PRACTICAL MANUAL

HOST PLANT RESISTANCE

ENT 507 2(1+1)

M.Sc. (Ag) Entomology

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Syllabus: Host Plant Resistance

Practical: Measurement of plant characters and working out their correlations with plant resistance; Testing of resistance in important crops; Bioassay of plant extracts of susceptible/ resistant varieties; Demonstration of antibiosis, tolerance and antixenosis.

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Practical No. 1

Objective: Study of field screening technique for measuring resistance

Activity: Visit field to observe screening technique and enlist it with brief description.

Description:……

Practical No. 2

Objective: Study of greenhouse screening technique for measuring resistance

Activity: Visit field to observe screening technique and enlist it with brief description.

Description:……

Practical No. 3

Objective: Study of laboratory screening technique for measuring resistance

Activity: Visit lab to observe screening technique and enlist it with brief description.

Description:……

Practical No. 4

Objective: Study of various bioassay techniques for measuring resistance

Activity: Visit lab to observe bioassay techniques and enlist it with brief description.

Description:……

Practical No. 5

Objective: To measure various plant key characters with the help of lab equipment

Activity: Visit the field and collect various plant parts to measure key plant characters

Materials required: ……

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Procedure:……

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Interpretation:……

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Practical No. 6

Objective: To study correlation of various plant key characters with plant resistance

Activity: Visit the field and collect various plant parts to measure key plant characters and correlate it with resistance

Materials required: ……..

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Procedure:……..……

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Interpretation:……..……

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Practical No. 7

Objective: Study of resistance in Rice.

Activity: Visit the field in your vicinity and test resistance by various methods (open and lab conditions) and describe it.

Procedure:……………………………………………………………………………………………

Practical No. 8

Objective: Study of resistance in Wheat.

Activity: Visit the field in your vicinity and test resistance by various methods (open and lab conditions) and describe it.

Procedure:…………………………………………………………………………………………………

Practical No. 9

Objective: Study of resistance in Mustard.

Activity: Visit the field in your vicinity and test resistance by various methods (open and lab conditions) and describe it.

Procedure:……………………………………………………………………………………………

Practical No. 10

Objective: Study bioassay of plant extracts of susceptible varieties

Activity: Visit the field and collects plant samples of susceptible varieties to prepare extract and insects pests and testing bioassay against insect pests.

Materials required: ……

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Procedure:……

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Result:……

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Practical No. 11

Objective: Study bioassay of plant extracts of resistant varieties

Activity: Visit the field and collects plant samples of resistant varieties to prepare extract and insects pests and testing bioassay against insect pests.

Materials required: ……

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Procedure:……

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Result:……

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Practical No. 12

Objective: Study to determine and demonstrate antibiosis

Activity: Collect plant samples and insects pests associated with it and observe and describe these mechanisms of resistance

Materials Required: ……………………………………………………………………………………

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Procedure:……………………………………………………………………………………………

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Practical No. 13

Objective: Study to determine and demonstrate antixenosis

Activity: Collect plant samples and insects pests associated with it and observe and describe these mechanisms of resistance

Materials required:

Procedure:

Practical No. 14

Objective: Study to determine and demonstrate antixenosis

Activity: Collect plant samples and insects pests associated with it and observe and describe these mechanisms of resistance

Materials required:

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Procedure:……

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Practical No. 15

Objective: Study to determine and demonstrate tolerance

Activity: Collect plant samples and insects pests associated with it and observe and describe these mechanisms of resistance

Materials Required: ……………………………………………………………………………………………

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Procedure:……………………………………………………………………………………………

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Practical No. 16

Objective: Study to determine and demonstrate tolerance

Activity: Collect plant samples and insects pests associated with it and observe and describe these mechanisms of resistance

Materials Required: ……………………………………………………………………

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Procedure:…………………………………………………………………………

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SCREENING TECHNIQUES FOR MEASURING RESISTANCE

Field Screening: The varieties to be evaluated or the segregating population to be screened for resistance may be raised in fields under natural infestation, either in endemic areas or by adopting techniques for increasing field infestation.

Cultural practices such as closer spacing to create the most desirable humid microclimate within the crop, application of additional dose of nitrogen and irrigation to induce vegetative growth may be adopted. The test material may be sown or planted early, before the adult emerge in an area already infested in the previous season.

In case of insects such as plant hoppers, flies etc. that have a tendency to move at a rapid speed, fibre glass mesh cages may be kept on microplots and artificially reared insects released at a specified number per plant.

The insect population should be able to infest the plant population uniformly so that plants that escape infestation are not graded as resistant. Highly susceptible plants can be interplanted as "spreader rows" along with rows of the test material. Insect attractants, such as fish meal for sorghum shoot fly may also be used to attract and increase the insect density in the field.

Green house screening: Screening the varieties or segregants in green houses providing conditions conducive for infestation is more rapid reliable than field screening. Special methods have been developed to increase the insect population to provide sufficient insect pressure for valid screening. Insects reared in the culture maintenance cages are released on plants raised in seed boxes kept in green house. Fibre glass screen cages are used for each seed box.

Depending on the crop on one hand and the insect pest on the other, the stage of the crop at which the insect is released, the stage of the insect, whether egg, larva or adult. The number of the insect population vary, as also the symptoms on the host to differentiate and grade the resistant plants from the susceptible.

Laboratory Screening: Laboratory screening for resistance can also be done using plant tips or leaf discs and allowing forced or free choice feeding by insects as in the case of lucerne weevil.

Bioassay techniques: Bioassay techniques are also used to screen resistance to insect pests such as *Heliothis* spp and pink boll worm in cotton. Lyophilized square powder is incorporated in an artificial diet and dispensed into two-ounce plastic containers. Late first or early second instar larvae of Heliothis are weighed and placed in the diet cup. By periodic observations, larval survival, growth and percent pupation are recorded. Dates on fecundity and longevity of emerging adults are obtained to screen resistant types.

MEASUREMENT OF PLANT CHARACTERS AND CORRELATIONS WITH PLANT RESISTANCE

Measuring plant characteristics is crucial for various fields such as botany, agriculture, and horticulture. Here are some key plant characters to measure and the methods commonly used:

- **Height-** Use a ruler or measuring tape. For taller plants, a yardstick or clinometer may be necessary.
- **Leaf Area-** Use a leaf area meter for precise measurements. Alternatively, you can trace the leaf outline on graph paper and calculate the area using grid counting.
- **Stem Diameter-** Measure at breast height (about 1.3 meters above ground) using a caliper or measuring tape.
- **Number of Leaves-** Count manually or use image analysis software for larger populations.
- **Flower and Fruit Count-** Count the number of flowers or fruits per plant or per specified area.
- **Root Depth Method-** Digging around the plant carefully or using a soil probe to measure root length.
- **Biomass-** Harvest the plant, dry it in an oven to a constant weight, and then weigh it.
- **Chlorophyll Content-** Use a chlorophyll meter or perform chemical extraction and spectrophotometry.

- **Leaf Thickness-** Use a micrometer or caliper to measure the thickness of leaves.
- **Growth Rate-**Measure height or biomass over a specific period and calculate the rate of growth.

Tips for Measurement

- **Consistency:** Use the same method for all plants in a study for comparability.
- **Replication:** Measure multiple samples to account for variability.
- **Environmental Conditions:** Record conditions (light, temperature, humidity) during measurement as they can influence results.

These measurements help in understanding plant health, growth patterns, and ecological interactions.

Correlation of plant characters with plant resistance: The correlation of plant characters with resistance to various stresses (such as pests, diseases, drought, or salinity) is a key area of research in plant science and agriculture. Here are some important plant characters often studied in relation to resistance, along with their potential correlations:

I. **Leaf Structure**

- **Thickness:** Thicker leaves may provide better resistance to herbivores and pathogens.
- **Trichomes:** The presence and density of trichomes (hair-like structures) can deter insect feeding and provide physical barriers.

II. **Chemical Composition**

- **Secondary Metabolites:** Compounds such as phenolics, alkaloids, and flavonoids can enhance resistance to pests and pathogens. Higher concentrations often correlate with increased resistance.
- **Tannin Content:** Higher levels of tannins can reduce palatability to herbivores and inhibit pathogen growth.

III. **Root Characteristics**

- **Root Depth and Architecture:** Deeper or more extensive root systems can enhance drought resistance by accessing water from deeper soil layers.
- **Mycorrhizal Associations:** Plants that form symbiotic relationships with mycorrhizal fungi may show increased nutrient uptake and resistance to soil-borne pathogens.

IV. **Growth Rate**

- **Rapid Growth:** Some studies suggest that faster-growing plants can escape herbivore pressure and quickly recover from damage, indicating a correlation with resistance.

V. **Leaf Area and Size**

- **Larger Leaf Area:** While larger leaves can capture more light and enhance photosynthesis, they may also attract more herbivores. The relationship can be complex and context-dependent.

VI. **Flowering Time**

- **Timing of Flowering:** Early or late flowering may help avoid peak pest populations, thus correlating with resistance.

VII. **Water Use Efficiency**

- **Stomatal Conductance:** Plants that efficiently manage water loss can exhibit greater drought resistance, which is critical in arid conditions.

VIII. **Disease Resistance Traits**

- **Genetic Markers:** Certain genetic traits are directly linked to disease resistance (e.g., R genes in crops). These can be measured through molecular techniques.

Correlation Analysis: To determine the strength and nature of correlations:

- **Statistical Methods:** Use correlation coefficients (e.g., Pearson or Spearman) to analyze relationships between different characters and resistance levels.
- **Field Trials:** Conduct controlled experiments to observe interactions under varying stress conditions.
- **Multivariate Analysis:** Explore the combined effects of multiple traits on resistance using techniques like principal component analysis (PCA).

TESTING OF RESISTANCE IN IMPORTANT CROPS

a. Testing pest resistance in rice

- **Infestation Trials:** Introduce pests (e.g., rice stem borers, leafhoppers) to plants in controlled environments or field conditions.
- **Damage Assessment:** Measure the extent of damage caused by pests, including feeding rates and survival rates.

➢ **Genetic Analysis**

- **Molecular Markers:** Use markers associated with resistance traits (e.g., SNPs, SSRs) to identify resistant genotypes.
- **QTL Mapping:** Conduct quantitative trait locus (QTL) mapping to locate regions associated with resistance traits.

➢ **Field Trials**

- **Multi-Location Trials:** Test rice varieties across different environments to assess performance under natural stress conditions.
- **Replicated Trials:** Use randomized complete block designs to ensure statistical validity.

➢ **Data Analysis**

- **Statistical Evaluation:** Use ANOVA and regression analyses to determine significant differences between resistant and susceptible varieties.
- **Correlation Studies:** Assess relationships between morphological, physiological, and yield traits under stress.

b. Testing pest resistance in wheat

➢ I**nfestation Trials:**

- Introduce pests such as aphids (e.g., *Sitobion avenae*) or the wheat stem sawfly in controlled environments or field plots.
- Measure parameters like pest population growth, feeding damage, and crop yield loss.

➢ **Resistance Mechanisms:** Examine physical traits (e.g., leaf pubescence) and chemical defenses (e.g., secondary metabolites) that may deter pests.

c. Testing pest resistance in mustard

➢ **Infestation Trials**

- **Controlled Infestation:**
  - Introduce pests (e.g., *Lipaphiserysimi* for aphids, *Plutellaxylostella* for diamondback moths) to plants in greenhouse or laboratory settings.
  - Use cages or enclosed environments to prevent escape and ensure proper infestation.
- **Natural Infestation:**
  - Conduct field trials in areas with known pest populations to observe how different mustard varieties respond to natural pest pressures.

2. Evaluation Methods

- **Damage assessment:**
  - Evaluate the extent of damage caused by pests through visual inspection, measuring leaf area loss, and assessing overall plant health.
  - Use scales (e.g., 0-5 scale) to categorize damage levels based on symptoms (e.g., leaf curling, feeding marks).
- **Population monitoring:**
  - Record the number of pests on plants at regular intervals to assess pest population dynamics and their relationship to plant resistance.

3. Morphological and Physiological Assessments

- **Physical Traits:** Assess characteristics such as leaf trichome density and thickness, which may influence pest feeding behavior.
- **Chemical Defenses:** Analyze levels of secondary metabolites (e.g., glucosinolates) that can deter herbivory. Higher concentrations may correlate with increased resistance.

BIOASSAY OF PLANT EXTRACTS OF SUSCEPTIBLE/ RESISTANT VARIETIES

Conducting a bioassay of plant extracts from resistant and susceptible varieties can help evaluate their effectiveness against pests or diseases. Here's a step-by-step guide to setting up and performing such a bioassay:

1. Preparation of Plant Extracts

- **Sample Selection:** Choose resistant and susceptible varieties of the target plant (e.g., mustard, rice).
- **Extraction Method:**
  - **Solvent Extraction:** Use solvents like ethanol, methanol, or water to extract phytochemicals.
    - Dry the plant material (leaves, seeds, etc.) and grind it to a fine powder.
    - Soak the powder in the solvent for 24-48 hours.
    - Filter the mixture and concentrate the extract using a rotary evaporator.
  - **Concentration:** Prepare series of dilutions to test different concentrations of extracts (e.g., 1%, 5%, 10 %).

2. Target Organism Selection: Select the pest or pathogen to test against (e.g., aphids, fungal pathogens).

3. Bioassay Setup

- **Methodology:**
  - **Direct Application:**
    - For pests, apply the extracts directly to the insects (e.g., by spraying or soaking).
    - For pathogens, inoculate the pathogen on the plant and then apply the extract.
  - **Petri Dish Method:**
    - For aphids or similar pests, place them on treated filter paper in Petri dishes.
    - For fungal pathogens, inoculate agar plates with the pathogen and apply the extracts.
- **Control Treatments:** Include control groups treated with a solvent (e.g., ethanol or water) without active extracts.

4. Data Collection

- **Pest Mortality:** Record mortality rates at regular intervals (e.g., 24, 48, 72 hours post-application).
- **Disease Severity:** For fungal pathogens, assess disease severity using standardized scoring systems (e.g., lesion size, growth rate).
- **Behavioral Responses:** Observe any changes in feeding behavior, movement, or growth of pests.

5. Statistical Analysis

- **Data Analysis:**
  - Use statistical methods such as ANOVA to compare the effectiveness of extracts from resistant vs. susceptible varieties.
  - Perform post-hoc tests to identify significant differences among treatments.

6. Interpretation of Results

- Analyze which extracts showed higher efficacy against pests or pathogens.
- Correlate the observed resistance with specific phytochemicals present in the extracts.

DEMONSTRATION OF ANTIBIOSIS, TOLERANCE AND ANTIXENOSIS

Determining antibiosis, antixenosis and tolerance is essential for understanding plant resistance mechanisms against pests. The methods used to evaluate them are as follows:

Antibiosis: Antibiosis refers to the detrimental effects of a plant on the growth, reproduction, or survival of pests through the production of toxic compounds or allelochemicals.

Methods to Evaluate Antibiosis:

- **Bioassays:**
  - **Feeding Trials:** Offer pests different plant varieties (resistant vs. susceptible) and measure parameters like weight gain, survival rate, and reproduction.
  - **Toxicity Tests:** Apply extracts or isolated compounds from plants to pests and assess mortality rates over time.

- **Growth Inhibition Tests:** Measure the growth of pests (e.g., length, weight) when fed on plant material from resistant varieties compared to susceptible ones.

Antixenosis: Antixenosis involves behavioral changes in pests due to plant traits that deter feeding or oviposition, such as leaf texture, color, or secondary metabolites.

Methods to Evaluate Antixenosis:

- **Choice Tests:** Set up trials where pests can choose between resistant and susceptible plant varieties. Measure preferences based on feeding or oviposition choices.
- **Feeding Trials:** Assess consumption rates of plant material from different varieties. Lower consumption on resistant varieties indicates antixenosis.
- **Observational Studies:** Monitor pest behavior on different plant varieties, recording time spent feeding or laying eggs.

Tolerance: Tolerance refers to the ability of a plant to withstand pest damage without a significant reduction in growth or yield.

Methods to Evaluate Tolerance:

- **Damage Assessment:** Expose plants to pests and measure the extent of damage (e.g., leaf area loss) along with growth parameters (e.g., height, biomass, and yield).
- **Stress Tests:** Compare the performance of plants under pest pressure versus non-infested controls to determine yield loss and recovery potential.
- **Performance Metrics:** Assess traits like root biomass, leaf area, and overall plant vigor after pest exposure to gauge tolerance levels.