Practical Manual

Techniques In Plant Protection

ENT 518 1(0+1)

M.Sc. (Ag.) Entomology

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Syllabus ENT 518 (0+1): Pest control equipments, principles, operation, maintenance, selection, and application of pesticides; release of bio-control agents; seed dressing, soaking, root-dip treatment, dusting, spraying, and pesticide application through irrigation water; application of drones in plant protection. Soil sterilization, solarization, deep ploughing, flooding, and techniques to check the spread of pests through seed, bulbs, corms, cuttings and cut flowers. Uses of light, transmission and scanning electron microscopy. Protein isolation from the pest and host plant and its quantification using spectrophotometer and molecular weight determination using SDS/PAGE. Use of tissue culture techniques in plant protection. Computer application for predicting/ forecasting pest attack and identification.

Name of Student .....

Roll No. .....

Session .....

Semester .....

Course Name: .....

Course No.: .....

Credit .....

CERTIFICATE

This is to certify that Shri./Km. .....ID No..... has completed the practical of course.....course No. ..... as per the syllabus of M.Sc. (Ag) Entomology ..... semester in the year.....in the respective lab/field of college.

Date: Course Teacher

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Appendix

Pest Control Equipment: Pest control equipment plays a critical role in the effective and efficient application of pesticides and other pest management techniques. The choice of equipment depends on factors such as the type of pest, the size of the area to be treated, the pesticide formulation, and the specific pest control strategy. Here is an overview of the key types of pest control equipment and their uses:

Sprayer:

Hand-operated Sprayers: These are manually operated sprayers used for small-scale applications such as in gardens, nurseries, or small farms. They consist of a tank for holding the pesticide solution, a pump (usually hand-operated), a hose, and a spray nozzle.

- **Knapsack Sprayers:** Carried on the back with a hand-operated pump to build pressure.
- **Compression Sprayers:** Hand-held with a small tank, operated by a pump to pressurize the pesticide inside the tank for spraying.

Power-operated Sprayers: These are motorized sprayers that use gasoline or electric engines to generate pressure, allowing for the spraying of pesticides over larger areas. They are equipped with a high-capacity tank and powerful nozzles for greater reach and efficiency.

- **Portable Power Sprayers:** Mounted on carts or carried by hand but powered by a motor. Suitable for medium-sized farms or orchards.
- **Tractor-mounted Sprayers:** Attached to tractors for large-scale operations in fields and orchards, covering extensive areas quickly.

Dusters: Dusters are devices used to apply powdered pesticides, such as insecticidal dusts or fungicides, to crops. They can be manually operated or powered by motors, depending on the size and type of application.

- **Manual Dusters:** Small, hand-operated devices where dust is manually blown or shaken onto crops.
- **Motorized Dusters:** Powered machines that blow dust more efficiently over wider areas, suitable for larger farms.

Fogging Machines: Fogging machines convert liquid pesticides into a fine mist or fog that can be easily dispersed over large areas. These machines are used for controlling pests in enclosed spaces or in areas with dense vegetation.

- **Thermal Foggers:** Use heat to vaporize the pesticide solution, producing a thick fog that can cover large areas and penetrate foliage.
- **Cold Foggers (ULV - Ultra-Low Volume Foggers):** Use high air pressure to atomize pesticides into a fine mist without the use of heat.

Principles of Operation: Each type of pest control equipment operates based on specific mechanisms designed to optimize pesticide application. The principles of operation vary depending on the equipment type:

- **Hand-operated Sprayers:** These sprayers work by manually creating pressure through pumping, which pushes the pesticide solution through the nozzle in a spray form. The spray pattern and droplet size depend on the type of nozzle used and the pressure maintained by the operator.
- **Power-operated Sprayers:** These use an engine (gasoline or electric) to pressurize the pesticide solution. The operator can adjust the pressure, flow rate, and nozzle settings to achieve a uniform spray pattern. Tractor-mounted sprayers are calibrated to cover large areas evenly, reducing the labor required.
- **Dusters:** Dusters operate by blowing fine pesticide powder onto crops. In motorized versions, a fan or air blower disperses the dust over the plants. The coverage is affected by wind direction, and care is taken to minimize drift.
- **Fogging Machines:** Foggers use either heat (thermal foggers) or air pressure (cold foggers) to convert liquid pesticides into a fine mist. The mist penetrates dense foliage or closed spaces, ensuring the pesticide reaches all areas. Thermal foggers create vapor by heating the liquid pesticide, while cold foggers atomize the pesticide under high pressure.

EQUIPMENT SELECTION AND MAINTENANCE

Equipment Selection:

- **Type of Crop and Pest:** The choice of equipment depends on the type of crop being treated and the type of pest infestation. For example, liquid sprayers are best for broad-spectrum pesticide application, while dusters are ideal for crops sensitive to moisture.
- **Size of Area:** For small-scale operations, hand-operated sprayers or small dusters may be sufficient. For large farms or orchards, power-operated or tractor-mounted sprayers are more efficient, as they can cover large areas quickly.
- **Pesticide Formulation:** Different pesticides require different equipment. Liquid formulations are best applied using

sprayers, while powders require dusters. Fogging machines are ideal for ultra-low-volume applications or pest control in enclosed environments.

- **Precision Requirements:** If the operation requires precise pesticide application, such as for localized pest hotspots or in sensitive crops, smaller, hand-operated sprayers or portable power sprayers are preferable.

Equipment Maintenance:

- **Cleaning:** After each use, equipment should be thoroughly cleaned to remove any residual pesticide, especially from the tanks, hoses, and nozzles. This prevents clogs and corrosion, ensuring consistent performance in future applications.
- **Calibration:** Sprayers and fogging machines should be calibrated regularly to ensure that the correct amount of pesticide is being applied. Calibration involves adjusting the pressure, flow rate, and nozzle settings to match the pesticide requirements.
- **Inspection for Wear and Tear:** Periodically check hoses, nozzles, seals, and other components for any signs of wear or damage. Replace any worn parts to maintain the equipment's functionality and prevent uneven pesticide application.
- **Engine Maintenance (Power Sprayers):** For power-operated sprayers, the engine should be maintained according to the manufacturer's guidelines. This includes checking the oil, air filters, and fuel systems regularly to ensure smooth operation.
- **Storage:** Store equipment in a dry, shaded area to prevent rust and deterioration. Protect the nozzles and other sensitive parts from dirt and physical damage. For long-term storage, empty any remaining pesticide from the tanks and flush the system with water.

MICROSCOPE

The microscope is an optical instrument used to magnify small objects or details, allowing for the observation of structures that are not visible to the naked eye. It typically consists of lenses and a light source to enhance visibility and clarity. Microscopes are used for pest and plant analysis, focusing on their importance, magnification capabilities, specific uses, and maintenance tips.

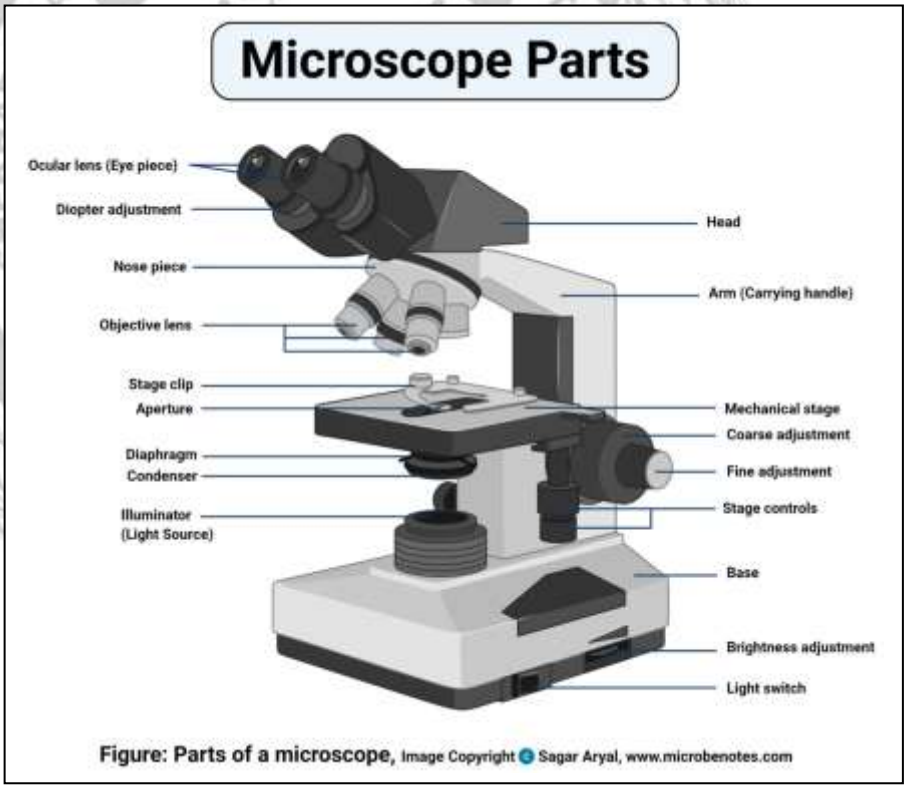


Figure: Parts of a microscope, Image Copyright © Sagar Aryal, www.microbenotes.com

Fluorescence Microscope

Importance:

- Enables visualization of specific components within cells using fluorescent dyes.
- Useful for studying dynamic processes in live cells.

Magnification:

- Typically ranges from 100x to 1000x, depending on the objective lens.

Use For:

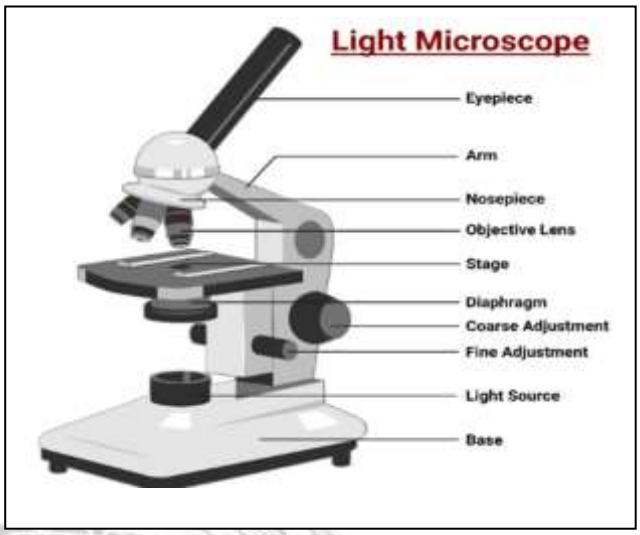
- Examining specific proteins or structures in pests and plants labelled with fluorescent markers.
- Useful in research areas like genetics and cellular biology.

Maintenance:

- Regularly check and replace light sources (e.g., LEDs, mercury bulbs).
- Clean optical components with appropriate solvents.

- Ensure filters are functioning and not damaged.

Light Microscope: A light microscope is a biology laboratory instrument or tool that uses visible light to detect and magnify very small objects and enlarge them.



Importance:

- Versatile for general biological studies.
- Widely available and user-friendly.

Magnification: Typically ranges from 40x to 1000x.

Use For:

- Observing cellular structures, tissues, and small organisms.
- Staining techniques can highlight specific cellular components.

Maintenance:

- Regularly clean lenses with lens paper.
- Keep the microscope covered when not in use.
- Check light source and replace bulbs as needed.

Stereo Microscope (Dissecting Microscope): These are also known as stereoscopic microscopes. This is a type of digital optical microscope designed with a low magnification power (5x-250x), by use of light reflected from the surface of the specimen, and not the light reflected the specimen. Its primary role is for dissection of specimens and viewing and qualitatively analyzing the dissected samples.

Importance:

- Provides a three-dimensional view of larger specimens.
- Ideal for dissection and manipulation of specimens.

Magnification: Usually ranges from 10x to 50x.

Use For:

- Observing larger pests (like insects) and plant structures.
- Useful for educational purposes and hands-on analysis.

Maintenance:

- Clean the lenses and stage after use.
- Check the illumination system and replace bulbs if necessary.
- Ensure proper alignment of optics.

Electron Microscope (Scanning and Transmission)

Importance:

- Provides extremely high-resolution images.
- Essential for detailed ultrastructural analysis.

Magnification:

- Scanning Electron Microscopes (SEM): up to 300,000x.
- Transmission Electron Microscopes (TEM): up to 1,000,000x.

Use For:

- Analyzing fine structural details of pests and plant cells at the nano-meter scale.
- Investigating surface morphology (SEM) and internal structures (TEM).

Maintenance:

- Regularly service the vacuum system and electron gun.

- Clean sample chambers and keep them free from contamination.
- Follow specific protocols for sample preparation to avoid damage.

PRINCIPLES OF PESTICIDE APPLICATION

Correct Timing: Pesticides should be applied at the most vulnerable stage of the pest's lifecycle for maximum effectiveness.

Proper Dosage: Applying the right amount of pesticide ensures efficacy while minimizing harm to the environment and non-target organisms.

Uniform Coverage: Ensuring even distribution of the pesticide across the target area helps prevent untreated areas where pests could survive.

Right Equipment and Technique: Using suitable equipment (sprayers, dusters, etc.) and techniques ensures the pesticide is applied effectively and safely.

Environmental Conditions: Weather factors such as wind, humidity, and temperature should be considered to minimize drift and maximize pesticide uptake.

CLASSIFICATION OF PESTICIDES

Based on Target Pest:

- **Insecticides:** Target insects (e.g., organophosphates, pyrethroids).
- **Herbicides:** Target weeds (e.g., glyphosate, atrazine).
- **Fungicides:** Target fungal diseases (e.g., sulfur, copper-based compounds).
- **Rodenticides:** Target rodents.

Based on Chemical Composition:

- **Organic Pesticides:** Derived from natural sources (e.g., neem, pyrethrin).
- **Synthetic Pesticides:** Man-made chemicals (e.g., DDT, malathion).

Based on Mode of Action:

- **Contact Pesticides:** Kill pests upon direct contact.
- **Systemic Pesticides:** Absorbed by the plant and ingested by pests.

Mode of Action

1. **Contact Action:** Pesticides that kill pests when they come into direct contact with the chemical. Examples include insecticides like pyrethroids, which attack the nervous system.
2. **Systemic Action:** These pesticides are absorbed by plants and translocated through the plant tissues, poisoning pests that feed on them. Examples include neonicotinoids.
3. **Stomach Action:** Pesticides ingested by pests through feeding (e.g., insecticidal baits). These disrupt digestion or poison the pest internally.
4. **Fumigant Action:** Gaseous pesticides that suffocate pests or disrupt their respiratory systems. Examples include methyl bromide.

Application Methods:

- **Seed Dressing**: Coating seeds with a pesticide before planting to protect against soil-borne pests and diseases. **Example:** Treating seeds with fungicides like **Thiram** to prevent fungal infections.
- **Soaking**: Immersing seeds in a pesticide solution for a set period to control seed-borne diseases or pests. **Example:** Soaking rice seeds in **carbendazim** solution to prevent seed-borne fungal infections.
- **Root-Dip Treatment:** Dipping plant roots into a pesticide solution before transplanting to protect against root-borne pests. **Example:** Dipping tomato seedlings in a **chlorpyrifos** solution to control nematodes.
- **Dusting:** Applying dry pesticide powder directly onto plants or soil to control pests. **Example:** Using **sulphur dust** to control powdery mildew on crops.
- **Spraying:** Applying liquid pesticides using sprayers for uniform coverage of crops. **Example**: Spraying glyphosate for weed control in fields.
- **Irrigation-Based Pesticide Application:** Adding pesticides to irrigation water for distribution through the soil to protect crops. **Example:** Applying **nematicides** via drip irrigation to control nematodes in vegetable crops.

Safety Guidelines:

- Study and follow safety precautions for handling and applying pesticides.
- Ensure operators are trained in equipment use and safety procedures.
- Use gloves, masks, goggles, and protective clothing to prevent pesticide exposure.

- ❖ Check for leaks, blockages, and worn-out parts before use.
- ❖ Properly calibrate sprayers and foggers for accurate pesticide application.
- ❖ Mix pesticides in ventilated areas and use proper measuring tools.
- ❖ Store equipment securely and clean thoroughly after use.
- ❖ Avoid spraying in high winds or rain to prevent drift and runoff.
- ❖ Keep first aid kits and emergency equipment nearby.

TECHNIQUES FOR RELEASING BIO-CONTROL AGENTS

Bio-Control Agents: Identification of predators, parasites, and pathogens used for biological pest control (e.g., *Trichogramma*, ladybird beetles).

Releasing Methods: Mass rearing and release of bio-control agents in the field.

Significance: Assess the ecological impact of bio-control agents in reducing pest populations.

DRONE TECHNOLOGY

The application of drone technology in agriculture, particularly for pest monitoring and pesticide application, has been gaining significant momentum due to its efficiency, precision, and environmental benefits. Here's how drones are being utilized in these areas:

Monitoring: Use drones to survey crops for pest infestations, capture aerial images, and analyze pest density.
Pesticide Application: Operate drones fitted with pesticide sprayers to target specific areas.
Advantages: Precision, reduced labor, and optimized pesticide usage.

TECHNIQUES FOR CONTROLLING SOIL-BORNE PESTS

Soil Sterilization: Apply chemical treatments (e.g., fumigants) to sterilize soil.
Solarization: Cover moist soil with transparent plastic sheets during hot seasons to trap heat and kill pests.
Deep Ploughing: Turn the soil deeply to disrupt pest life cycles.

PREVENTING SPREAD OF PESTS THROUGH SEEDS, BULBS, CORMS, CUTTINGS & CUT FLOWERS

Inspection and Certification: Learn procedures for inspecting and certifying pest-free plant materials.
Quarantine Measures: Implement quarantine treatments for imported and exported plant materials.
Cleaning and Treatment: Seed treatment and post-harvest treatments (e.g., hot water or chemical treatments) for bulbs and corms.

PROTEIN ISOLATION AND QUANTIFICATION

These are fundamental techniques in biological research, often used to analyze protein content in plants, pests, or any other biological samples. Spectrophotometers are widely used in the quantification phase because they provide a quick and reliable method to measure protein concentration based on light absorbance. Here's an overview of the entire process, from protein isolation to quantification using spectrophotometers.

Protein Isolation: Protein isolation involves extracting proteins from a biological sample, such as plant tissues, pest bodies, or microbial cultures. The isolation procedure typically involves the following steps:

Steps for Protein Isolation:

- **Tissue Homogenization**: The biological sample is ground or homogenized using a buffer (usually containing a salt solution like Tris-HCl) to break open the cells and release the proteins.
- **Cell Lysis**: Cell disruption can be achieved mechanically (grinding or sonication) or chemically (using detergents and enzymes). This releases proteins into the solution.
- **Centrifugation**: The homogenate is centrifuged to separate soluble proteins from cell debris, membranes, and other insoluble materials. The supernatant (liquid containing proteins) is collected.
- **Precipitation (optional)**: Some protocols may include steps to concentrate proteins by precipitation (e.g., using ammonium sulfate or trichloroacetic acid).
- **Purification**: Depending on the research goal, purification techniques like dialysis, ion-exchange chromatography, or gel filtration may be used to isolate specific proteins.

Once the proteins are isolated, they are quantified using a spectrophotometer.

Protein Quantification Using Spectrophotometers: A spectrophotometer measures the absorbance or transmission of

light by a sample at specific wavelengths. In protein quantification, spectrophotometers can determine protein concentration based on absorbance at particular wavelengths, usually after protein samples are treated with specific reagents. Several methods are commonly used for protein quantification with a spectrophotometer.

COMMON PROTEIN QUANTIFICATION METHODS

UV Absorbance (Direct Measurement): Proteins absorb light in the ultraviolet (UV) range, particularly at 280 nm, due to the presence of aromatic amino acids (tryptophan, tyrosine, and phenylalanine).

- The protein sample is placed in a cuvette, and the absorbance is measured at 280 nm using a UV spectrophotometer.
- The concentration of the protein can be calculated using the **Beer-Lambert law:**

$$A = \varepsilon \cdot c \cdot l$$

where:

- A is the absorbance,
- ε is the molar extinction coefficient (specific to the protein),
- c is the concentration,
- l is the path length (usually 1 cm).

Bradford Assay: The Bradford assay is one of the most commonly used colorimetric methods for protein quantification. It is based on the binding of Coomassie Brilliant Blue dye to proteins, which causes a shift in absorbance.

Principle: The dye binds primarily to arginine residues in proteins, causing a shift in the absorbance maximum of the dye from 465 nm (unbound) to 595 nm (bound).

Procedure:

- Add Bradford reagent to the protein sample.
- Incubate the mixture for a few minutes.
- Measure absorbance at 595 nm using a spectrophotometer.

Bicinchoninic Acid (BCA) Assay

The BCA assay is another colorimetric method that quantifies proteins based on their ability to reduce Cu^{2+} ions to Cu^{1+} under alkaline conditions. The Cu^{1+} ions then react with BCA to form a purple complex, measurable at 562 nm.

Procedure:

- Mix protein sample with BCA reagent.
- Incubate at 37°C for 30 minutes to develop color.
- Measure absorbance at 562 nm with a spectrophotometer.

Lowry Assay: The Lowry assay is a traditional protein quantification method based on the reduction of copper ions in an alkaline solution (similar to BCA) and subsequent reaction with Folin-Ciocalteu reagent to produce a blue color.

Procedure:

- Mix protein sample with Lowry reagent.
- Incubate with Folin-Ciocalteu reagent to develop color.
- Measure absorbance at 750 nm using a spectrophotometer.

Standard Curve Preparation: Most colorimetric assays (Bradford, BCA, and Lowry) require the preparation of a standard curve to accurately determine protein concentration. The standard curve is generated using a known protein standard (usually bovine serum albumin, BSA).

Procedure:

- Prepare a series of dilutions of the standard protein.
- Measure the absorbance of each dilution at the appropriate wavelength.
- Plot absorbance against protein concentration to create a standard curve.
- Use the standard curve to determine the concentration of unknown protein samples by comparing their absorbance.

SODIUM DODECYL SULFATE POLYACRYLAMIDE GEL ELECTROPHORESIS (SDS-PAGE)

This is a widely used technique for determining the molecular weight of proteins. It separates proteins based on their size by denaturing them and forcing them to move through a gel matrix under an electric field. Here's a detailed explanation of how SDS-PAGE works and how it is used to determine the molecular weight of proteins:

Principle of SDS-PAGE: The SDS-PAGE method involves the following key components:

Denaturation of Proteins with SDS

- **Sodium Dodecyl Sulphate (SDS)** is a detergent that binds to proteins and coats them with a negative charge. SDS binds uniformly along the length of the protein, masking its intrinsic charge and causing it to unfold and linearize.
- This denaturation process eliminates the effects of the protein's native structure (such as folds or tertiary/quaternary structure) and charge, so that the proteins differ only in **size**.

Polyacrylamide Gel Matrix

- **Polyacrylamide gel** is used as a sieving matrix. The gel has a network of pores whose size can be controlled by adjusting the concentration of acrylamide.
- Smaller proteins move faster through the gel matrix, while larger proteins encounter more resistance and thus move more slowly.

Electric Field

- An electric field is applied across the gel, with a positive electrode (anode) at the bottom and a negative electrode (cathode) at the top.
- Since SDS-coated proteins are negatively charged, they migrate toward the positive electrode, with the speed of migration inversely proportional to their size.

Procedure of SDS-PAGE

Step 1: Sample Preparation

- **Protein Denaturation**: Proteins are treated with SDS and a reducing agent (e.g., **β-mercaptoethanol** or **dithiothreitol**, DTT), which breaks disulfide bonds between cysteine residues, further ensuring that proteins are fully linearized.
- **Heat Treatment**: The sample is typically heated (at 95°C for a few minutes) to complete the denaturation process.

Step 2: Gel Preparation

- **Gel Composition**: A polyacrylamide gel is prepared with a stacking gel (with larger pores) and a resolving gel (with smaller pores). The stacking gel allows the proteins to enter the gel in a focused manner, while the resolving gel separates the proteins based on size.
- **Acrylamide Concentration**: The acrylamide concentration in the resolving gel is adjusted depending on the size of the proteins being analyzed. Lower percentages (e.g., 7.5%) are used for separating larger proteins, while higher percentages (e.g., 15%) are used for smaller proteins.

Step 3: Gel Loading and Electrophoresis

- **Loading the Gel**: The denatured protein samples are loaded into wells at the top of the gel. A **protein ladder** (a mixture of proteins with known molecular weights) is loaded into one lane to serve as a reference for determining molecular weights.
- **Running the Gel**: An electric current is applied, and the proteins begin migrating through the gel. Smaller proteins move faster, while larger ones migrate more slowly.

Step 4: Staining the Gel

- Once the electrophoresis is complete, the proteins are stained to visualize their migration. Common staining methods include:
  - **Coomassie Brilliant Blue**: A widely used stain that binds to proteins and turns them blue.
  - **Silver Staining**: A more sensitive method for detecting smaller amounts of protein.

Step 5: Visualizing the Gel

- After staining, the gel is visualized under appropriate light (e.g., white light for Coomassie staining). Bands representing individual proteins appear at different positions in the gel, depending on their molecular weight.

MOLECULAR WEIGHT DETERMINATION

To determine the molecular weight of proteins using SDS-PAGE, the migration distance of the protein bands is compared to that of the standard protein ladder.

Step-by-Step Process for Molecular Weight Determination:

Run the Protein Ladder: Include a lane in the gel for the protein ladder (molecular weight marker). This ladder consists of proteins with known molecular weights.
Measure Migration Distance: Measure the distance each protein band (both unknown samples and ladder bands) has migrated from the top of the resolving gel. This distance is inversely related to the logarithm of the protein's molecular weight.
Plot a Standard Curve: Plot the **log of the molecular weights** of the proteins in the ladder against the migration distances of the corresponding bands. This graph will produce a standard curve, which is usually linear within a certain molecular

weight range.
Determine the Molecular Weight of Unknown Proteins: Measure the migration distance of the unknown protein bands. Use the standard curve to determine the molecular weight by interpolating the corresponding molecular weight from the migration distance of the unknown protein.

Factors Affecting SDS-PAGE Results

- **Acrylamide Concentration**: The pore size of the polyacrylamide gel is determined by the concentration of acrylamide. Higher concentrations are used for smaller proteins, while lower concentrations are suitable for larger proteins.
- **Buffer System**: The pH of the running buffer and stacking buffer plays a role in ensuring proteins are properly focused and separated during electrophoresis.
- **Sample Preparation**: Incomplete denaturation or reduction of disulfide bonds may lead to anomalous migration patterns, as some proteins may retain partial structure or aggregate.
- **Post-Translational Modifications**: Modifications like glycosylation or phosphorylation can alter the apparent molecular weight of a protein, causing it to migrate differently than expected.

Applications of SDS-PAGE for Molecular Weight Determination

- **Protein Purification**: SDS-PAGE is used to assess the purity of protein preparations by determining whether only one band (representing a single protein) is present or multiple bands (representing impurities) are visible.
- **Protein Identification**: The molecular weight obtained from SDS-PAGE can help identify unknown proteins by comparing them to known proteins of similar molecular weights.
- **Protein-Protein Interactions**: By analyzing protein complexes under reducing and non-reducing conditions, SDS-PAGE can provide insights into whether proteins form complexes through disulfide bonds.
- **Verification of Recombinant Proteins**: When expressing recombinant proteins, SDS-PAGE is commonly used to verify that the protein has the expected molecular weight, confirming successful expression and purification.

6. Interpreting SDS-PAGE Results

- **Single Band**: If a protein sample results in a single band, it indicates the presence of one predominant protein of a specific molecular weight.
- **Multiple Bands**: If multiple bands are present, the sample may contain multiple proteins, proteolytic fragments, or contaminants.
- **Smearing**: Protein smearing across the gel can indicate overloading, poor sample preparation, or improper gel conditions.

Example: Determining Protein Molecular Weight

- **Step 1**: Prepare a sample of the protein of interest and run it on an SDS-PAGE gel alongside a molecular weight marker.
- **Step 2**: After electrophoresis and staining, measure the distance traveled by the unknown protein bands and the protein standards in the marker.
- **Step 3**: Plot the log of molecular weights of the marker proteins against their migration distances to generate a standard curve.
- **Step 4**: Use the standard curve to estimate the molecular weight of the unknown protein based on its migration distance.

TISSUE CULTURE TECHNIQUES

These are widely applied in plant protection for the production of disease-free, pest-resistant, and high-yielding plants. These biotechnological methods allow for rapid multiplication and propagation of plants with desirable traits. Below is a comprehensive look at the **applications of tissue culture in plant protection**:

Production of Disease-Free Plants: One of the key uses of tissue culture is in generating **pathogen-free plants**, especially for species prone to viral, bacterial, or fungal infections. Tissue culture techniques ensure that plants are clean and healthy, providing a significant advantage in plant protection.

Methods and Applications:

Meristem Culture: Infected plants can often be cleaned of systemic pathogens by culturing their meristem, which is less likely to harbor viruses. This is particularly useful for crops like potatoes, bananas, and sugarcane e.g., in potato cultivation, **meristem culture** has been instrumental in producing virus-free seed potatoes, preventing the spread of viral diseases like potato virus Y (PVY).
Somatic Embryogenesis: This method involves regenerating plants from somatic cells that are not involved in the reproductive process. Somatic embryogenesis can be used to produce disease-free plantlets for crops like coffee and oil palm.

Development of Pest-Resistant Plants: Tissue culture can be integrated with genetic engineering to produce plants that

are resistant to pests and diseases, reducing reliance on chemical pesticides and promoting sustainable agricultural practices.

Methods:

Somaclonal Variation: Tissue culture induces genetic variability (somaclonal variation) in plants. This variation can be screened for resistance to specific pests or diseases. Plants with desirable traits can then be selected and propagated e.g., Somaclonal variation has been used in sugarcane and wheat to develop lines resistant to **rust fungi** and **stem borers**.

Protoplast Fusion: Protoplast fusion combines different plant species or varieties at the cellular level, creating hybrid plants with pest-resistant traits. This technique has been applied to create disease-resistant plants that are otherwise difficult to achieve through conventional breeding e.g., Resistance to bacterial wilt in tomatoes has been improved using protoplast fusion with wild relatives that carry resistance genes.

RAPID MULTIPLICATION OF PEST-RESISTANT VARIETIES

Tissue culture is used for **micropropagation**, which is a method to rapidly multiply pest-resistant or disease-resistant varieties on a large scale. This technique ensures uniformity and helps meet large-scale agricultural demands.

Methods:

Micropropagation (Clonal Propagation Plants with pest-resistant traits, once identified through breeding or genetic engineering, can be rapidly multiplied through tissue culture. This technique allows for the mass production of elite clones with consistent pest-resistant traits e.g., in banana cultivation, tissue culture has been used to propagate varieties resistant to **banana wilt** and **Black Sigatoka**, which are devastating fungal diseases.

CRYOPRESERVATION FOR GERMPLASM CONSERVATION

Cryopreservation is a tissue culture technique used to store plant genetic material (germplasm) at ultra-low temperatures (typically in liquid nitrogen at -196°C). This method plays a key role in the long-term conservation of pest-resistant plant lines and other valuable genetic resources.

Application: Resistant plant varieties, including those with resistance to pests, diseases, or environmental stresses, can be cryopreserved and regenerated when needed. Cryopreservation ensures the availability of pest-resistant plant material for future breeding programs.

In Vitro SCREENING FOR DISEASE AND PEST RESISTANCE

Tissue culture offers a controlled environment to **screen plant lines** for resistance to specific diseases or pests before they are grown in the field. By inoculating plants with pathogens or pests in vitro, researchers can identify resistant lines early in the development process.

Methods:

Callus Culture Screening: Plant tissues (callus) can be exposed to pathogens or pests in vitro to assess their response. Resistant tissues can be selected for further development e.g., Callus cultures of rice have been screened for resistance to the rice blast fungus.

In Vitro Pathogen Challenge: Whole plants or tissues grown in vitro can be inoculated with pathogens to evaluate their resistance. This technique is faster and less expensive than field testing e.g., in grapevine and tomato, in vitro screening for resistance to **fungal pathogens** like **Fusarium** and **Verticillium** has been effective.

GENETIC ENGINEERING FOR PEST AND DISEASE RESISTANCE

Tissue culture is an essential platform for **genetic transformation** in plants. Using tissue culture techniques, plants can be genetically modified to express traits like pest resistance through the introduction of specific genes.

Methods:

Agrobacterium-mediated Transformation: This is a common method for inserting foreign genes into plant cells. After transformation, the modified cells are cultured to regenerate whole plants with new traits, such as resistance to insect pests or herbicides e.g., **Bt cotton** and **Bt maize** are genetically modified to express the **Bacillus thuringiensis (Bt)** toxin, which is effective against various insect pests.

Gene Editing (CRISPR/Cas9): Tissue culture techniques are also used in conjunction with gene-editing tools like **CRISPR/Cas9** to create targeted modifications in the plant genome, allowing precise introduction of pest resistance traits

e.g., Gene editing has been used in tomatoes to improve resistance to viral diseases and nematode pests.

7. Restoration of Endangered Species and Wild Relatives

Tissue culture techniques like **embryo rescue** and **in vitro propagation** are used to restore endangered plant species or wild relatives that harbor pest-resistant genes. These species can then be crossed with cultivated plants to transfer resistance traits.

Application: Wild relatives of crops often possess resistance to pests and diseases, and their genes can be introduced into cultivated species through breeding or genetic engineering. Tissue culture allows the propagation of difficult-to-cultivate species and hybrids that carry valuable pest-resistant traits.

PREDICTING AND IDENTIFYING PEST ATTACKS

This is crucial for effective pest management in agriculture, and there are several software tools and platforms that assist in this task. These tools use various technologies like artificial intelligence (AI), machine learning (ML), remote sensing, and geographic information systems (GIS) to forecast pest outbreaks, monitor crop health, and identify pests in real-time. Here is a list of some of the leading software for **pest attack prediction, forecasting, and identification**:

Data Collection & Monitoring

- **IoT Sensors & Drones**: Collect real-time data on weather, humidity, temperature, crop health, and pest presence using sensors, drones, and satellite imagery.
- **Tools**: Climate FieldView, Trapview, FarmBeats.

Data Integration

- **GIS & Remote Sensing**: Use Geographic Information Systems (GIS) and remote sensing to integrate spatial data on crops and pest populations.
- **Tools**: ArcGIS, ENVI, OpenDroneMap.

Modeling & Pest Forecasting

- **Pest Prediction Algorithms**: Analyze climate data and pest lifecycle models to forecast outbreaks.
- **Tools**: DSSAT (Decision Support System for Agrotechnology Transfer), RIMpro, Zoner.

Machine Learning & AI

- **AI/ML for Pest Prediction**: Machine learning algorithms analyze historical pest data and environmental factors to predict potential attacks.
- **Tools**: Agrio, Farm AI, PEAT (Plantix).

Pest Identification

- **Image Recognition**: AI-powered image recognition tools identify pests and diseases from photos of affected crops.
- **Tools**: Plantix, PlantVillage, Crop Doctor.

Decision Support

- **Recommendations**: Based on forecasts, provide tailored recommendations on pest control (e.g., pesticide application, biological control).
- **Tools**: Cropwise, Field Climate.

Rotary Duster

Knapsac power operated duster

Bucket sprayer with single cylinder

Knapsac sprayer

Rocker Sprayer

Pedal Pump Sprayer

Pneumatic Hand Sprayer

Pneumatic Knapsac Sprayer

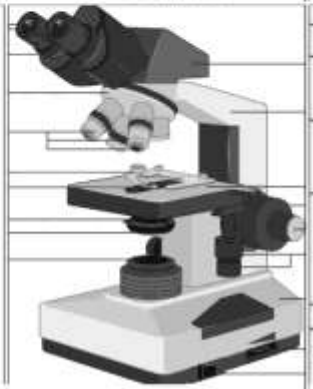
Power operator Mist Blower

Granule applicator

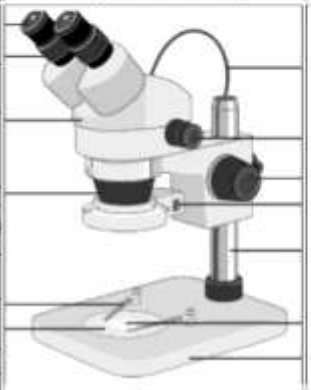
Thanjavur bow rat trap

Flame Thrower

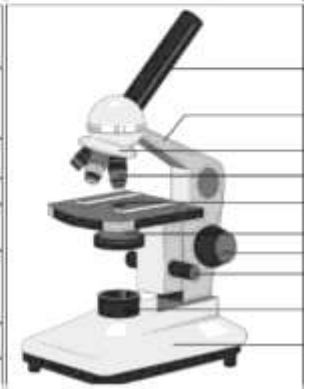
Fogging machine



Binocular Microscope



Stereo Microscope



Light Microscope