Laboratory Manual

Course- Principles of Integrated Pest and Disease Management

Course Code- 13A.311

B.Sc. (Hons.) Agriculture, Vth Semester

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Experiment 1: Methods of diagnosis and detection of various insect pests, and plant diseases

A. Methods of diagnosis and detection of various insect pests

Symptoms of crop damage caused by different phytophagous insects based on their different types of mouth parts

Crop plants suffered most damage from different phytophagous insects is a result of direct feeding on above-ground and below-ground plant parts as they utilize the plants to secure food and derive their nutrition or as a shelter. Almost all portions of plant viz., roots, stem, bark, leaves, buds, flowers and fruits are attacked by these insects which causes economic losses to the farmers. The type of feeding injury or damage caused by insect pests is related to the type of mouthparts of the insect. The mechanism of mouth parts and type of feeding determine to a larger extent the pest management strategies including the type of pesticide to be used. The nature and symptoms of damage caused by phytophagous insects based on their feeding habits according to the modification of their mouthparts is described below.

I. Biting and Chewing type: This type of mouth parts is supposed to be the most primitive type as the other types are believed to be evolved from biting and chewing type of mouth parts. They are adapted for biting and chewing of the plant material. Biting and chewing pests bite into and chew the leaves, stems, buds, flowers, and even the roots of plants. Some examples are grasshoppers, cutworms, caterpillars, beetles etc. Based on the symptoms of damage due to feeding of different plant parts, chewing insects can be classified into different groups as mentioned below.

1. Stem borers: Larvae bore the stem and feed on internal contents. As a result, damaged part is cut off from the main plant and affected part wilts, dries up and exhibits symptoms like dead heart during vegetative stage and white ear heads during reproductive stage in case of paddy due to larval feeding inside the stem and they can be easily pulled out and bunchy top in case of sugarcane (destruction of growing point results in the activation of side buds, just below the growing point and produces a bunch of side shoots called bunchy top). Example- stem borers of paddy, millets, sugarcane and brinjal.

2. Shoot borers: Larvae infest and feed tender shoots and bore inside during vegetative stage of crop growth and cause wilting, drooping of terminal plant parts which later dries up. Example- shoot borers of brinjal, okra, cotton and castor.

3. Defoliators/Skeletonizers: Larvae feed on the leaves completely leaving only midrib/veins or scrape the chlorophyll content of leaves or cause numerous holes. Example- castor semilooper, ash weevils, tobacco caterpillar, epilachna beetle on brinjal.

4. Leaf miners: Larvae mine leaves/leaflets between the epidermal layers and feed on greenish matter, resulting in the appearance of translucent mines/white patches/zig-zag galleries. Example- leaf miners of citrus, cashew and rice hispa.

5. Leaf webbers: Larvae web leaves/ leaflets by means of silken threads and feed on the chlorophyll content by remaining within the web. Often faecal pellets/frass are found within the web. Example- leaf webbers on gingelly, ground nut, sapota, mango and cashew shoot webber.

6. Leaf folders: Larvae fold leaves from tip to base/longitudinally/margin to margin there by giving appearance of a fold/roll. Example- rice leaf folder, cotton leaf folder.

7. Gall makers: Larvae feeding inside the stem/tiller/leaf/flower bud stimulates excessive growth of cells at the affected portion and distorts normal growth. It results in malformation of plant parts, exhibiting gall formation and provides shelter to the pest. Example- paddy gall midge, tobacco stem borer, cotton stem weevil etc.

8. Pod/capsule borers/boll worms: During the reproductive stage of the crop larvae enter in to the pods, capsules and feed on the seeds/lint exhibiting symptoms like webbed condition of pods /bolls or web few pods/capsules with frass and excreta or holes of different sizes and shapes/damaged tissues (chilli/lint on Cotton). Example- spotted pod borer, capsule borers of castor and gingelly, red gram pod fly, tobacco caterpillar, gram caterpillar, pink boll worm etc.

9. Fruit borers: Larvae enter into the tender fruits and feed on fresh matter/pulp and plug the larval burrow with excreta. Example- fruit borer of brinjal/okra/tomato, mango stone weevil, cashew apple and nut borer.

10. Bark borers: Larvae remain in a small tunnel at the axils of branches, under the bark constructing galleries of frassy web on the stem and near bark/angles of branches and move about, conceal inside the silken gallery and feed on the bark by scraping. Example- bark eating caterpillars of citrus, mango, guava etc.

11. Tree borers: Larvae bore deep into the tree trunk, make the tunnels in zig-zag manner and feed on inner tissues, arresting translocation of sap to top portions of tree, there by the trees exhibit symptoms like yellowing, withering of leaves, drying of twigs or complete drying of tree. Sometimes, gummy material oozes from the affected portion on the tree trunk. Example- tree borers of mango, cashew, coconut red palm weevil etc.

12. Root feeders: Larvae feed on root/root nodules or nymphs and adults suck sap form the roots resulting in stunted growth/poor tillering/drying of plants in isolated patches. Example- white grubs, termites, rice root weevil and ragi root aphid.

13. Seed feeders (Stored grain pests): Grubs/larvae and adults feed on stored seeds either internally or externally by webbing the food particles. Example- rice weevil, red flour beetle, rice moth etc.

II. Sap Suckers or Feeders: These pests have part or all of the mouthparts modified into a piercing proboscis or stylet. Sap is sucked either from the phloem or xylem or from general tissues of foliage, roots or fruits. This type of mouth parts is found in planthoppers, leafhoppers, thrips, paddy gundhi bug, red cotton bug, sorghum ear head bug, aphids, mealy bugs, scales, whiteflies, mites etc. Nymphs and adults suck sap from the base of the plant/leaves/tender terminal plant parts/ flowers, thereby affect the vigour and growth of the plants or from developing ovaries/milky grains resulting in the formation of shriveled or chaffy grains. Different insects exhibit different symptoms. In case of severe infestation, sooty mould develops on the plant parts covered with honey dew excreted by insects while feeding.

1. Hopper burn, complete drying of leaves and plants in patches, giving scorched appearance: paddy brown planthopper, white backed plant hopper, paddy leafhopper.

2. Curling of leaf margins/mottling/necrotic patches: cotton leafhopper

3. Upward curling of leaves: chilli thrips

4. Downward curling of leaves/elongation of petioles of older leaves/reduction in leaf size and clustering at tip of branch/brittleness: chilli mites

5. Leaf drying from top to bottom: onion thrips

6. White/yellow blotches on upper surface of leaves: mites on castor/coconut/okra

7. Reduced vigour/sooty mould/square/flower drop: cotton whiteflies

8. Yellowing/crinkling of leaves: thrips on groundnut and pulses

9. Reduced vigour/stunted growth/yellowing/sooty mould: aphids

B. Methods of detection and diagnosis of plant diseases

Objectives: To study the different detection techniques of plant diseases.

Materials Needed: Paper and/or data sheets, disease identification books, hand lens, zip lock bags if specimens are to be brought for identification in laboratory, microscope and other necessary equipment.

Steps involved in Detection and diagnosis:

1. Field survey: This is the first step of detection and diagnosis of plant diseases. We should familiarize our-self with symptoms of diseases. Field survey is a procedure conducted over a defined period of time to determine the characteristics of a disease population or to determine which pathogen species occur in an area.

2. Sample collection and packaging: Second step is sample collection. Samples submitted to the lab must be representative of the symptoms observed in the field. Specimens must be fresh. Samples should be packaged to prevent contamination during transport. Proper packaging may include a combination of layering with damp paper towelling, use of paper bags or enclosure in a plastic bag. If a sample cannot be transported immediately, we need to keep it in refrigerator and out of direct sunlight.

3. Visual estimation from symptoms: All the plant pathogens can detect through characteristics symptoms which they have produced in different plant parts. For proper visual identification, we can use the reference books on symptoms or we can go through internet.

4. Detailed examination of samples in laboratory by microscope: Microscopic examination is generally required to enable identification of the pathogen and diagnosis of the disease. The identification of fungal pathogens is based initially on morphological features, such as spores and spore-forming structures. For example, most fungal pathogens that cause leaf diseases produce spore forming structures; perithecia, pycnidia, acervuli, sporangiophores or conidiophores with the fungus body (mycelium) that can be readily examined microscopically. The shape, size, color and manner of arrangement of spores on the sporophores or in the fruiting bodies as well as the shape and color of the sporophores or fruiting bodies are sufficient characteristics used to identify the taxonomy of fungi, the class, order, family, and genus.

5. Study of cultural and morphological characteristics: For a correct identification of the causal agent of plant diseases and make reliable conclusions, appropriate isolation procedure must be followed. Obtaining of pure culture will be helpful to identify the pathogen on the basis of morphological and cultural characteristics.

6. Pathogenicity testing: Pathogenicity test can be done by following the steps of Koch's Postulates. These steps includes i)Association ii)Isolation iii)Purification iv) Re-inoculation v) Comparison of the symptoms of re-inoculated plants with symptoms of infected plants.

7. Serological methods: Serological methods are based on production of antibodies and reaction between antigen and antibodies. These are ELISA and its variant: Direct antigen coated, double antibody sandwich ELISA.

8. Molecular approaches: The recent and advanced method of detection and diagnosis of the pathogen is the molecular detection method at species level identification. Some of the nucleic acid based techniques includes direct visualization of entire genomes, restriction digestion and electrophoresis of DNA, hybridization of DNA and RNA and the polymerase chain reaction (PCR). Molecular approaches are having their reliability and accuracy for identification of the pathogen but these are cost effective methods.

9. Final identification and confirmation of the pathogen: Once the genus of the fungus has been determined, descriptions of the known species are found in monographs of genera or in specific publications in research journals. Books, keys and manuals should be kept as resources in diagnostic laboratories. Many scientific publications on taxonomy can be accessed via the internet for proper identification.

Work to be done: We will be learnt the section cutting and teasing process of diseased sample followed by slide preparation of diseased sample in laboratory.

Materials required: Sample (Diseased Plant tissue), razor blade, watch glass, distilled water, blotting paper, camel hair brush, slide, cover slip, lacto phenol, cotton blue stain, compound microscope.

Procedure:

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 root.

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 and observe under microscope.

Experiment 2: Methods of insect pests and plant disease measurement

1. Introduction

Effective pest and disease management requires accurate and timely measurement. This manual presents standard field and lab methods for quantifying insect pests and plant diseases in agricultural systems.

2. Objectives

- To identify standard tools and techniques for measuring insect pests and plant diseases.
- To practice quantitative data collection and assessment.
- To support pest and disease monitoring for Integrated Pest Management (IPM).

3. Materials and Tools Required

- Hand lens or magnifying glass
- Sweep net
- Light trap
- Sticky traps (yellow, blue)
- Quadrat frame (1 m^2)
- Field notebook and data sheets
- Camera or smartphone
- GPS device (optional)
- Plant disease rating scales
- Personal Protective Equipment (PPE)

4. Insect Pest Measurement Methods

4.1 Direct Counting

- **Method**: Count insects manually on plants.
- **When to Use**: Low-mobility insects (e.g., aphids, scale insects).
- **Procedure**:

1. Select random plants or leaves.
2. Count insects per leaf, stem, or plant.
3. Record the average number.

4.2 Sweep Net Sampling

- **Method**: Sweep net through crop canopy.
- **Best For**: Flying or jumping insects (e.g., leafhoppers).
- **Procedure**:
    1. Perform 10–25 sweeps per plot.
    2. Transfer insects to a bag or vial.
    3. Count and identify later.

4.3 Light Trap Collection

- **Method**: Use light to attract nocturnal insects.
- **Best For**: Moths, beetles.
- **Procedure**:
    1. Set trap at dusk.
    2. Collect insects in the morning.
    3. Identify and count.

4.4 Sticky Trap Monitoring

- **Method**: Use colored adhesive cards.
- **Best For**: Whiteflies, thrips, aphids.
- **Procedure**:
    1. Place traps at crop canopy level.
    2. Count insects per cm^2 or per trap.
    3. Replace weekly.

4.5 Quadrat Sampling

- **Method**: Use a 1m x 1m quadrat to define sampling area.

- **Procedure**:
    1. Randomly place quadrat in the field.
    2. Count pests or damage in the square.
    3. Repeat and calculate average.

4.6 Damage Assessment

- **Method**: Assess pest symptoms (e.g., leaf holes, defoliation).
- **Procedure**:
    1. Use rating scales (e.g., 0–5 or % damage).
    2. Record number of damaged leaves/plants.

5. Plant Disease Measurement Methods

5.1 Visual Scoring

- **Method**: Assess visible symptoms (spots, wilting, blights).
- **Tools**: Disease rating scale.
- **Procedure**:
    1. Choose representative plants.
    2. Score based on severity scale.

Scale	Description
0	No symptoms
1	1–10% infected area
2	11–25%
3	26–50%
4	51–75%
5	>75% or plant dead

5.2 Disease Incidence and Severity

- **Incidence** (%):

Disease Incidence=Number of infected plants×100/Total number of plants

Severity (%):

Disease Severity=Area of plant affected×100/Total plant area

5.3 Area Under Disease Progress Curve (AUDPC)

- **Purpose**: Measures disease over time.
- **Formula**:

$$AUDPC=\sum[(Y_i+Y_{i+1})/2] \times(t_{i+1}-t_i)$$

  - Y_i: Disease severity at time i
  - t_i: Time at observation i

6. Sample Data Sheet Templates

Insect Count Sheet

Date	Plot No.	Crop Stage	Pest Name	No. per Plant	Damage (%)	Notes

Disease Scoring Sheet

Date	Plant No.	Symptoms Observed	Severity Score (0–5)	% Area Affected	Comments

Experiment 3: Assessment of crop yield losses

Objective: To understand the methods of assessment of crop yield losses due to insect damage which will help in deciding the timing of control measures in order to avoid indiscriminate use of insecticides.

❖ **CROP: RICE**

1. Chewing insects (damage is assessed in 10 randomly selected hills)

Rice stem borer: Assessment is based on eggs and larval damage. Presence of yellowish brown egg mass near the leaf tip and presence of dead heart at vegetative stage or white ear at reproductive stage.

Eggs in the nursery: Number of egg masses/m^2 (ETL: 2).

Larval Damage: count the total tillers and affected tillers in a unit area and arrive at a percentage

Percent Dead Heart = □□□□□□ □□ □□□□□□□□□ □□□□□□□ □□ □□□□*h* □□□□×100/ T□□□□□□ □□□□□□□

Percent White Ear = □□□□□□ □□ □□□□□□□□□ □□□□□□□ □□ □*h* □□□ □□□□×100/ T□□□□ □□□□□□□□□

Percent Silver Shoot = □□□□□□ □□ □□□□□□ □*h* □□t×100/ T□□□□ □□□□□□ □□ □□□□□□□

Gall midge: Assessment is based on damage on damage, silver shoot or onion shoot (ETL- 10% silver shoot).

Percent Silver Shoot = □□□□□□ □□ □□□□□□ □*h* □□t×100/ T□□□□ □□□□□□ □□ □□□□□□□

Leaf Folders: Assessment is based on damage, folded and scrapped leaves in 10 randomly selected plants (ETL- 10% at vegetative stage or 5 % at flowering stage)

Percent Infested Leaves= □□□□□□ □□ □□□□□□□□ □□□□□s×100/T□□□□ □□□□□□ □□ □□□□s

Whorl maggot: Assessment is based on damage, marginal blotching and yellow patches on the leaves in 10 randomly selected plants (ETL- 25% infested leaves)

Percent Infested Leaves= □□□□□□ □□ □□□□□□□□ □□□□□s×100/ T□□□□ □□□□□□ □□ □□□□s

2. Sucking Insects:

Thrips: Feeding results in longitudinal curling and yellowing with pointed leaf tips mostly in the nursery. Leaves may dry in due course. Pass wet palm or table tennis bat over the seedling in five places and count the number of thrips (ETL- 25/5 passes or 10% of affected seedlings).

Green leafhopper (GLH): Feeding on leaves results in yellowing. It is the vector for rice tungro virus disease (RTV). Count the number of insects per seedling in the nursery (ETL- 50/100 seedling) or number per hill in the field (ETL- 5/hill at vegetative stage, 10/hill at reproductive stage, 2/hill in RTV endemic area). Sweep net can also be used for sampling (ETL- 60/25 sweeping).

Brown plant hopper (BPH): Feeding on stem just above water level results in hopper burn. Count the total number of insects in 10 hills selected random in one square meter area (ETL- 1/tiller or 2/tiller if predatory spider is present).

Earhead or Gundhi bug: Black spot at feeding point on the grain and individual chaffy grains. Insects emit stinky odour. Count the number of bugs in 100 earheads selected at random (ETL- 5 bugs at flowering stage or 16 bugs at milky stage/100 panicles).

Percent Chaffy Grains= N□□□□□ □□ □ℎ □□□□ □□□□□s×100/T□□□□ □□□□□□ □□ □□□□□□ □□ 10 □□□□□□□□ □□□□□□□□ ℎ □lls

Experiment 4: Calculations based on economics of IPM

After assessing the insect population, decision regarding the types of control measures to be followed is done based on the Economic Threshold Level (ETL) and Economic Injury Level (EIL) of the pest. The beginning point of EIL is known as Gain Threshold.

Gain threshold= Cost of management (Rs. per ha) /Market value of commodity (Rs. per kg)=…..kg/ha

EIL is also known as Action Thresholds Levels: The EIL concept is flexible and may vary from area to area, crop to crop depending upon the specific agronomic practices. The EIL decreases as the value of the crop increases.

Economic Injury Level (EIL) or Action Threshold Level: Stern *et al*., 1959 defined EIL as the lowest population density that will cause economic damage. It is the lowest population at which the pest will cause economic damage or it is the pest level at which the damage can no longer be tolerated and therefore it is the level at or before which the control measures are initiated. EIL is usually expressed as the number of insects per unit area.

EIL= C/VID

EIL= Number of injury equivalents per production unit (insect/ha)

C= Cost of management activity per unit of production (Rs./ha)

V= Market value per unit of product (Rs./tonnes)

I= Crop injury per pest density

Whether expressed as numbers or injury equivalents, the EIL is governed by five primary variables: cost of the management tactic per production unit, (C), market value per production unit (V), injury units per pest (I), damage per injury unit (D), and the proportional reduction in pest attack (K). If the relationship of these variables is linear or roughly so, the EIL can be given as:

EIL = C/VIDK

where, EIL = Economic injury level in insects/production (or) insects/ha

C = Cost of management activity per unit of production (Rs./ha)

V = Market value per unit of yield or product (Rs./tonne)

I = Crop injury per insect (Per cent defoliation/insect)

D = Damage or yield loss per unit of injury (Tonne loss/% defoliation)

K = Proportionate reduction in injury from pesticide use (if the proportionate reduction of injury is 60%, the K value is taken as 0.6)

Worked examples of EIL:

Calculate EIL in terms of pest population/ha with following figures

C = Management cost per unit area = Rs.3,000/- per ha

V = Market value in Rs./unit product = Rs.1,000/tonne

I = Crop injury/pest density = 1% defoliation/100 insects

D = Loss caused by unit injury = 0.05 tonne loss/1% defoliation

K = Proportionate reduction in injury by pesticide application = 0.8 (80% control)

EIL = C/VIDK = $3000/(1000\times0.01\times0.05\times0.8)$ =7500 insects/ha

Economic Threshold Level: ETL is defined as the population density at which control measures should be implemented to prevent an increasing pest population from reaching the economic injury level or refers to number of insects per unit area when management action should be taken to prevent the pest from reaching EIL. Although measured in insect density, the ET is actually a time to take action, i.e., numbers are simply an index of that time. ETL is experimentally designed as follows

➢ Measure the yield of crop for a range of pest densities, including zero (control plot) under controlled experiments.

➢ Measure the yield and total crop revenues in the same type of experiments for each management practices to be analyzed.

➢ Compute total crop revenue for each management at each pest density (Yield × Price per unit of output).

➢ Compute net revenue (subtract cost of each management action from crop revenue at each pest density).

➢ The pest density where the net revenues under controlled and uncontrolled conditions are equal is the Economic Threshold Level.

Or

The pest density where Marginal crop revenue is equal to the management action cost is also ETL (a hypothetical example is given in the table).

Marginal crop revenue = Crop revenue from taking action – Crop revenue from not taking action It is clear that crop value and management cost are the two important factors deciding the EIL and ETL. In the given example if the price of fruit goes down to Rs. 1 per kg the management cost also should reduce by same proportion i.e. 10.40-5.20 to maintain the same ETL. In the given case if the management cost remains at the same level of Rs. 10.40, then the ETL will come down.

Table 1: Influence of crop value and management cost on ETL for a moringa plant affected by moringa fruit fly, *Gitona distigma*

Pest density (% affected fruits)	**Yield/Plant (Kg)**	**Management cost @ Rs. 10.40 per plant**	**@ Rs. 2/kg of fruit**	
			Revenue	**Net Revenue**
0	**40.00**	**It is uniform for all plants**	**80.00**	**69.60**
1	**39.60**		**79.20**	**68.80**
2	**39.20**		**78.40**	**68.00**
3	**38.80**		**77.60**	**67.20**
4	**38.40**		**76.80**	**66.40**
5	**38.00**		**76.00**	**65.60**
6	**37.60**		**75.20**	**64.80**
7	**37.20**		**74.40**	**64.00**
8	**36.80**		**73.60**	**63.20**

9	**36.40**		**72.80**	**62.40**
10	**36.00**		**72.00**	**61.60**
11	**35.60**		**71.20**	**60.80**
12	**35.20**		**70.40**	**60.00**
13	**34.80**		**69.60**	**59.20**
14	**34.40**		**68.80**	**58.40**
15	**34.00**		**68.00**	**57.60**
16	**33.60**		**67.20**	**56.80**
17	**33.20**		**66.40**	**56.00**
18	**32.80**		**65.60**	**55.20**
19	**32.40**		**64.80**	**54.40**
Uncontrolled	**32.00**		**64.00**	**64.00**

7 per cent affected fruits gives a net revenue of Rs. 64 which is equal to net revenue from uncontrolled condition.

Marginal crop revenue at 7% of affected fruits = Crop revenue from taking action – Crop revenue from not taking action

= 74.40-64.00 = Rs. 10.40

So, the marginal crop revenue at 7% of affected fruits is Rs. 10.40 which is equal to the management cost.

Therefore, ETL is 7% affected fruits per plant.

Experiment 5: Identification of bio control agents, different predators and natural enemies

1. Ladybird beetle: *Cheilomenes sexmaculata* (Coccinellidae: Coleoptera) *Cheilomenes sexmaculata* is a very important, polyphagous predator of aphids and other soft bodied insects. It has been recorded in most crop ecosystems, particularly where aphids are serious pests. It has been produced in the laboratory and used for the suppression of *A. craccivora* on groundnut.

2. Ladybird beetle: *Cryptolaemus montouzieri* (Coccinellidae: Coleoptera) The adults and larvae of these insects eat scale insects, especially mealybugs. Females lay their eggs among the egg sack of mealybugs. Larvae feed on mealybug eggs, young crawlers and their honeydew. They become adults in 24 days, after three larval stages and a pupal stage. The life span lasts two months.

3. Green lace wing: *Chrysoperla carnea* (Chrysopidae: Neuroptera) Larvae are important predators of insect pests viz., aphids, mealy bugs, eggs and smaller larvae of various insects of agricultural importance and mites. Each larva has potential to feed on average 12 aphids/day or about 120 aphids during the entire developmental period.

4. Egg parasitoid: *Trichogramma sp*. (Trichogrammatidae: Hymenoptera) *Trichogramma* sp. are of common occurrence and distributed throughout the world. They parasitise eggs of Lepidopteran mainly but are also reported from Coleoptera, Neuroptera and Diptera. In India it is commercially available for the pest suppression of sugarcane, cotton, sorghum, maize and paddy borers.

5. Larval parasitoid: *Bracon hebetor* (Braconidae: Hymenoptera) It is a well known external, gregarious larval parasitoid of several lepidopteran pests.

6. Pupal parasitoid: *Tetrastichus israeli* (Eulophidae: Hymenoptera) The pupal parasitoid was observed to parasitise the pupae of *Opisina arenosella* and an average 90 adult parasitoids emerged from a single pupa under natural conditions. It can be mass reared on fresh pupae of *S. litura*, *H. armigera*, *Plusia sp*., or *Ergolis sp*.

Experiment 6: Mass multiplication of *Trichoderma*

Objective: To learn the techniques of mass production of *Trichoderma* sp.

Material required: Pure culture plate of *Trichoderma* sp., cork borer, distilled water, conical flasks, autoclave, jaggery, yeast extract, cotton plugs, streptocyclin, inoculation needle, muslin cloth, talcum powder, carboxy methyl cellulose, rubber bands.

Procedure: The whole process will be done by two steps-

Step 1: Preparation of mother culture:

1) Prepare Molasses yeast medium by mixing 5gm yeast powder and 30 gm molasses into 1000 ml water.

2) Dispensed the prepared medium into conical flasks and sterilized at 15 lb pressure for 15 minutes in an autoclave.

3) Inoculate the cooled media with 10 days old fungal disc of *Trichoderma* and incubate for 10 days for fungal growth.

Step 2: Mass multiplication:

1) Molasses yeast medium is prepared in fermentor and sterilized as described earlier.

2) Then after the medium is cooled, the mother culture is added to the fermentor @ 1.5 lit / 50 lit of the medium and incubated at room temperature for 10 days.

3) The fungal biomass collected from fermentor is mixed with talc powder at 1:2 ratio.

4) The mixture is air dried in shade and mixed with carboxy methyl cellulose (CMC) @ 5 g / kg the product.

5) It is packed in polythene bags and should be used within 4 months.

Experiment 6: Mass multiplication of *Pseudomonas*

Objective: To learn the techniques of mass production of *Pseudomonas* sp.

Materials required:

- Pure culture of Pseudomonas sp.
- Distilled water
- Conical flasks
- Autoclave
- Cotton plugs
- Inoculation loop
- Talcum powder
- Rubber bands
- Peptone
- Dipotassium phosphate (K_2HPO_4)
- Magnesium sulphate ($MgSO_4$)
- Glycerol

Procedure: The whole process will be done by two steps-

Step1: Preparation of mother culture

1) Prepare king's B medium by mixing necessary components (Peptone: 20.0g K_2HPO_4:1.5g $MgSO_4$:1.5g Glycerol: 10ml) into 1000 ml water.

2) Dispensed the prepared medium into conical flasks and sterilized at 15 lb pressure for 15 minutes in an autoclave.

3) Inoculate the cooled media with a loop of Pseudomonas sp. and incubate for 2 days.

Step 2: Mass multiplication:

1) The king's B medium is prepared and poured into the fermentor and sterilized at 15 lb pressure for 15 minutes.

2) After the broth has cooled, the mother culture of Pseudomonas sp. is added to the king's B medium in the fermentor at the rate of 3 lit for 40 lit of the broth.

3) Then it is incubated in the fermentor for 2 days with frequent mixing of the broth by operating the stirrer.

4) Then the broth containing the bacterial growth is collected in plastic buckets and used for mixing with talc powder (1:2 ratio) for commercial formulation.

Experiment 7: Mass multiplication of *Trichogramma*

The genus *Trichogramma* is cosmopolitan in distribution and present in all terrestrial habitats and is one of 80 genera in the family Trichogrammatidae. *Trichogramma* primarily parasitize eggs of Lepidoptera, but parasitism also occurs in eggs of other orders such as Coleoptera, Diptera, Hemiptera, Hymenoptera and Neuroptera. In India it is commercially available for the pest suppression of sugarcane, cotton, sorghum, maize and paddy borers.

(a) Biology

Fecundity: 20-200 eggs

Egg period: 1-2 days

Larval period: 3-4 days

Pupal period: 4-5 days

Adult longevity: Male: 5-7 days, Female: 5-20 days

(b) Materials required: *Corcyra cephalonica* eggs, Nucleus culture of *Trichogramma*, Polythene bags, Rubber bands, Scissors, Gum, Tea strainer, Brush, Mesh sieve (40 mesh size), Tricho cards, 50% honey solution, Stapler, Refrigerator/fridge, B.O.D. incubators and UV lamp/LED light.

c) Rearing: In India, *Trichogramma* sp. are reared on the eggs of rice meal moth. Freshly collected eggs of Corcyra are cleaned of the scales, mites and other foreign matter associated with these and are glued on the Trichocard with uniformly thin layer using 2 per cent gum Arabic in distilled water (W/V). The sprinkling of the eggs is done either with camel hair brush or a fine sieve which does not allow more than one or two eggs to pass through its hole at a time. Thus 18000-72000 (1 ml) frozen host eggs are glued on a trichocard (15 x 7.5 cm). If the eggs were not frozen the trichocard should be exposed to UV lamp for about 10 minutes. The card is further divided through punching into 6 strips each of 7.5 x 2.5 cm size which can be easily pressed and separated. A strip containing glued eggs on it was inserted into a glass tube (10 x 2.5 cm) having newly emerged adults. The adult parasitoids are provided with honey streaks (50% honey dissolved in water) drawn on inner side of the tube and secured tightly with muslin cloth and rubber bands. The card is changed after 24 hours and replaced with fresh card. Thus, continuity of changeover is maintained for 3 to 4 days or till female survive and remain productive. The host eggs oviposited by female turns black after 3 days of parasitization. The parasitoid completes its life cycle in 7-9 days at 27 ± 2°C and 75 ± 5% RH.

Precautions:

1) If host eggs are not frozen/treated with UV rays to kill the embryo, the moth's larvae may hatch out from the unparasitized eggs. These larvae should be brushed out gently since they eat away the unparasitized eggs.

2) Avoid super parasitism either by exposing host eggs upto 8 hours or providing 6 eggs for one parasitoid.

3) Maintain pure species of different species of Trichogrammatids through proper handling and regular examination.

4) Do not offer frozen eggs to T. japonicum as it does not develop well on such eggs.

5) Do not rear T. brasiliensis at the temperature exceeding 26°C where undesired male formation is more.

6) Do not cold store parasitized eggs at 5-10°C for more than 15-20 days as beyond this storage biological attributes of the parasitoids are affected.

7) Use healthy eggs of host for healthy parasitoid.

8) Do not put excess gum while sprinkling the host eggs.

9) Do not rely on super parasitized parasitoids as they are normally weak and unfit for the production of healthy progeny.

Experiment 9: Mass multiplication of NPV

Among viruses of the group baculoviridae, nuclear polyhedrosis viruses are utilized for the successful suppression of various insect pests of many agricultural and horticultural crops. Nuclear polyhedrosis viruses of Helicoverpa armigera and Spdoptera litura are highly specific to their respective live hosts for multiplication. So, production of viruses for use as insecticides needs mass production of their hosts as a first step.

Basic steps in the production of nuclear polyhedrosis viruses of any insect are-

1. Mass culturing and maintenance of host insects

2. Host inoculation with viruses

3. Harvesting of viruses

4. Purification

5. Storage

A) Mass culturing of SlNPV

Materials required: Plastic tub, Conical flask, Nucleus of SlNPV solution, Distilled water, Disinfectant, Brush, Black cloth, Blender, Centrifuge, Beaker, Funnel, Sieves, Filter paper, Castor leaves, Wash bottle, Cotton wool, Compound microscope, Haemocytomter, Staining chemicals (Eosine), Sticker.

Procedures:

➢ Collect 3rd instar larvae. Starve larvae for 4 to 5 hours before feeding.

➢ Prepare 1x108 POB/ml NPV suspension or dilute solution in plastic tub and add sticking agent. Pluck castor leaves with the petiole & dip in the virus solution. Dry the leaves in shade.

➢ Keep the newspaper at the bottom of bucket & provide the treated leaf for feeding & cover mouth of bucket with cloth.

➢ Repeat twice after 24 hours, 4 days after inoculation, disease symptoms start to appear and larvae will die within a week.

➢ Take 250 ml conical flask, add 150 ml distilled water, 100 diseased larvae in it and tighten the mouth with cotton and keep the flask for 15 days for purification.

➢ Blend the solution for homogenization and filter through muslin cloth. Centrifuge the filtrate for 5 minutes at 500 rpm.

➢ Collect the supernatant and again centrifuge at 4000 rpm for 30 minutes.

➢ Collect sediment with distilled water and keep in glass bottle. Store in refrigerator until use.

➢ Field Use: Dosage: 450 LE/ha two to three times at 10-15 days interval. Use 0.05% Teepol as the sticking agent.

B) Mass culturing of HaNPV

Materials required: Pupation box Blender, Nucleus Ha-NPV solution, Distilled water, Centrifuge, Beaker, Measuring cylinder, Bengal gram/Bhendi, Haemocytometer, Sticker.

Procedures:

➢ Collect 3rd instar or early 4th instar larvae. Starve larvae for 3 to 5 hours before feeding.

➢ Prepare bhendi vegetable for larval feed. Introduce piece of the virus contaminated diet in each container with pre-starved larvae.

➢ Pour 2-3 drops of Ha-NPV suspension in the penicillin vial and feed to the larvae. Replace uneaten part of diet after 2-3 days & clean the containers.

➢ Repeat the inoculation process twice after 24 hours. Within 4 days disease symptoms appear and larvae die within a week.

➢ Take the diseased larvae in distilled water. Keep the flask for 15 days for putrefaction. Filter the solution through muslin cloth.

➢ Centrifuge the filtrate for 5 minutes at 500 rpm. Collect the supernatant and again centrifuge at 4000 rpm for 30 minutes.

➢ Collect the sediment with distilled water and keep it in glass container. Store the pure Ha-NPV in refrigerator until use.

➢ Field Use: Dosage: 450 LE/ha two to three times at 10-15 days interval. Use 0.05% Teepol as the sticking agent.

Experiment 10: Identification and nature of damage of important insect pests and diseases and their management

Observation in field:

i. **Pest or disease common name-**
ii. **Scientific name or causal organism-**
iii. **Nature of damage-**
iv. **Major damage symptoms-**
v. **Management practices-**
    - Cultural methods-
    - Physical methods-
    - Mechanical methods-
    - Biological methods-
    - Chemical methods-

Experiment 11: Crop (agro-ecosystem) dynamics of a selected insect pest and diseases

Rice is the principle food crop in India. Rice crop is prone to stress throughout the crop growth period due to onslaught from different pests such as insects, nematodes, diseases, weeds and rats. Brown plant Hopper (*Nilaparvata lugens*) causes serious insect-pest problem during Kharif season. Brown plant Hopper is a pest of national significance.

Agro- Eco System Analysis (AESA) is a process in which farmers observe the crop, analyze the field situation and take crop management decisions based on field observations. The health of a plant is determined by its environment which includes physical factors (i.e. sun, rain, wind and soil nutrients) and biological factors (i.e. pests, diseases and weeds). All these factors can play a role in the balance which exists between herbivore insects and their natural enemies. Understanding the intricate interactions in an ecosystem can play a critical role in pest management. Focus in AESA based IPM is on pest-defender dynamics, abilities of plants to compensate for the damage caused by pests and the influence of abiotic factors on pest build up.

The basic components of AESA are-

➢ Plant health at different stages

➢ Built-in-compensation abilities of the plants

➢ Pest and defender population dynamics

➢ Soil conditions

➢ Climatic factors

➢ Farmers past experience

❖ Pest dynamics: Brown Plant hopper is the major problem in rainfed and in irrigated wetland environments and also occurs in the areas with continuous cultivation, submerged conditions in the fields, high shade, use of chemical fertilizers /pesticides and humidity. Closed canopy of the rice plants, densely seeded crops excessive use of nitrogen and early season insecticides spraying also favours insect development.

❖ Monitoring and surveillance: Continual monitoring and accurate forecasting of pest population during the crop growing period could be useful in protecting rice crops against BPH. Accurate and timely forecasting of the pest incidence would support in planning effective mitigation. BPH infestation mostly starts from 1st week of September, hence monitoring should start from 1st week of September. To monitor BPH, the basal parts of some rice plants are to be disturbed mildly with a stick so that the insects jump to standing water from which their occurrence or ETL can be ascertained.

1. Roving survey: Undertake roving survey at every 10 km distance at 7-10 days intervals (depending upon pest population). Everyday at least 20 spots should be observed.

Field scouting: Field scouting for pests and bio-control fauna by extension agencies and farmers once in 3-5 days should be undertaken to workout ETL.

• The Economic Threshold Level (ETL) for the pest is: BPH: 5-10 insects/ hill in non-endemic area and 2/hill in endemic area.

• If the insect pest population is above ETL, apply any recommended chemical insecticide if the rice crop is at late vegetative or panicle initiation stage. Pest defender ratio (P:D) 2:1 may be useful to avoid application of chemical pesticides against plant hoppers. Wherever rice crop has become mature or when grain hardening is completed, no insecticide should be applied.

❖ Field Observations: Field observations on insect pest infestations are to be initiated after 20 days of transplanting. In each field select five spots randomly (four in the corner, at least 5 feet inside the border and one in the centre). At each spot select four hills randomly for recording observations (total 20 hills/field).

❖ Plant growth (weekly): Measure the height of hill, count the number of tiller per hill and number of leaves.

❖ Crop situation:

- Plant health: Observe the crop stage and deficiency symptoms etc.
- Count BPH population at different places on the plant.
- Natural enemies: Count parasitoids and predators.
- Soil condition
- Irrigation
- Weather conditions After assessing all the above mentioned aspects, the suitable and recommended pest management practices for BPH are then to be followed.

Experiment 12: Plan & assess preventive strategies (IPM module) and decision making

1. Grow a healthy crop:

• Select a variety which is resistant/tolerant to major pests.

• Treat the seeds/seedlings/planting material with recommended pesticides, especially bio-pesticides.

• Select healthy seeds/seedlings/planting material.

• Improve soil health by nutrient management especially organic manures and bio fertilizers based on soil test results. If the dosage of nitrogenous fertilizers is too high the crop becomes too succulent and susceptible to insects and diseases. If the dosage is too low, the crop growth is retarded. So, the farmers should apply adequate amount for best results. Phosphate fertilizers should not be applied every season as the residual phosphate of the previous season will be available for the current season also.

• Proper irrigation.

• Crop rotation.

2. Observe the field regularly: Farmers should monitor the field situations at least once a week (soil, water, plants, pests, natural enemies, weeds, weather factors etc.); make decisions based on the field situation and Pest: Defender (P: D) ratio and take necessary action (e.g. remove infested plants etc.).

3. Plant Compensation Ability: Compensation is defined as the replacement of plant biomass lost to herbivores and has been associated with increased photosynthetic rates and mobilization of stored resources, for example from roots and remaining leaves.

4. Understand and Conserve defenders: Know about natural enemies to understand their role through regular observations of the agroecosystem and avoid use of chemical pesticides.

5. Insect zoo: Various types of insects are present in the field where some are beneficial some may be harmful. Generally farmers are not aware about this. The concept of Insect zoo can help in enhancing farmers ‘skill to identify beneficial and harmful insects. In this method, unknown predators are collected in plastic containers from the field. Each predator is placed inside a plastic bottle together with parts of the plant and some known insect pests. Insects in the bottle are observed to determine whether the test insect is a pest (feeds on plant) or a predator (feeds on other insects).

6. Pest: Defender ratio (P: D ratio): The natural enemies of crop pests include parasitoids, predators and pathogens. Identifying the pests and beneficial insects helps farmers make appropriate pest management decisions. Sweep net, visual count etc. can be adopted to arrive at the numbers of pests and defenders. The P: D ratio can vary depending on the feeding potential of natural enemy as well as the type of pest. The general rule to be adopted for management decisions relying on the P: D ratio is 2:1. However, some of the parasitoids and predators can control more than 2 pests. Whenever the P: D ratio is found to be favorable, there is no need for adoption of other management strategies. In cases where the P: D ratio is found to be unfavorable, the farmers can be advised to resort to release of parasitoids/predators depending upon the type of pest. In addition, bio pesticides such as insect growth regulators, botanicals etc. can be used before resorting to chemical pesticides.

❖ **Pest Management Decision Making**: From the above, we can see that decisions need to be taken by the farmer as to how to implement pest management in the most effective and economic manner. Many of these decisions are taken a long time before any pests appear, such as the crop rotation, time of planting, amount of fertilizer, etc. During the growing season, however, continual decisions need to be made regarding pest management. The pest management decision making process is a continuous cycle that can be summarized as:

• **Detection:** Continual monitoring to see what pests and beneficial insects are present in the crop. This is usually called scouting. Pests are detected before they cause economic loss, and spot treatments can possibly be made. If detection is too late, control will be less effective, more costly, and crop losses will occur.

• **Identification:** The organism must be identified to see if it is a pest, a beneficial insect, or of no importance. If the organism is an insect pest, it is necessary to identify the stage in the life cycle so that control methods can be directed at the most vulnerable stage.

• **Economic significance:** Is the pest causing, or, if left uncontrolled, will it cause, economic damage or loss? Economic damage is the amount of damage that justifies the costs of control. Crop growth stage and economic value also need to be taken into consideration. It should be noted that biological damage (for example, holes in leaves) often occurs without there being any yield or economic loss. The potential (controlling) impact of beneficial species also needs to be considered.

• **Decision:** If the pest level is below the threshold, then no pesticide treatment should be applied. If the pest level is above the threshold, then control treatment is required.

• **Selection of control methods:** The method of control must be effective, practical, economic and safe. More than one control method may be involved for a single pest. For example, the application of an insecticide spray to kill yellow stem borer of rice and clipping of top leaf portion containing egg masses, while at the same time encouraging biocontrol agents to minimize future infestations.

• **Evaluation:** The follow-up of the control method to evaluate the effectiveness. This is a continuation of the monitoring procedure at the beginning of the decision making process, and starts the decision making cycle again.

Experiment 13: Crop monitoring attacked by insect, pest and diseases

A. Crop monitoring attacked by insect pests:

1. Survey/Field Scouting: The objective through roving surveys is to monitor the initial development of pests in endemic areas. Therefore, in the beginning of crop season survey routes based upon the endemic areas are required to be identified to undertake roving surveys. Based upon the results of the roving surveys, the state extension functionaries have to concentrate for greater efforts at block and village levels as well as through farmers to initiate field scouting. Therefore, for field scouting farmers should be mobilised to observe the insect pest and disease occurrence at the intervals as stipulated hereunder. The plant protection measures are required to be taken only when insect pests and diseases cross Economic Threshold Level (ETL) as per results of field scouting. (i) Roving survey: Undertake roving survey at every 10 km distance at 7-10 days intervals (depending upon pest population). Every day at least 20 spots should be observed. (ii) Field scouting: Field scouting for pests and bio-control fauna by extension agencies and farmers once in 3-5 days should be undertaken to workout ETL.

2. Pest monitoring through pheromones/light traps etc.: Majority of insect population can be monitored by fixing and positioning of pheromones or light traps at appropriate stage of crop. The State Department of Agriculture can initiate this action at strategic locations at village level as per the following details:

(i) Pheromone trap monitoring: 5 traps per ha may be used to monitor yellow stem borer and moth population.

(ii) Light trap: Chinsurah light trap or any other light trap can be operated for two hours in the evening to observe photo-tropic insect pests.

(iii) Sweep nets or water pans: Besides visual observations sweep-nets and water pans may also be used to assess the population of insect pests, and biocontrol agents to determine the type of pesticides to be recommended or used.

B. Crop monitoring of diseases:

1. Introduction to Crop Disease Monitoring

- Definition: Crop monitoring is the regular observation and analysis of crops to detect signs of stress, including pest and disease attacks.
- Importance: Early detection helps reduce yield losses, minimize chemical use, and improve crop health.

2. Common Signs of Crop Disease

- **Visual symptoms**:
  - Leaf discoloration (yellowing, browning)
  - Wilting or stunted growth
  - Spots, blights, mildew, molds
  - Abnormal growth (galls, cankers)
- **Environmental clues**:
  - High humidity, poor drainage, warm temperatures (ideal for fungal/bacterial growth)

3. Tools and Techniques for Monitoring

A. Field Scouting

- Regular walking through the fields
- Using a smartphone or notebook to record symptoms
- Sampling diseased plants for lab analysis

B. Remote Sensing & Drones

- Drones with multispectral cameras detect stress before visible symptoms appear.
- NDVI (Normalized Difference Vegetation Index) maps to identify affected zones.

C. Mobile Apps and IoT Sensors

- Mobile apps (e.g., Plantix, FarmLogs) use AI to diagnose disease via photos.
- Soil moisture sensors, leaf wetness sensors – detect conditions favorable for disease.

D. Laboratory Diagnosis

- Send samples to lab for accurate identification:
  - Microscopic analysis
  - Culture tests
  - PCR/ELISA for virus/bacteria

4. Data Collection and Record Keeping

- Use of spreadsheets, apps, or farm management software
- Recording:
  - Date of symptom detection
  - Weather conditions
  - Crop stage
  - Control measures applied

5. Integrated Disease Management (IDM)

- Based on monitoring results, apply IDM:
  - **Cultural**: crop rotation, resistant varieties
  - **Mechanical**: removal of infected plants
  - **Biological**: use of biopesticides or beneficial organisms
  - **Chemical**: targeted use of fungicides/pesticides

6. Practical Case Study Example

Crop: Tomato
Problem: Early blight
Monitoring:

- Weekly scouting revealed small black spots on lower leaves
- Drone survey showed reduced NDVI in central field zone
- Lab confirmed *Alternaria solani*

Actions Taken:

- Removed infected plants
- Sprayed mancozeb fungicide
- Improved spacing and airflow between plants
- Scheduled weekly monitoring

7. Preventive Monitoring Tips

- Start monitoring early in the season
- Monitor after rain or irrigation
- Keep scouting logs
- Educate farm workers on symptom recognition

8. Conclusion

- Crop disease monitoring is vital for early detection and effective control.
- Combining traditional and modern methods ensures better results.
- Continuous learning and use of technology improve disease management outcomes.

Experiment 14: Awareness campaign at farmers' fields

Objectives:

- Train students to plan and conduct awareness campaigns.
- Educate farmers on pest and disease identification and management.
- Promote sustainable, integrated pest management (IPM) practices.
- Improve communication and extension skills of students.

Field Campaign at Farmers' Fields

Activity	Description
Welcome & Introduction	Introduce the students and purpose of the campaign to farmers
Field Demonstration	Show pest & disease symptoms, biological control agents, and traps
IPM Session	Students explain cultural, mechanical, biological, and chemical controls
Safe Pesticide Use Demonstration	Proper sprayer calibration, protective gear, safe handling
Diagnostic Walk (Field Visit)	Joint scouting with farmers, pest and disease identification practice
Distribution of IEC Materials	Leaflets, charts, and posters in local language
Feedback & Interaction	Farmers ask questions; students collect feedback for report

Tools and Materials Required:

- Pest & disease identification charts
- Sprayers (knapsack/hand-held)
- Biological control agents (*Trichogramma* cards, neem oil, etc.)
- Traps (pheromone, yellow sticky traps)
- Personal Protective Equipment (PPE)
- Posters, leaflets (preferably in the local language)
- Stationery (notebooks, pens for feedback collection)

Post-Campaign Assignment:

Each student (or group) must submit a field report covering:

- Campaign objectives and location
- Farmer turnout and engagement
- Pest and disease problems observed
- Methods demonstrated
- Farmer feedback and suggestions
- Personal reflection and learning