ……..

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Disease severity:…….……

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Disease severity

Disease grade	Total number of samples	Total rating

Results:……

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Yield loss: ……

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Calculations:……

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Experiment No. 16

Objective: To learn about the mass multiplication of *Trichoderma*

Materials Required:

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Procedure:……

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Precautions:

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Experiment No. 17

Objective: To learn about the mass multiplication of *Pseudomonas*

Materials Required:

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Procedure:……

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Precautions:……

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STEPS IN DIAGNOSIS OF INSECT DAMAGE

I. Define the problem

A. **Plant identification and characteristics:** Establish what the normal plant would look like at this time of year. Describe the abnormality, symptoms and sign

B. **Examine the entire plant and its community:** Determine the primary problem and part of the plant where initial damage occurred.

II. Look for patterns

A. Non–uniform damage pattern (Scattered damage on one or only a few plant species) is indicative of living factors (Pathogens, insects etc.)

B. Uniform damage pattern Over a large area and uniform pattern on the individual plant and plant parts indicates non living factors (Mechanical, Physical and Chemical factors)

III. Development of Damage pattern

A. Progressive spread of the damage on a plant, onto other plants, or over an area with time indicates damage caused by living organisms.

B. Damage occurs, does not spread to other plants or parts of the affected plant. Clear line of demarcation between damaged and undamaged tissues. These clues indicate nonliving factors.

IV. Determine the cause of the plant damage

A. Distinguish among living factors

- Symptoms and signs of Pathogens
- Symptoms of signs of Insects, Mites and other Animals.

B. Distinguish among nonliving factors

- Mechanical Factors
- Physical Factors
- Chemical Factors

CROP LOSS ASSESSMENT

Crop loss can be summarized as the difference between the attainable yield from the healthy crop and that obtained from the diseased crop and is expressed as percentage mostly in terms of money. The most important purpose of disease appraisal is the assessment of crop loss. Crop loss can be summarized as the difference between the attainable yield from the healthy crop and that obtained from the diseased crop and is expressed as percentage mostly in terms of money.

- The most important purpose of disease appraisal is the assessment of crop loss.
- Various attempts have been made to utilize disease assessment data for estimation of loss.
- However, such conversion is not easy.
- There is no straight forward way to determine the amount of yield loss.
- While calculating the yield loss from a disease, its nature, extent of damage in terms of yield, quality and loss of market value are to be considered.
- Diseases like smuts, root rots, ergot etc. cause almost 100 per cent damage to the crop and the loss estimates are rather easy to make.
- However, those causing damage to the foliage and other debilitating diseases, thereby affecting the yield partially to different extents pose a great difficulty in assessing losses.
- Sometimes, the crop stage, when the crop is attacked becomes critical in this respect.

ESTIMATION OF YIELD LOSS

- For estimating yield loss due to diseases, comparisons between crops grown in different years or localities are not reliable as other factors are not the same.
- For valid comparisons, disease free plots are to be compared with those nearby with varying amount of disease.
- Disease free plots are mostly obtained by use of fungicides with little or no phytotoxicity.
- If the yield loss is to be estimated on a regional basis, data on disease incidence obtained from the disease surveys would be utilized using formulae based on fungicide trials.
- These data are usually employed in the ‘critical point’ models which are actually the regression equations.
- ‘Multiple point’ models in which the loss estimation is based on many diseases appear to be more reliable.
- These data are used to produce a multi-dimensional model whose dimensions include the date of disease onset, shape of the disease progress curve, the host cultivar and the yield loss as the dependent variables.

ECONOMICS OF IPM

Successful IPM programs have produced many benefits. These include (i) lower production costs (at farm level), (ii) enormous savings for governments from reduced pesticide imports and subsidies for pesticide use, (iii) reduced environmental pollution, particularly improved soil and water quality, (iv) reduced farmer and consumer risks from pesticide poisoning and related hazards, and (v) ecological sustainability by conserving natural enemy species, biodiversity, and genetic diversity.

The acceptability of a farming practice is primarily determined by short-term profitability. IPM attempts to integrate available pest control methods to achieve a farmer's most effective, economical, and sustainable combination for a particular local situation.

Economic Injury Level (EIL). The smallest number of insects (amount of injury) that will cause yield losses equal to the insect management costs.

Economic threshold Level (ETL). The pest density at which management action should be taken to prevent an increasing pest population from reaching the economic injury level."

The goal of the economic threshold is to prevent a pest population from reaching the point where its damage causes monetary losses that are equal to the cost of control. This "break-even" point is referred to as an economic injury level, and is computed using the following formula:

EIL = C/VIDK, where

C = cost of control

V = value of the commodity

I = injury (e.g. defoliation or damaged fruit) based on a given density of insects

D = economic damage (e.g. bushels lost or quality discount) caused by a given level of injury

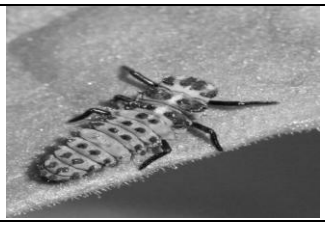
K = proportion of reduction in injury resulting from a control measure (usually the proportion of the insect population that is killed, or "percent control")

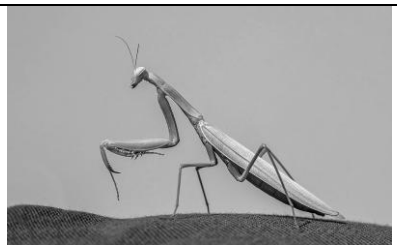
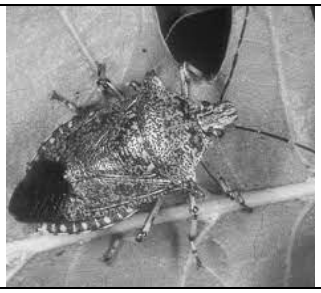
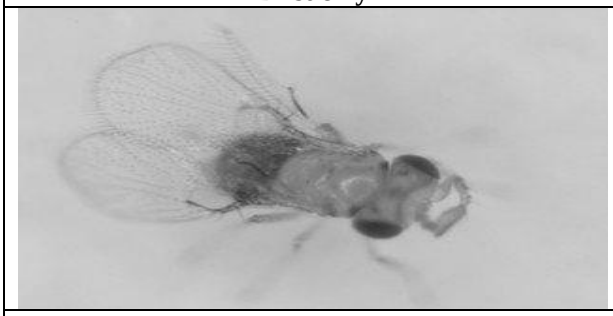
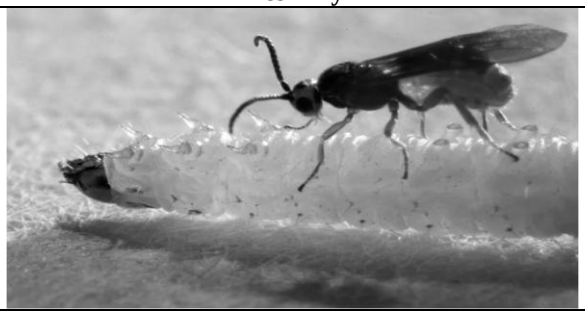
These variables are discussed in detail in a variety of research papers, including a thorough review (Pedigo, 1986).

The cost of control and the value of the commodity are (usually) pretty straightforward, while the remaining values must be determined for individual pest species through applied research. Let's consider a hypothetical example: an imaginary species of beetle that feeds directly on apples. In this case, you can expect 5 apples to have feeding injury for every beetle that you find per tree (I = 5 damaged apples per beetle). Each injured fruit means a loss of 0.5 pounds of apples (D = 0.5 pounds per damaged apple). You can sell a pound of apples for $1.50 (V = $1.50 per pound) at the local farmer's market. An insecticide that costs $20 to apply to a tree (C = $20 per tree) will reduce the damage by 90% (K = 0.9). In this scenario, you would calculate the EIL as:

$$EIL = \frac{20}{(1.50) \times (5) \times (0.5) \times (0.9)} = \mathbf{5.9\ beetles\ per\ tree}$$

BIOCONTROL AGENTS

INSECT PREDATORS	
	
Green Lacewings Larvae & Adult	
	
Lady Bird Beetle Grub & Adult	

<table>
<tr><td>  </td><td>  </td></tr>
<tr><td>Preying Mantid</td><td>Predator Bug</td></tr>
<tr><td>  </td><td>  </td></tr>
<tr><td colspan="2">Syrphid fly Larvae & Adult</td></tr>
<tr><td colspan="2">INSECT PARASITOIDS</td></tr>
<tr><td>  </td><td>  </td></tr>
<tr><td>Bristle fly</td><td>Flesh Fly</td></tr>
<tr><td>  </td><td>  </td></tr>
<tr><td>Trichogramma chilonis</td><td>Bracon brevicornis</td></tr>
<tr><td>  </td><td>  </td></tr>
<tr><td>Xanthopimpla punctata</td><td>Elasmus nephantidis</td></tr>
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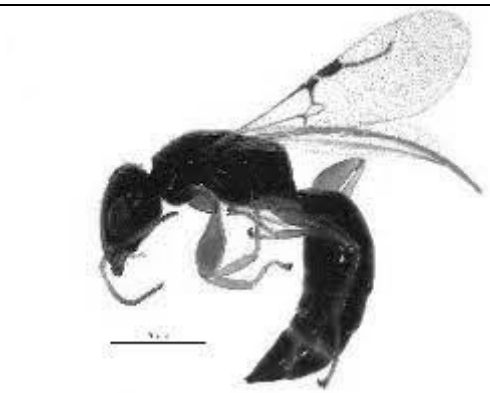
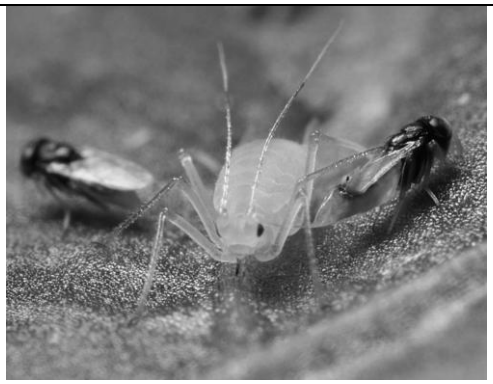
	
Goniozus nephantidis	***Apelinus mali***

MASS PRODUCTION TECHNIQUE OF *TRICHOGRAMMA* SPP.

Materials required

- Sterilized sorghum
- Corcyra rearing boxes/trays/jars made up of plastic or wood with lid provided with wire mesh for aeration
- Corcyra egg laying cage
- Black cloth
- Mosquito net
- Table
- Racks for placing Corcyra cages
- Honey
- Glycerin
- Tubes for collecting Corcyra moth
- Measuring cylinder
- Plastic tubs for egg laying purpose
- Brush
- Roasted ground nut powder - 100 g
- Yeast - 5 g
- Wettable sulphur - 5 g
- Streptomycin sulphate - 0.05 g

Preparation of egg laying cage of *Corcyra cepholonica*: Take a plastic bucket with lid. Cut the lid in circular shape leaving space for providing /fixing wire mesh for egg laying purpose in the (circular wire mesh). Make a hole on the centre of bottom of the plastic bucket to pour the collected adults in the bucket. Keep bucket inverted in the plastic tub for egg laying purposes.

Steps for production of *Corcyra cephalonica*

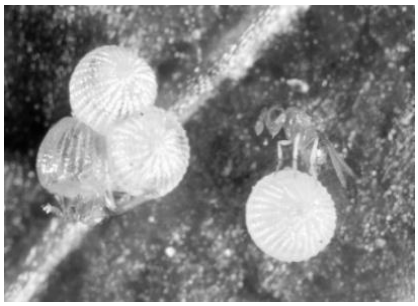
- Sterilize the rearing boxes (if wooden) in hot air oven for 100 degree centigrade for 30 minutes
- If plastic trays are used, wash them before use
- Dry broken grains of jowar in sunlight properly
- Pour sterilized grain - 2.5 kg/box/tray
- Add 100 g of roasted ground nut powder, 5 g of yeast, 5 g of wettable sulphur, 0.05g of streptomycin sulphate in each box or tray
- Mix well all ingredients
- Sprinkle 1 cubic centimeter of *Corcyra* eggs/box/tray on the top of mixture (culture medium)
- Cover the box with lid, label the date of inoculation
- Keep these boxes in racks protected by ant pans
- Favourable temperature for rearing is 28+/-2 degree centigrade and Relative humidity, 75% +/- 5%
- The moth starts emerging on 40th day
- Bring the boxes ready for moth emergence and collect moths inside the net by glass tubes
- Transfer the moths to egg laying chamber
- Provide cotton soaked 20% honey+ vitamin E solution as adult food in the egg laying chamber
- Collect the eggs daily
- Pour the eggs in a paper by tilting slightly downward so eggs come down side where as dust particles remain in upper side
- Clean the eggs further by passing through different size sieves to 10, 15 and 40 meshes
- Discard the moth after 4 days
- Utilize the *Corcyra* eggs for *Trichogramma* production (or) host culture or store them in refrigerator at 10 degree centigrade for 7 days, if required.

MASS PRODUCTION OF *TRICHOGRAMMA*

Materials required

- *Corcyra* eggs
- Nucleus culture of *Trichogramma*
- Polythene bags
- Rubber bands
- Scissors
- Gum
- Brush
- Tea strainer
- Tricho cards
- 50% honey solution
- Stapler
- Refrigerator
- UV lamp

Methodology



Clean fresh *Corcyra* eggs by passing through 15, 30 and 45 mesh sieves. Prepare "Trichocard" by cutting card board sheet to the size of 10 x10 cm which can accommodate 1 cc of eggs. Apply gum on the card and sprinkle the cleaned eggs uniformly. Remove the excess eggs from the cards by using brush. Allow the card for shade drying for 30 minutes. Treat the eggs under UV lamp for 30 minutes. Take polythene bag, insert UV treated "Trichocard" and nucleus card at the ratio of 6:1 (6 *Corcyra* egg cards: 1 *Trichogramma* nucleus card) and provide 50% honey vitamin E in a soaked cotton swab. Remove the Tricho cards after 2 days *Corcyra* eggs changes black colour on 3rd day indicates the parasitization of eggs. Release the parasitized egg cards immediately in the fields (or) store them in refrigerator at 10 degree centigrade up to 21 days. Place/tie/staple parasitized cards on leaf sheath of plant.

MASS PRODUCTION TECHNIQUE OF NPV

Mass production of NPV of *Spodoptera litura*

S. litura can be mass cultured using the natural diet, castor leaves under laboratory condition in plastic buckets. The steps involved in the production of NPV are as follows:

- Pre starve 4th instar larva-overnight
- Prepare virus suspension containing 108POB/ml in water containing 0.1% teepol
- Dip clean castor leaves in virus suspension and shade dry
- Allow the caterpillar to feed for two days and subsequently on untreated leaves
- Collect the diseased larvae in distilled water
- Allow to putrefy for 5days
- Polyhydra settles at bottom as white layer
- Sediment contain POB
- Suspend in distilled water
- Centrifuge for 1 min at 500RPM
- Supernatant containing POBs
- Centrifuge at 2500RPM for 15 min
- Collect pellet (POB's)
- Resuspend in distilled water
- Repeat differential centrifugation
- Pure POB's
- The dose of virus is expressed as larval equivalent (LE)

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

TEMPORARY MOUNTS AND STAIN

Procedure:

1. First prepare a clean glass slide and cover slip and place a drop of water on the slide.
2. Add the specimen to the drop of water. The specimen is then, properly aligned on the slide with dissecting needles. In many cases, specimens must be torn and teased apart with needles.
3. The cover slip is then, placed on top of the preparation. This is done by placing one edge of the cover slip on the glass slide in contact with the drop of water. Using the tip of a dissecting needle, gently lower the cover slip into position. If this procedure is done correctly, the mount should be free of air bubbles.

Fungal Stain:

Lactophenol Cotton Blue: It is used as a general-purpose staining and mounting agent for the staining of the fungal structures. It has the following constituents:

Phenol (pure crystals)	-	20 gm
Lactic acid	-	20 gm
Glycerine	-	40 gm
Water	-	20 ml.
Cotton Blue	-	In traces (0.5%)

Mounting Agent:

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

Use of Stain:

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

Precautionary Measures:

1. The most common error in making temporary mounts occurs from using too much on too thick material on the slide. Only very thin objects can be studied with the compound microscope.
2. The cover slip must lie flat.
3. The specimen and area under the cover slip must be flooded with the mounting medium. Avoid the presence of water on the rest of the slide or top of the cover slip.

PREPARATION OF POTATO DEXTROSE AGAR MEDIUM

Materials Required: For the preparation of 1 liter of potato dextrose agar medium the following ingredients in different quantities are used

(i) Peeled potato slices	-	200g
(ii) Dextrose	-	20g
(iii) Agar- agar	-	20g
(iv) Distilled water	-	1000 ml

Method:

(1) Potato slices are cooked in 500 ml of water.
(2) Then filtered with the help of muslin cloth.
(3) Agar-agar is melted in 500 ml of water.
(4) Potato juice is added to the melted agar.
(5) Volume is made 1000 ml by adding required water.
(6) Again, lit is filtered through muslin cloth.
(7) Dextrose is added in this mixture and shaken well.
(8) Medium is sterilized in an autoclave at 1.1kg/cm2 pressure for 20 minutes at temperature of 121.6°C.

ISOLATION OF PATHOGEN

From Leaves

i) Fungi

1. Cut across several small sections of 5 to 10 mm square size from the margin of the infected lesion so that they contain both diseased and healthy-looking tissue.
2. Place them in 1% sodium hypochlorite or 0.1 % mercuric chloride solution, for about 15 to 30 seconds, and the sections are then washed in three changes of sterile water; blotted dry on clean sterile blotter paper, and are finally placed on the medium pored Petri plate, usually three to five bits per petri dish aseptically.
3. Incubate the inoculated petri dishes at 25±1˚C for 3-5 days, mycelium growth is seen. The spore can be observed under microscope.

ii) Bacteria: The serial dilution method is often used to isolate pathogenic bacteria from diseased tissues contaminated with other bacteria. After surface sterilization of sections of diseased tissues from the margin of the infection, the sections are ground aseptically but thoroughly in a small volume of sterile water and then part of the homogenate is diluted serially in equal volumes or 10 times the volume of the initial water. Finally, plates containing nutrient agar are streaked with a needle or loop dipped in each of the different serial dilutions, and single colonies of the pathogenic bacterium are obtained from the higher dilutions that still contain bacteria.

From soil: The method involves the transfer of microbial propagules from soil to the culture medium with the aim of obtaining pure culture. Different methods of isolation from soil are as follows:

a) Soil-Dilution Plate Method: Place 10g of soil in a sterile flask with 90 ml sterile water and stir with a magnetic stirrer for 20 to 30 min. to this solution, withdraw 10ml soil water and add to 90 ml of sterile water in a sterile bottle. Shake for 1min and transfer 10ml of the suspension to another 90 ml sterile water. Repeat the process until the desired dilution is obtained. A proper dilution allows 50 to 150 colonies per culture plate. In general, suitable dilutions are 10-3 to 10-4 for actinomycetes, 10-5 to 10-6 for bacteria and 10-4 to 10-5 for fungi. Spread 1ml of a dilution on a suitable agar surface by an inclined rotary motion of the plate.

b) Soil-Plate Method: Suitable small amounts of soil (0.005g to 0.15g) are taken from the mean soil sample using a sterile needle with a flattened tip. Each small, sub-sample is placed in a drop of sterile water in a

Petridish and dispersed. Eight to ten ml of molten agar medium are added to each Petridish, the dish being rotated gently to allow disperion of the soil particle in the nutrient medium.

c) Immersion-tube Method: Introduction of glass tube, with 4-6 spirally arranged invaginated capillaries filled with agar into soil. After 7-14 days, the tube is removed and fungi are isolated by removing the soil core and then cutting it into portions, which are plated out.

d) Isolation by Baiting: A variety of micro-organisms may be isolated from the soil by the use of some preferential substrate. The desired organism is able to develop on the bait to the exclusion of other organisms, thus allowing isolation by ordinary method. Agro-bacterium tumefaciens and Thielaviopsis basicola are isolated from soil by the use of carrot discs. Rhizoctonia with cellophane and a number of watermolds are isolated by the addition of boiled hemp seed to pond water or soil water mixtures.

From stems, fruits, seeds, and other aerial plant parts: Almost all the methods described for isolating fungal and bacterial pathogens from leaves can also be used to isolate these plant pathogens from superficial infections of stems, fruits, seeds, and other aerial plant parts. Entire seeds can be plated. In addition to these methods, however, plant pathogens can often be isolated easily from infected stems and fruits in which they have penetrated fairly deeply. This is accomplished by splitting the stem or breaking the fruit from the healthy side first and then tearing it apart toward and past the infected margin, thus exposing tissues not previously exposed to contaminants and not touched by hand or knife and therefore not contaminated. Small sections of tissue can be cut from the freshly exposed area of the advancing margin of the infection with a flamed scalpel and can be plated directly on the culture medium.

KOCH's POSTULATES

Robert Koch was the first to show in 1876 that anthrax a disease of sheep and other animals, including humans, was caused by a bacterium that he called as *Bacillus anthracis*. Based on the studies related to anthrax, he set out the four steps that must be satisfied before a micro-organism isolated from a diseased human, animal or plant can be considered as the cause of the disease.

Four steps of Koch Postulates:

1. The suspected causal agent must be present in every diseased organism examined.
2. The suspected causal agent must be isolated from the diseased host organism and grown in pure culture.
3. When a pure culture of the suspected causal agent is inoculated into a healthy susceptible host, the host must reproduce the specific disease.

E. F. Smith amended these postulates and put forth the fourth postulate.

4. The same causal organism must be recovered again from the experimentally inoculated and infected host i.e., the recovered agent must have the same characteristics as the organism in step 2.

FUNGICIDE FORMULATION

There are 6 formulations so far available in the market:

1. **Wettable powder:** It is a very common formulation for most of the fungicides, which is used for spray mixtures. The modern wettable powders are water-dispersible which have the quality to wet easily and disperse well in water. They are also called as Water-Dispersible Powders (WDP). The active ingredient is incorporated, usually at the rate of 30-80%, with a finely ground inert dust (filler) such as Kaolin, a wetting agent and a suspending agent.
2. **Dust formulations:** It is usually containing 1-10% active ingredient for direct application in dry forms. They are manufactured in such a way that they are light enough to be carried by a slight breeze for a considerable distance. The finely divided particle of active ingredient is carried on a carrier particle. The commonly used carriers (diluents) are attapulgite, kaolin, talc, pyrophylite, diatomaceous earth, bentonite, calcium silicate, hydrated silica, calcium carbonate, magnesium carbonate, gypsum, lime etc.
3. **Water dispersible Powders (WDP):** The active ingredient is incorporated, usually at the rate of 30-80%, with a finely ground inert dust (filler) such as Kaolin, a wetting agent and a suspending agent. The commonly used suspending agents are sodium lignin sulphonate (Sulphite dye), methyl celluloses, polyvinyl acetate and aluminium silicate. In addition, spreader-sticker is sometimes desirable, especially on plants with glossy or waxy leaves. Agitation is generally necessary to keep uniform suspension.
4. **Granules (Pellets):** Pellets are the formulations of the fungicide with inert materials formed into particles about the size of coarse sugar. The granules normally contain 3-10% of the active ingredient. Due to their size, the granules do not drift but have limited application being confined to soil and seed treatments. Granules have the advantage they can be measured in dry form more easily and accurately than dusts or wettable powders. These are formulation in which a dry form of the active ingredient is mixed with a liquid. Such formulations usually contain a high percentage of active ingredient similar to wettable powders. They

are mixed with water for final use and require agitation. These are mostly used as seed dressers in seed processing companies.

5. **Solutions:** True solutions are formulations in which active ingredient or a combination of active ingredients and a solvent is dissolved in water solutions. This has the advantage of requiring no agitation after formulation is added in water.
6. **Suspension or slurries:** These are formulation in which a dry form of the active ingredient is mixed with a liquid. Such formulations usually contain a high percentage of active ingredient similar to wettable powders. They are mixed with water for final use and require agitation. These are mostly used as seed dressers in seed processing companies.

Preparation of fungicidal solutions

Bordeaux mixture: One kg of copper sulphate is powdered and dissolved in 50 litres of water. Similarly, 1 kg of lime is powdered and dissolved in another 50 litres of water. Then copper sulphate solution is slowly added to lime solution with constant stirring or alternatively, both the solutions may be poured simultaneously to a third contained and mixed well.

Merits:

1.Its natural tenacity to the plants
2.Its relative cheapness
3.Its utility in controlling wide variety of diseases
4.Somewhat non-toxic to human beings and cattle

Demerits:

1.Its phytotoxic nature on certain plants like paddy, apples, peaches etc.
2.It causes delay in ripening of fruits
3.The preparation is not very much practicable under field conditions
4.It's corroding action on metallic containers of spraying equipment.

It is very much useful against a number of diseases like downy mildews, bacterial citrus canker etc.

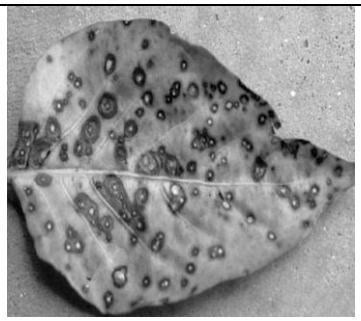
Bordeaux paste: Bordeaux Paste consists of same constituents as that of Bordeaux mixture, but it is in the form of a paste as the quantity of water used is too little. It is nothing but 10 per cent Bordeaux mixture and is prepared by mixing 1 kg of copper sulphate and 1 kg of lime in 10 litres of water. The method of mixing solution is similar to that of Bordeaux mixture. It is a wound dresser and used to protect the wounded portions, cut ends of trees etc., against the infection by fungal pathogens.

Burgundy mixture: It is prepared in the same way as Bordeaux mixture, except the lime is substituted by sodium carbonate. So, it is called as 'Soda Bordeaux'. It was developed Burgundy (France) in 1887 by Mason. The usual formula contains 1 kg of copper sulphate and 1 kg of sodium carbonate in 100 litres of water. It is a good substitute for Bordeaux mixture and used in copper-sensitive crops.

Cheshunt compound: It is compound usually prepared by mixing 2 parts of copper sulphate and 11 parts of ammonium carbonate. This formula was suggested by Bewley in the year 1921. The two salts are well powdered, mixed thoroughly and stored in a air tight container for 24 hours before being used. The ripened mixture is used by dissolving it in water at the rate of 3 g/litre. The mixture is dissolved initially in a little hot water and volume is made up with cold water and used for spraying.

Chaubattia Paste: It is another wound dressing fungicide developed by Singh in 1942 at Government Fruit Research Station, Chaubattia in the Almora district of Uttar Pradesh. It is usually prepared in glass containers or chinaware pot, by mixing 800g of copper carbonate and 800g of red lead in litre of raw linseed oil or lanolin. This paste is usually applied to pruned parts of apple, pear and peaches to control several diseases. The paste has the added advantage that it is not easily washed away by rain water.

DIFFERENT TYPES OF DISEASE SYMPTOMS PRODUCED BY PATHOGEN

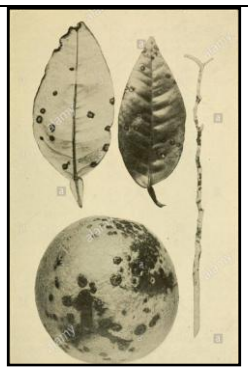


Leaf spot

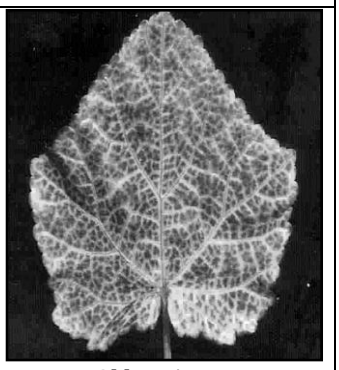
Leaf blight

Anthracnose

Damping off

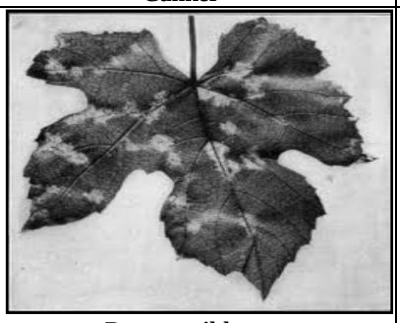


Canker



Chlorosis

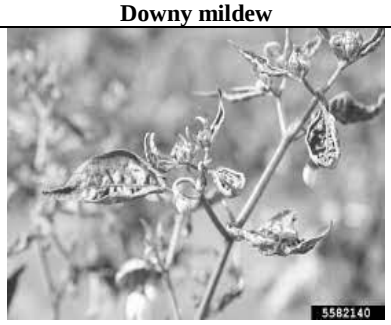
Vein banding



Downy mildew

Powdery mildew

Leaf distortion

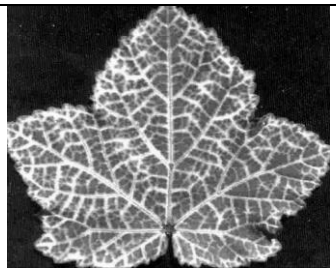


Leaf curl

Wilt

Phyllody

Mottle

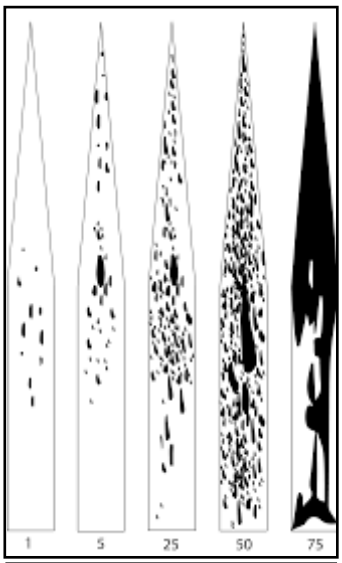


Vein clearing

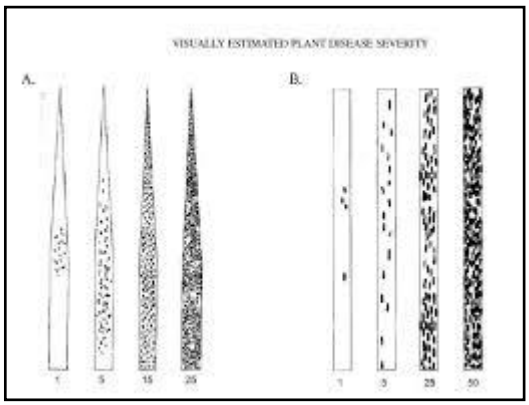
DISEASE ASSESSMENT

Plant disease assessment, or phytopathometry (Large, 1966), involves the measurement and quantification of plant disease and is therefore of fundamental importance in the study and analysis of plant disease epidemics. Disease can be measured using direct methods (i.e. assessing disease in or on plant material) or indirect methods (e.g. monitoring the spore population using spore traps). Direct quantitative methods are largely concerned with measurements of incidence or severity, defined as follows.

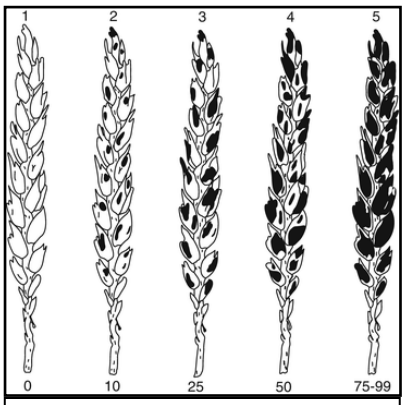
Examples of pictorial assessment keys for estimating disease severity



Yellow rust of wheat



Leaf and Stem rust of cereals



Septoria blotch of wheat

Disease severity= (area of diseased tissue /total tissue area) x 100

$$\text{Disease index} = \frac{0(X_0) + 1(X_1) + 2(X_2) + \ldots\ldots + n(X_n)}{(X_0+X_1+X_2+\ldots\ldots+X_n) \times (\text{maximum grade})} \times 100$$

Where,

X: No. of entries within the grade

0-n: Grades of disease as per disease rating scale

Yield losses = control yield-treatment yield/ control yield *100