MANAGEMENT OF TREE INSECT PESTS AND DISEASES

Practical Manual

M. Sc., Forestry

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Experiment No: 1 **Date:**

Insect collection techniques

Objectives:

1. To study on different insect collection techniques.

A large variety of methods have been devised for collecting insects and arachnids. Some methods are suitable for collecting a wide range of arthropod groups that occur in many different habitats, whereas others are designed for catching specific types of insects and arachnids in particular habitats. The collecting method chosen will depend on the particular species or groups that are being sought, and whether live or dead specimens are required.

Use different methods for insect collection given below:

1. **Collecting bag:** A lightweight bag with a shoulder or waist strap is required for carrying collecting equipment in the field.
2. **Aspirators**
   - An aspirator (also called a pooter) is used for catching small specimens by sucking them into a container. An aspirator consists of a glass or Perspex vial, with a stopper pierced by two flexible tubes.
   - The end of one of the tubes is covered by a small piece of gauze to prevent specimens from being drawn into the operator's mouth.
   - Specimens are collected by sucking on the end of the gauze-covered pipe while holding the end of the other tube close to them.
   - A piece of absorbent paper should be placed in the vial to absorb moisture.
   - Specimens that have been caught in the aspirator can be killed by introducing a small piece of cotton wool dipped in ethyl acetate into the vial.
   - This ball of wool can be blown down the open-ended tube into the vial, to avoid having to remove the stopper and risk specimens escaping.
   - Aspirators are commercially available but can easily be home-made.
3. **Hand collecting**
   - Sedentary or slow-moving arthropods may be collected by hand.
   - As many insects and arachnids can bite or sting, forceps should be used to pick them up, unless one is certain that they are harmless.
   - A wide variety of specimens can be found by searching on plants, which are a food source, refuge or place to lay eggs for many species.
   - Specimens occur on or in various parts of the plant, such as leaves, roots, stems, seeds, fruit and flowers.
   - These places are habitats for spiders, adult and immature insects like moths, flies, butterflies and wasps as well as mites, harvestmen, pseudoscorpions, scale insects, planthoppers, stick insects and mantids.

4. **Collecting nets:** There are three basic types of nets

    1. **Aerial nets**: Aerial nets are used to collect flying insects like butterflies, antlions, flies, dragonflies, grasshoppers, wasps and bees. Aerial netshave a circular frame made from an aluminum strip to which the net is attached. Holes are made at regular intervals in the metal frame for tying on the net, as shown. A frame can also be made of thick wire. A band of cloth, with a deep hem to allow the frame of the net to pass through, is sewn around the top of the net. This band should be of durable material, like calico, to withstand knocks of the frame against stones, trees and other hard objects. The handle of the net can be of wood or aluminum, and should have a comfortable grip. Specimens can be removed from the net with the fingers if harmless, or directed into a killing bottle or vial of preservative. An aspirator is convenient for collecting small specimens caught in the net.

    2. **Sweep nets**: A most effective way of collecting large numbers of specimens, especially the many small bugs, beetles, pseudo-scorpions, spiders and parasitic wasps found in grass and plant foliage, is by means of a sweep net. These types of nets are swung through the vegetation to dislodge the specimens, which are knocked off plants into the bag of the net. Sweep nets are sturdier than aerial nets, with a wider diameter. They usually have a hexagonal shape which allows better contact with the foliage being swept. As with aerial nets, a handle extension can also be used for sampling foliage which would otherwise be out of reach

    3. **Aquatic nets:** An aquatic net should offer minimum resistance when dragged through water, but have a fine enough mesh to capture small specimens. The bag of the net need not be deep, and should be made of a synthetic mesh such as nylon. Transparent material is preferable, to make viewing of the catch possible. The band at the top of the net, as in other nets, must be of strong material. The arthropods caught in the net can be tipped into a white tray or similar receptacle to facilitate sorting.

Other materials required for insect collections are

a. Beating sheets: Well-camouflaged or hidden species on plants are best collected with a beating sheet. This method is useful for collecting sessile or wingless groups such as some beetles and bugs, stick insects, caterpillars, pseudoscorpions, spiders and mites. These are knocked from the vegetation, by beating it with a stick, onto a sheet placed beneath the plants. A hand-held beating sheet is especially convenient for sampling vegetation. This consists of a shallow canvas bag, preferably white, stretched over a folding frame.

b. Knock-down sprays: Knock-down sprays enable the collector to sample areas of a plant that are inaccessible to a net or beating sheet. White sheets are placed beneath a tree or shrub, which is then sprayed with a fast-acting pesticide, such as a synthetic pyrethroid. The dead specimens fall from the tree onto the sheets. This technique does not work well for fast-flying insects that are easily disturbed, such as wasps, flies and grasshoppers.

c. Traps: Use different types of traps for insect attractant and collection from different ecological condition.

- ✓ Yellow-pan traps: Yellow-pan traps are used mainly to collect aphids. They can be made from trays measuring about 40 cm in diameter and painted yellow. These are filled with water containing a little detergent to break the surface tension.
- ✓ Sticky traps: Small flying insects such as aphids, wasps, psyllids, thrips and flies can be collected using sticky traps. These usually consist of a small yellow plate about 15 cm square, or a yellow cylinder with a diameter of about 15 cm and 20 cm long. The plate or cylinder is covered with a sticky substance, such as 'Flytac', and attached to a pole.
- ✓ Paper-band traps: Spiders and insects that live in crevices in the bark of trees, and species that migrate up tree trunks after overwintering in the ground, can be caught using
- ✓ Pitfall traps: Containers such as small plastic buckets, plant pots, glass jars or jam tins are sunk into the ground to trap flightless, ground-living insects and arachnids, especially beetles (ground beetles and toktokkies), cockroaches, crickets, spiders, solifugids, pseudoscorpions, harvestmen and mites.
- ✓ Pheromone traps: Pheromones are chemical substances emitted by insects for communication. Sexual pheromones can be used in traps to attract specimens of the opposite sex. Live females of some species, like emperor moths, that are confined in a trap will attract males over a long distance.
- ✓ Suction traps: Suction traps draw specimens into a receptacle or net by creating a down-draught. They are used to collect ballooning spiders and small flying insects such as flies, aphids and wasps. Although mostly used in quantitative ecological studies, museum specimens can also be collected this way.
- ✓ Light traps: This is the most common method of collecting nocturnal specimens that hide or rest during the day in places where they are unlikely to be seen. Large numbers of insects and a wide variety of species can be caught at night using light traps. The simplest light trap consists of a suspended white sheet with a light hung in front of it. Specimens that settle on the sheet can be collected in killing bottles or with an aspirator

Activates:

1. To collection of insect pest specimens from agro-forestry area.
2. To prepared yellow stick traps for aphid attraction.
3. To prepared pit fall traps and install in the field for observed insect pest population.

Experiment No: 2 **Date:**

Insect killing and temporary storage techniques

Objectives:

2. To study on insect killing method
3. To study on insect preservation techniques.

Once insects and arachnids have been collected, they must be killed and stored until they are mounted and/or preserved permanently. Killing and storage methods vary according to the type of arthropod that has been collected. These procedures are explained below.

Procedure

Use of liquid

- All arachnids, soft-bodied insects (e.g. aphids and termites) and all eggs and larvae must not be allowed to dry out once they are dead. They should either be placed directly into a liquid preservative (usually 70–95 % ethyl alcohol) or, where required, into a fixative such as Pampel's fluid. Large spiders and whip-spiders should first be killed with chloroform or ethyl acetate before being transferred to alcohol. Formalin (formaldehyde) should not be used for storing insects and arachnids as it makes specimens hard and difficult to examine.
- Robust, non-hairy arthropods like beetles, scorpions and ticks can be killed by immersing them in near-boiling water. Scorpions should be left in the water for approximately 5 seconds, until the metasoma straightens. (They can also be placed directly into alcohol, but this may cause contortion of their bodies).
- Once dead they should then be stored in Kahle's fluid. The chelicerae may be pulled forwards and one of the fingers opened to facilitate subsequent examination. Small delicate specimens should be placed in a glass tube before immersion in hot water. Larvae of Lepidoptera and Coleoptera should be placed live into near-boiling water to denature their body proteins and prevent decay. They should then be placed in a fixative like Pampel's fluid before being transferred to a preservative. Each sample should be collected into a separate vial.

Composition for Pampel's fluid: 95 % ethanol (750 ml), distilled water (1375 ml), 40 % formalin (250 ml), glacial acetic acid (125 ml)

Composition for Kahle's fluid: 6 parts formalin**,** 15 parts isopropyl or n-propyl alcohol (99 %)**,** 1 part glacial acetic acid**,** 30 parts distilled water

Freezing: Insects and arachnids can be killed by placing them in a freezer. This method is particularly suitable for reared moths and butterflies. Care should be taken to ensure the specimens are dead before removing them from the freezer, which may take up to 48 hours.

Pinching: Larger butterflies can be stunned or killed by pinching the thorax between the thumb and fore-finger.

Activities:

4. To prepared insect preservative chemicals for store immature stages of insects.
5. To collected immature stages of insect pests and stored in preservative chemicals.

Experiment No: 3 **Date:**

Insect killing bottle and pinning techniques

Objectives:

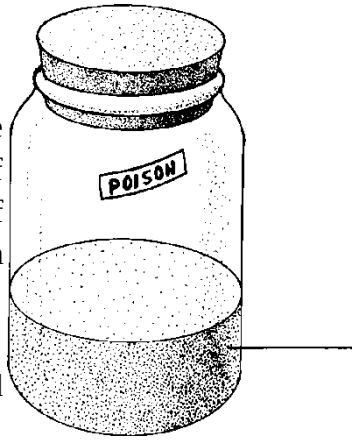
4. To study on insect killing bottle.
5. To study on insect pinning techniques.

Killing bottles:

- Most insects and small arachnids can be killed in a killing bottle. The bottle should be wide-mouthed and made of glass, polypropylene or polyethylene (ethyl acetate dissolves many other plastics). Absorbent paper should be placed inside the bottle to soak up condensation, regurgitated or defaecated liquid and to prevent insects from damaging each other.
- Many species, such as large beetles, can take several hours to die after becoming immobile in the killing bottle, and should not be removed too soon. Note that delicate specimens can be damaged if left too long in the killing bottle.
- Delicate specimens, and all butterflies and moths, should be killed in separate bottles from other insects, otherwise they may be damaged by more robust specimens such as large beetles. Other types of insects will also be covered in scales if placed in the same killing bottle as Lepidoptera.
- The poisons used in killing bottles are hazardous and should be handled with great care. Bottles should be held away from the face when opening them, and care must be taken to avoid inhaling their fumes. They should be kept away from foodstuffs and preferably cleaned outdoors. All killing bottles should be labelled POISON and kept out of reach of children.
- Cyanide killing bottles that are no longer effective should be disposed of by burying them.

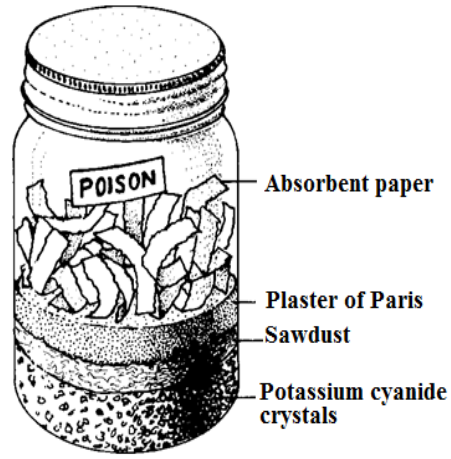
1. **Ethyl acetate bottles**

Procedure



- Make a paste of plaster of Paris and water, and place a thick layer on the bottom of a bottle. A pad of cotton wool can also be used in place of plaster of Paris, but it should be covered with a tight-fitting piece of cardboard to prevent specimens from becoming entangled in the cotton fibres.
- Allow the plaster of Paris to dry in a well-ventilated place.
- Saturate the plaster of paris with ethyl acetate. Any excess liquid should be poured off.
- Place crumpled absorbent paper on top of the plaster of Paris.

- Let the bottle dry out before recharging it with ethyl acetate.
- Ethyl acetate bottles are easy to prepare and are less toxic to humans than potassium cyanide bottles. Green insects should be removed from the bottle as soon as they are dead, as ethyl acetate discolours them.
- Ammonia, benzene, chloroform, carbon tetrachloride and trichloroethylene can also be used, but most of these are hazardous to the collector's health.



Cyanide killing bottles

2. Potassium cyanide bottles:

Procedure:

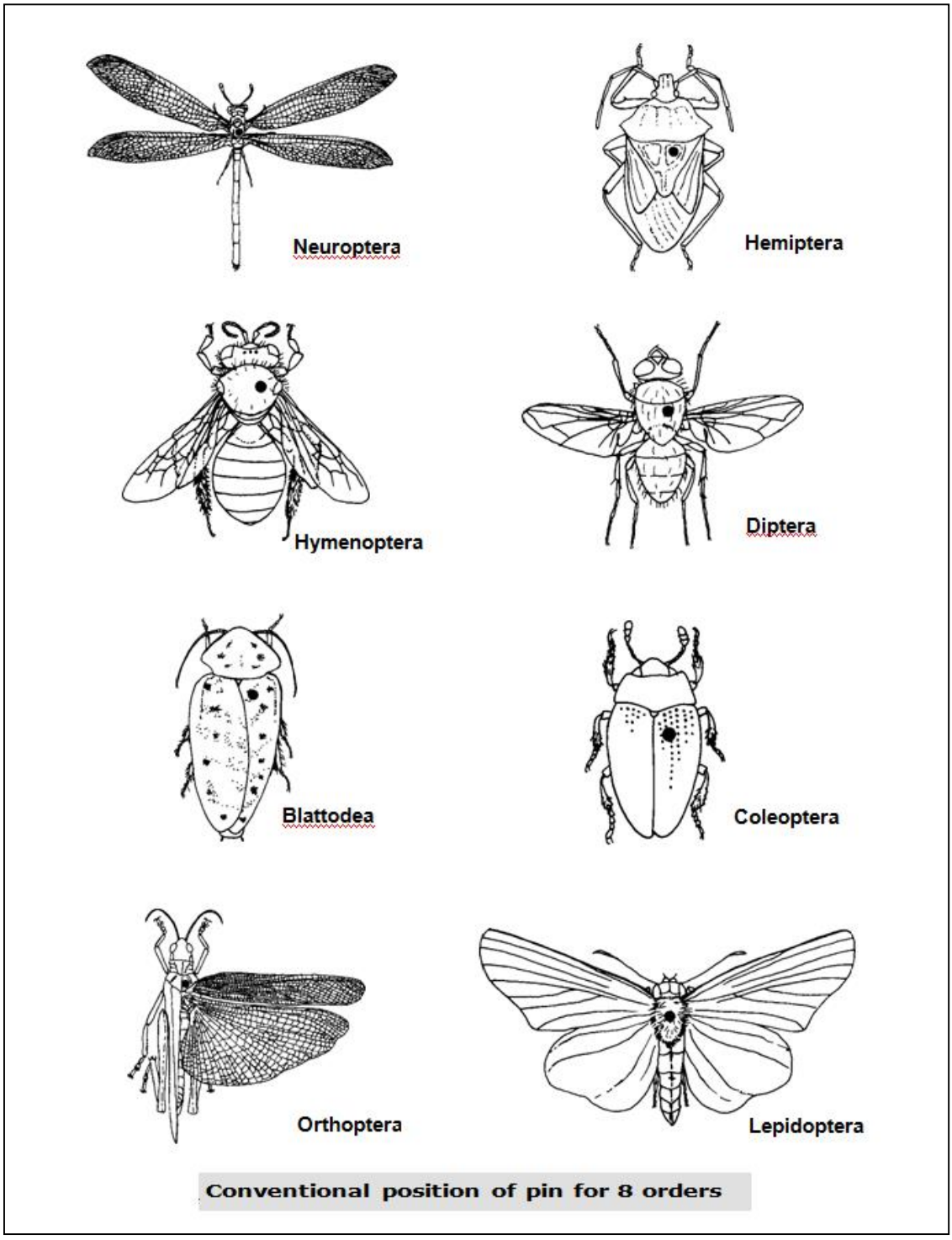
- Place crushed potassium cyanide crystals mixed with sawdust or cotton wool to a depth of 15 mm in a suitable bottle.
- Cover the crystals with a fairly thick layer of plaster of Paris mixed into a paste. A removable porous stopper, made from foam plastic can also be used, which allows for periodic recharging with cyanide.
- Allow the plaster of Paris to dry in a well-ventilated place.
- Add crumpled absorbent paper to the bottle and close the bottle.
- Add a few drops of water to the plaster of Paris (or foam stopper) to activate the bottle an hour or so before use. The water will react with the crystals to produce hydrogen cyanide gas.

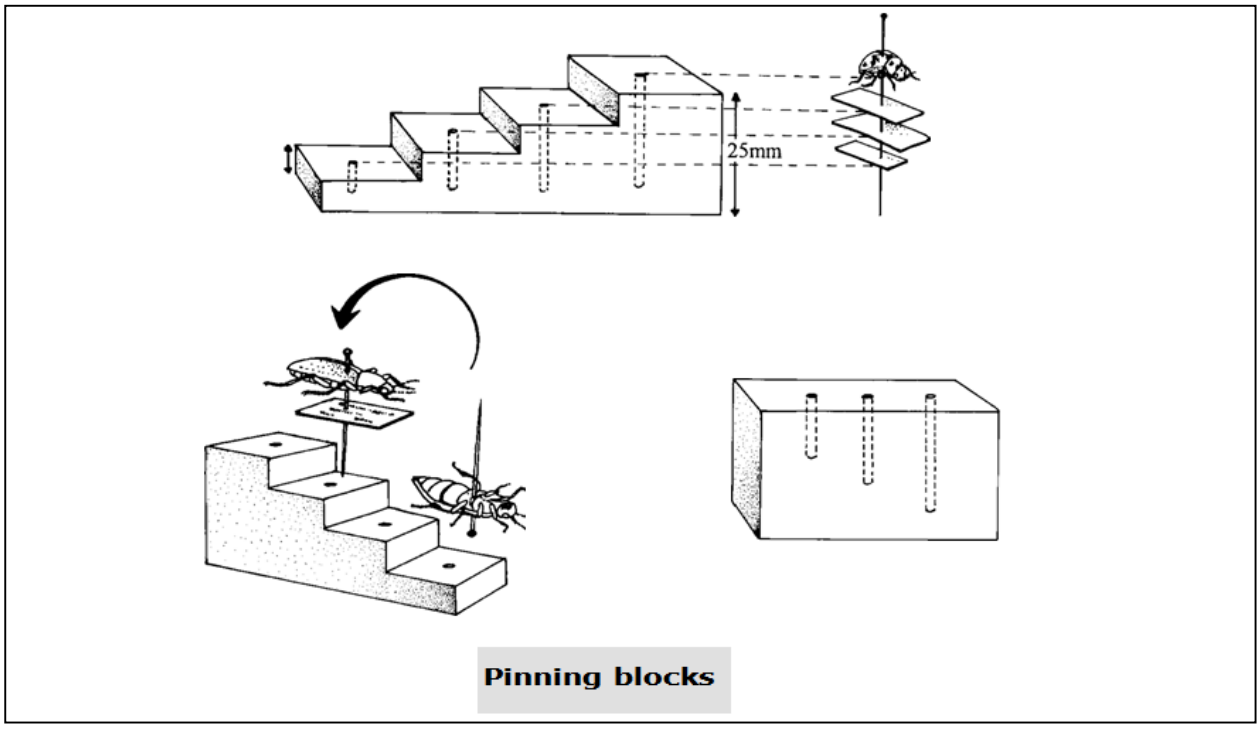
Cyanide bottles are effective for killing most insects except certain Coleoptera. Besides being very toxic to humans, cyanide has a tendency to cause brittleness in specimens and discoloration of yellow-colored bees and wasps. If a killing bottle is used only occasionally, cyanide is not recommended.

Insect pinning

- Prepare a mounting board made of polystyrene covered with paper or expanded polyethylene ('EPX'), at least 30 mm thick.
- Push the pin vertically through the thorax, avoiding the legs as the point of the pin emerges on the underside of the body). The pin should be inserted slightly to the right of the centre of the mesothorax
- Using a pinning block (constructed from hard wood with vertical holes drilled to different depths, adjust the specimen on the pin at a height which leaves the top 8–10 mm of the pin projecting above the insect, to facilitate handling.
- Push the pin with the insect into the mounting board until the underside of the body rests on the board.

- Arrange the legs and antennae close to the body (to reduce the likelihood of damage) and secure them in their positions with bracing pins. Most insects are pinned with their wings folded.
- Secure a data label next to the insect.
- Remove the bracing pins when the specimen is dry.





Activities:

1. To prepared a temporary killing bottle.
2. To collected adults stages of different insect and pinning on suitable point.

Experiment No: 4 **Date:**

Methods of diagnosis and detection of various insect pests

Objectives:

To know the pest and its damage in crop plants for the effective pest management

Diagnosis of pest damage

- Insect and non-insect pests cause a particular type of damage to the plant parts often characteristic to particular pests. The pest mostly insect not present on the site of damage makes it difficult to know the causal agent. Sometimes, the symptoms of damage caused by insects may closely resemble to those resulted due to pathogens or due to nutritional disorders. So, practical experience makes familiar about the correct diagnosis on the basis of visual symptoms of damage to take appropriate control measures.
- The pest mostly insect not present on the site of damage makes it difficult to know the pest. Sometimes, the symptoms of damage caused by insects may closely resemble to those resulted due to pathogens or due to nutritional disorders. So, practical experience makes familiar about the correct diagnosis on the basis of visual symptoms of damage to take appropriate control measures.
- It is very important to know and study about the symptoms of damage by various phytophagous insect pests for proper, effective and economic management. Insect-pests found to be cause injury to plant either directly or indirectly to secure food, their development and further generations. Insect-pests attack on various parts of plant viz., root, stem, bark, leaf, bud, flower and fruit. Based on the nature and symptoms of damage, insect-pests can be classified into different groups as mentioned below.
- Moreover, the process of diagnosing a plant health problem without any specialized laboratory equipment is called field diagnosis. This is the situation at a plant clinic and when making a farm visit. Many times farmer brought the plant sample to us, they may have collected the wrong part of the plant or the sample may have deteriorated in transit. It may be necessary to visit the field to see fresh symptoms and to gain other information on the pest. If you intend to send a sample to a colleague or a formal diagnostic support service, it is usually a good idea to visit the field yourself and to select a fresh sample of your own.

General points to take in consideration during diagnosis

- Don’t be in too much of a hurry. Slow down, cut open the plant and have a look inside.
- Use a hand lens to look for small insects and their stages.
- Most importantly, talk to the farmer and ask questions relating to what you are looking at and the ideas that are forming in your mind.

The following is a summary of what to do when visiting a field to observe the symptoms in the context of the entire crop.

Step 1: Get in close

- What parts are affected?
- Describe symptoms using the correct terminology.
- Observe changes in shape, colour and growth.
- Look for visible signs of insect pests and non-insect pests.

Step 2: Look at the whole plant (including Roots)

- Where are the symptoms within the plant?
- Which growth stages are affected?
- How do the symptoms progress from early to late stages?
- How severe is the attack?

Step 3: Examine groups of plants

- Incidence: how many plants are affected?
- Distribution: random, edge of the plot only, in patches, pattern caused by use of machinery?
- Remember: consider plant variety, age and how it is grown.

Step 4: Speak To farmers and other local extension workers

- When did the problem appear? Is this the first time?
- Record local name for the problem.
- Consider soil type and climate (patterns).
- Obtain information on the varieties used, recent history of chemical inputs used, etc.

Classification of insect pests based on the damage with special reference to the parts they damage for effective insect pests diagnosis

1) **Root feeding insects**: Insect larvae feed on roots, root nodules; nymphs and adults suck sap from roots, resulting in stunted growth, poor tillering, drying of plants in isolated patches etc. White grubs, grubs of rhinoceros beetles, termites, rice root weevil & ragi root aphid.
2) **Stem borers**: Larvae enter into the shoot of main stem, tillers and feed on the central growing point. As a result, nutrient supply from the main plant beyond the infested part is affected leading to withering, wilting and drying up exhibiting symptoms such as dead heart / white ear / over growths of bunchy top etc. Stem borers of paddy, millets, sugarcane, brinjal, bhendi, cotton etc. 3
3) **Shoot borers**: Larvae attack tender shoots and bore inside during the vegetative stage of crop growth and cause wilting, dropping of terminal plant part which later dries up. Shoot fly of sorghum, early stem borer in sugarcane, stem fly in black gram/ French bean, soybean, shoot borers of brinjal, bhendi, cotton, castor etc.
4) **Tree borers**: Larvae bore deep into the tree trunk, tunnels in zigzag manner and feed on inner tissues, affecting nutrient and translocation of sap to upper portions of branches / tree exhibiting symptoms such as withering of leaves, drying of twigs or complete dying of tree. Presence of fresh powdered material, ooze of gummy, exudations etc. from the affected portion on the tree trunk is also seen in some cases. Tree borers of mango, cashew; coconut red palm weevil etc.

5) **Bark borers**: Larvae enter into the bark and tunnel into the branches. The larvae remain hiding in the galleries formed from floss / fecal matter and silken saliva on the stem and continue to scrape the bark. Larval feeding results in drying of branches and breaking of affected portion with wind or gale. Bark eating caterpillars of citrus, mango, guava, casuarinas, jack etc.
6) **Gall formers**: Larvae/nymphs feeding inside the stem! tiller /leaf/ flower bud affect the tissue by nibbling the meristamitic tissues and secretion of auxins that results in excessive growth of cells at the affected portion leading to distorted growth and malformation of plant parts known as 'Gall'. Paddy gall midge, chilly midge, gingelly midge, cucurbit stem borer, mango malformations, tobacco stem borer, cotton stem weevil, mango inflorescence midge, chilli midge etc.
7) **Leaf folders:** Larvae fasten the margins of individual leaves from margins / fold longitudinally or roll leaves into bell shape and feeds within by scrapping the chlorophyll. Rice/ maize/leaf folder, cotton leaf roller, red gram / black gram leaf folder.
8) **Leaf miners**: Larvae fasten the leaves /leaflets by means of silken threads (derived from saliva) and scrape the chlorophyll content by remaining within the web. Fecal pellets / frass remain present in the web. Leaf webbers on groundnut / gingelly, Webbers of mango / sapota/Cashew.
9) **Leaf webbers**: Larvae fasten the leaves /leaflets by means of silken threads (derived from saliva) and scrape the chlorophyll content by remaining within the web. Fecal pellets / frass remain present in the web. Leaf webbers on groundnut / gingelly, Webbers of mango/sapota/Cashew.
10) **Defoliators/ Skeletonizers**: Larvae feed on the leaves completely leaving only midrib / veins or scrape the chlorophyll content of leaves or cause numerous holes. Castor semilooper, red hairy caterpillar, bihar hairy caterpillar, snakegourd semilooper, ash weevils, tobacco caterpillar, brinjal epilachna beetle.
11) **Pod/Capsule borers**: During the reproductive stage of crop, larvae bore into the flowers, pods, capsules and fasten the adjacent plant parts with silken threads, fross and excreta and feed on the internal contents within the web. Spotted pod borer in legumes, capsule borers of castor / gingelly; pod borer complex in pulses, gram caterpillar, pink bollworm, tobacco caterpillar, chilly pod borer etc.
12) **Fruit borers/ Bollworms**: Larvae enter into the tender fruits bolls and feed on internal content /pulp and plug the larval burrow with excreta. Fruit borer of brinjal/bhendi/tomato, mango fruit borer, fruit fly, mango stone weevil, cashew apple and nut borer, Anar/ guava fruit borer, Cotton bollworm etc.
13) **Seed feeding insects/ stored grain pests**: Larvae feed on stored seeds either as internal/ external feeders / by webbing the food particles. Rice weevil, lesser grain borer, red rust flour beetle, rice moth, cigarette beetle, saw ^ toothed beetle etc.
14) **Sap sucking insects/ Feeders**: Nymphs and adults suck sap from the base of the plant/leaves / tender terminal plant parts and affect the vigor and growth of the plants. Different insects exhibit different symptoms. Most of the sap suckers suck; sap in excess of their requirement and excrete honey dew, which is rich in sugars, a

resource for sooty mold development. Aphids, leafhoppers (jassids), plant hoppers, white flies etc on important crops.

Activities:

1. To collect the insect specimens from different plantation crops plant parts.
2. To collect the insect specimens from different field crops.

Experiment No: 5 **Date:**

Botanical insecticides

Objectives:

1. To study on different botanical insecticides.
2. To prepare different botanical insecticides under laboratory.

Natural compounds called botanical pesticides work well against viruses, nematodes, bacteria, fungus, and insect pests. They are abundantly available in the environment, highly biodegradable, have a variety of mechanisms of action, are less harmful to humans, and are not pollutants. Or

One of the naturally occurring chemicals found in plants is referred to as botanical pesticides. Nature-oriented pesticides can be used as an alternative to synthetic formulations, but they are usually claimed to be more toxic to humans. Some of the most lethal carcinogenic substances, like deadly toxins, develop quickly and thrive in nature.

Major insecticides

Generic Name	Oral LD50	Dermal LD50	Signal Word
Pyrethrins	1,200-1,500	>1,800	Caution
Rotenone	60-1,500*	940-3,000	Caution
Nicotine	50-60	50	Danger
d-Limonene	>5,000	—	Caution
Linalool	2,440-3,180	3,578-8,374	Caution
Neem	13,000	—	Caution

1. **Pyrethrum and Pyrethrins**: Pyrethrum is the powdered, dried flower head of the pyrethrum is daisy, *Chrysanthemum cinerariaefolium*. Most of the world's pyrethrum crop is grown in Kenya. The term "pyrethrum" is the name for the crude flower dust itself, and the term "pyrethrins" refers to the six related insecticidal compounds that occur naturally in the crude material, the pyrethrum flowers. They are extracted from crude pyrethrum dust as a resin that is used in the manufacture of various insecticidal products.
2. **Rotenone:** Rotenone is insecticidal compound that occurs in the roots of *Lonchocarpus* species in South America, Derris species in Asia, and several other related tropical legumes. Commercial rotenone was at one time produced from Malaysian Derris. Currently the main commercial source of rotenone is Peruvian *Lonchocarpus*, which often is referred to as cube root.
3. **Nicotine:** Nicotine is a simple alkaloid derived from tobacco, *Nictiana tabacum*, and other Nicotiana species. Nicotine contitutes 2-8% of dried tobacco leaves. Insecticidal formulations generally contain nicotine in the form of 40% nicotine sulfate and are currently imported in small quantities from India.

4. **Neem:** Neem products are derived from the neem tree, *Azadirachta indica* that grows in arid tropical and subtropical regions on several continents. The principle active compound in neem is azadirachtin, a bitter, complex chemical that is both a feeding deterrent and a growth regulator. Meliantriol, salannin, and many other minor components of neem are also active in various ways. Neem products include teas and dusts made from leaves and bark, extracts from whole fruits, seeds, or seed kernels, and oil expressed from the seed kernel. The product known as "neem oil" is more like vegetable or horticultural oil and acts to suffocate insects. Neem and neem oil are often confused.
5. **Citrus Oil Extracts: Limonene and Linalool:** Crude citrus oils and the refined compounds d-limonene (hereafter referred to simply as limonene) and linalool are extracted from orange and other citrus fruit peels. Limonene, a terpene, constitutes about 90% of crude citrus oil, and is purified from the oil by steam distillation. Linalool, a terpene alcohol, is found in small quantities in citrus peel and in over 200 other herbs, flowers, fruits, and woods.

❖ **Standard Procedures for the Preparation and Application of Neem Extracts**

- Select healthy neem leaves that are free from diseases.
- When storing the plant parts for future usage, make sure that they are properly dried and are stored in an airy container (never use plastic container), away from direct sunlight and moisture. Make sure that they are free from moulds before using them.
- Use utensils for the extract preparation that are not used for your food preparation drinking and cooking water containers. Clean all the utensils properly before and after use.
- Do not have direct contact with the crude extract while in the process of the preparation, and during the application.
- Make sure that you place the neem extract out of reach of children and house pets while leaving it overnight.
- Harvest all the mature and ripe fruits on the crop to be sprayed before neem application.
- Always test the plant extract formulation on a few infested plants first before going into large scale spraying. When adding soap as an emulsifier, use a potash-based one such as gun soap (Kenya).
- Wear protective clothing while applying the extract.
- Wash your hands after handling the plant extract.

❖ **Detailed recipe to prepare 10 litres of Neem Seed Kernel Extract (NSKE):**

- Grind 500 grams (g) of neem seed kernels in a mill or pound in a mortar.
- Mix crushed neem seed with 10 litres of water.
- It is necessary to use a lot of water because the active ingredients do not dissolve easily.
- Stir the mixture well.
- Leave to stand for at least 5 hours in a shady area.
- Spray the neem water directly onto vegetables using a sprayer or straw brush.
- Neem water can be stored and will remain effective for 3 to 6 days if it is kept in the dark.
- It has been estimated that 20 to 30kg of neem seed (an average yield from 2 trees), prepared as neem water can treat 1 hectare of crop.

1. **Neem seed kernel powder:** Collect neem fruits locally, remove the outer skin along with the pulp, clean the seeds and dry in shade. After a week, remove the seed coat and grind the resultant contents into fine powder. Sieve it and preserve in glass jars for further use.
2. **Pongamia leaf extract:** Farmers of Karnataka collect the leaves of pungam (*Pongamia* spp.) which grow on river sides, road sides and in forests and incorporate them in water-logged paddy fields before transplanting. The leaves get thoroughly mixed during the puddling operation, and once they mix well in the field they decay within 2 or 3 days.
3. **Ipomoea leaf extract:** Clip and pool 5 Kg of fresh leaves of Ipomoea, leaving the waste materials like waste bark and dried stem. Clip the fresh leaves and make them into fine paste. Mix the paste with equal volume of water and boil. Now add little quantity of cow urine to this boiling mixture. When mixture is at one fourth of its actual volume, remove from the burner and allow it to cool for overnight. Filter the solution in the morning and repeat the washing with cow urine and make the volume to 4.5 litre. Add 250g of soap powder in 500 ml of water and mix the extract with the soap solution and use 500 ml in 10 litre of water for an acre.
4. **Vitex leaf extract:** This is commonly available on the road sides. Pluck the fresh leaves, clean and chop them well using grinder and make them into paste. Soak this in 3 litre of clean, filtered cow urine for overnight. In the next day morning, stir the soaked solution and boil at 70-80°C. For about 3-4 hours. Add fresh distilled water when the solution in concentrated too much allow it to cool overnight and filter using kada cloth. Mix 150 g of soap powder in 250 ml of water and add to the filtrate. Dilute 50 ml of final solution mixture in 1 litre of water to spray.
5. **Calotropis leaf extract:** Collect calotropis leaves, 1 kg with latex, clean well from impurities, chop and make them into fine paste and mix this paste with 50 l. of water and filter the suspension and spray.
6. **Tinospora plant extract:** Chop vines of *Tinospora cordifolia* Boerl, grind into paste with water and stir thoroughly. Rice seedlings are soaked in this liquid for whole night before transplanting. About 10-15 kg of chopped vines is needed for the treatment of seedlings for planting in one hectare. This is effective against the rice green leafhopper. The vines are cut into 30 cm long pieces or tie it to couple of bamboo pole on both ends and keep them in the field. Sometimes, smaller pieces are stuffed in net type shopping bag. The bag as such is kept at the water inlet of the field. However, the vine pieces should be removed before the grain sets.
7. **Jatropha plant extract:** Clean stem and leaves of Jatropha plant with pure water and grind well, soak this oily paste, 5 kg in 5 litre of cow urine for overnight and boil it for 3-4 hours in 2 litre of water. The material is concentrated to 5 litre and filtered in coarse kada cloth and the filtrate is sprayed.
8. **Tobacco waster extract:** Collect the tobacco waste from the field after harvest or form shops, cut them (5 kg) into pieces and soak in cow urine of 5 litre for 7 days. Filter the suspension and mix 150 g of soap and 250 ml of water and spray @ 50 ml/litre of water.
9. **Agave flesh extract:** Agave plants which grow on the road sides are cut, cleaned, chopped into pieces and then made into paste by grinding well. Soak it in pure water for 24 hour. the suspension must be mixed well and filter it using muslin cloth. About 500 ml of cow urine may also be added at every spray to 100 ml of the extract and 10 g of soap solution.
10. **Garlic clove extract:** Chop clean garlic bulbs, make them into paste and soak with equal volume of kerosene and allow the mixture to stand still for overnight. Mix it and

filter with fine kada cloth. Add 150 g of common soap powder to the above mix and store in a clean glass bottle.

11. **Green chilli and garlic extract:** Wash destalked fresh green chillies in distilled water, make them into fine paste, mix in 10 litre of water and boil at 70-80°C till the contents are reduced to half. Chop clean garlic cloves, make them into paste and soak with equal volume of kerosene and then allow the mixture to stand still for 12-24 hours and later mix it and filter using fine kada cloth.Mix about 150 g of common soap powder with 250 ml of water and mix the above three extracts together, spray 20 ml of the extract per 1 litre of water.
12. **Aqueous solution of kernels of *Melia azedarach*:** *Melia azedarach* kernels are soaked in a liter of water and boiled for half an hour and kept undisturbed for 24 hours.1 litre of cow urine is mixed with 159 g of soap powder and filtered. The filtrate is mixed with Melia azedarach kernel extract and stored for future use.
13. **Nerium seed extract:** Seeds of Nerium indicum are collected, shade-dried for a few days, made into powder, sieved, and then soaked in water to be left undisturbed for overnight, filter with kada cloth and filtrate is sprayed.
14. **Mahuva oil spray:** Mix 30 ml of the Mahuva oil and 5 g of soap with 1 litre of water for spray.
15. **Fish oil rosin soap solution:** Fish oil rosin soap 1 litre is added to water and tobacco extract and soap are added. Spraying is done in morning hours.
16. **Cigar end filtrate:** Boil non-filter-tipped cigarette ends or half of filters in 9 litre of water for half an hour. Strain through muslin cloth and heat the clear brown liquid with one of soft soaps till it is dissolved. Mix 1 part of brown fluid with 4 parts of water for spraying.
17. **Cow dung extract:** Collect fresh cow dung, dissolve it at the rate of 100-g in 100 ml of water, filter with kada cloth and spray.
18. **Neem leaf powder:** Collect fresh neem leaves, clean well to ensure free of dirt and dusts, and dry it in shade for 15 days. Then, crush the leaves to powder form and treat the seeds.
19. **Adathoda leaf dust:** Collect Adathoda leaves locally, clean from impurities and shade dry for a week. Crush the shade-dried leaves into fine powder and store for future use.
20. **Cannabis leaf dust:** Collect cannabis leaves, shade dry, crush into fine powder and use.
21. **Nochi leaf dust:** Collect Nochi leaves locally, shade dry for 15 days, crush into fine powder and use.
22. **Tobacco leaf dust:** Dry the cured tobacco leaf in the shade, make it into fine powder and use.
23. **Ipomoea leaf dust:** Collect Ipomoea leaves from the plants found on the waste lands and irrigation canals, dry for 15 days, make it into fine powder and use.
24. **Tobacco dust:** Collect tobacco plant waste materials from the field as well as shops, shade-dry for about 15 days and then powder finely.
25. **Neem and Datura leaf powder:** Collect Neem and Datura leaves, clean and dry them under shade. After a week, crush the dried leaves into powder and store for future use. Neem and Datura are mixed in equal amounts.
26. **Turmeric powder:** Prepare Turmeric powder out of turmeric rhizome gathered from the local market and store in glass bottles.
27. **Vasambu rhizome powder:** Prepare vasambu rhizome collected from the local market, into fine powder after removing its outer skin and use.

28. **Rice bran and kerosene mixture:** Sieve the rice bran gathered from the rice mill to get fine particles and mix it (14 kg) thoroughly in 2 l. of kerosene and dust the mixture in the morning hours.
29. **Rice and saw dust:** Rice bran and saw dust gathered from the rice mill and saw mill are used.
30. **Saw dust:** 15 kg of sieved saw dust is mixed with 2 l. of kerosene and used as dust.
31. **Cycas cone pieces:** The cycas cone is available in the western ghats of the Tamilnadu border at Pulayari in Tirunelveli District and Ariyankkavu in Kerala state. The male cones when become matured, emit the fragrant smell, which is insecticidal in action. These cones are cut into small pieces and kept in a bunch of wet paddy straw. This is placed on the top of stick kept in the field to the level of plant canopy. The paddy straw is continuously kept wet to enhance the odour emission.
32. **Tree bark pieces:** Mix barks of various trees like cinnamomum zeylanicum, and Eucalyptus in a ratio of 1:4 and keep the bags of paddy seeds. The barks are broken in a reasonable size and then used.
33. **Common Ash:** Prepare ash from the common fire wood and dry leaves after burning, grind ad sieve to get fine powder and use.
34. **Brick kiln ash:** Collect ash from brick kiln and sieve for fine particles and use.
35. **Wood ash dust:** The ash is collected from the residues of fire wood and cleaned well by sieving. The fine powder thus collected is stored in separate clean containers for future use.
36. **Fly ash:** Grind the lignite fly ash available from Neyveli Lignite Corporation, Neyveli into fine powder. Sieve it to get fine powder and store in glass containers for future use.
37. **Sand:** River sand is sieved to fine and used in seed treatment.
38. **Leaves of Dhumas (*Combretum ovalifolium* Spreng.):** Collect leaves of Dhumas, a shrub found along farm boundary and in waste lands. About 5-7 persons stand in a row and keep leaves of this plant in individual bags on their back. They start moving from one side of the field to the other in the direction of wind. In the way, they catch 1-2 bugs from the air and crush them with 2 or 3 leaves by rubbing palms of hands. Peculiar smell comes from the mixture of leaves and insects. According to user of this practiced, the pest will fly away in the direction of wind due to peculiar odour. As soon as the smell is over (or) got reduced i.e., after 2-4 min. fresh insects and leaves are crushed continuously till all the insects fly away in one direction quickly.
39. **Calotropis stem scare:** The farmers remove the leaves and then split the stems of calotropis vertically into the two halves with a sickly and cut them into small pieces (10 to 15 cm long). Scatter these dieces in the field along the border and on bunds at a distance of 2 to 3m. In such a way that their top sides remains up. The stem surface which resembles the skin of the snake ocally found is believed to scare the rats away.
40. **Glyricidia rat killer:** Glyricidia sepium, a fast growing leguminous tree is a "rat killer". Farmers grind the bark and leaves, mix it with damp wheat or smear it on banana slice and use it for the purpose. A mixture of cereals and the leaves of Glyricidia are ground together and allowed to ferment. The fermented solution is used as bait for the rats.Bunch or throny thistle flower is tied to the ends of the beam holding food shift deters the rat by pricking them and so the rats do not try to cross over the thorns.
41. **Turmeric cooked rice:** Turmeric powder-mixed cooked rice, which will be yellowish is placed in the field for infestation by the worms and the semiloopers and birds are attracted to this. They will be picked by the birds sitting on poles set in the field. Approximately 1 kg rice is needed for an acre. Small umps of the yellow coloured rice

are placed in the field, at 5 m apart in early morning or late evening, this practice initiated when significant numbers of larvae begin to appear. It is repeated continuously for 2 or 3 days. The first day usually passes as a baiting day, but from the second day large number of birds, attracted by the rice, will prey upon the larvae. The food is offered in a thin dish made from the cross section of a banana leaf sheath balanced delicately on a slated, when the birds attempt to take the food, accidentally tilts and the food falls down to the ground. When the birds go for the fallen food, they find the caterpillars then and eat them too.

Activities:

1. To prepared neem seed kernel extract.
2. To collect the plants have insecticidal properties.

Experiment No: 6 **Date:**

Pesticides formulations

Objectives:

To study on different pesticide formulation.

Insecticides formulation

Formulation: A formulation is a mixture of ingredients prepared in a certain way and used for a specific purpose.

Formulation Classification

1. **Solid formulations:** Dust, wettable or water dispersible powder, granules, capsules, baits etc.
2. **Liquid formulations:** Solution, emulsifiable concentrate, ultra low volume formulations, suspension etc.
3. **Gaseous formulations:** Fumigant, aerosol, foams, smokes, mists and fog.

Different formulations

1. **Dusts**: Dusts are manufactured by the sorption of an active ingredient onto a finely-ground, solid inert such as talc, clay, or chalk. They are relatively easy to use because no mixing is required and the application equipment (e.g., hand bellows and bulb dusters) is lightweight and simple. Dusts can provide excellent coverage, but the small particle size that allows for this advantage also creates an inhalation and drift hazard. Dusts are generally applied as spot treatments for insect and disease control outside. Commercial pest control operators use dusts effectively in residential and institutional settings for control of various insect pests. Indoors, this type of formulation permits the delivery of an insecticide into cracks and crevices, behind baseboards and cabinets, etc. Thus, the insecticide is placed into the pest's habitat and away from contact by people and pets.
2. **Granules:** The manufacture of granular formulations is similar to that of dusts except that the active ingredient is sorbed onto a larger particle. The inert solid may be clay, sand, or plant materials. A granule is defined by size: Granule-sized products will pass through a 4-mesh (number of wires per inch) sieve and be retained on an 80-mesh sieve. Granules are applied dry and usually are intended for soil applications where they have the advantage of weight to carry them through foliage to the ground below. The larger particle size of granules, relative to dusts, minimizes the potential for drift. There is also a reduced inhalation hazard, but some fine particles are associated with the formulation. In addition, granules have a low dermal hazard. The primary drawbacks of granules are their bulk, the problems they present in handling, and the difficulty inherent in achieving uniform application. Granules also may have to be incorporated into the soil to work, and they are sometimes attractive to non-target organisms such as birds.
3. **Pellets:** Pellets are very similar to granules, but their manufacture is different. The active ingredient is combined with inert materials to form slurry (a thick liquid

mixture). This slurry is then extruded under pressure through a die and cut at desired lengths to produce a particle that is relatively uniform in size and shape. Pellets are typically used in spot applications. Pelleted formulations provide a high degree of safety to the applicator. They do have the potential to roll on steep slopes and thereby harm non-target vegetation or contaminate surface water.

4. **Wettable Powders**: Wettable powders are finely divided solids, typically mineral clays, to which an active ingredient is sorbed. This formulation is diluted with water and applied as a liquid spray. The mixture forms a suspension in the spray tank. Wettable powders will likely contain wetting and dispersing agents as part of the formulation. These are chemicals used to help wet the powder and disperse it throughout the tank. Wettable powders are a very common type of formulation. They provide an ideal way to apply (in spray form) an active ingredient that is not readily soluble in water. Wettable powders tend to pose a lower dermal hazard in comparison to liquid formulations, and they do not burn vegetation as readily as oil-based formulations. This formulation can present an inhalation hazard to the applicator during mixing because of the powdery nature of the particles. Furthermore, there are a series of disadvantages associated with all formulations that form a suspension in the spray tank: They require agitation to prevent settling out; they can be abrasive to equipment; and they may cause strainers and screens to plug.
5. **Dry Flowables**: Dry flowables—or water dispersible granules, as they are sometimes called—are manufactured in the same way as wettable powders except that the powder is aggregated into granular particles. They are diluted with water and applied in a spray exactly as if they were a wettable powder. Dry flowables form a suspension in the spray tank; they have basically the same advantages and disadvantages as wettable powders, with several important exceptions. During the mixing process, dry flowables pour more easily from the container and, because of their larger particle size, reduce inhalation hazard to the applicator. Note: The labels of some dry flowables do permit application of the product in the dry state.
6. **Soluble Powders**: Soluble powders, although not particularly common, are worth mentioning in contrast with the wettable powders and dry flowables. Their lack of availability is due to the fact that not many solid active ingredients are soluble in water; those that do exist (formulated as soluble powders) are mixed with water in the spray tank, where they dissolve and form a true solution prior to spraying. Soluble powders provide most of the same benefits as wettable powders, without the need for agitation once dissolved in the tank. They are also nonabrasive to application equipment. Soluble powders, like any finely divided particle, can present an inhalation hazard to applicators during mixing.
7. **Liquid Flowables**: The manufacture of liquid flowables (also called flowables or suspension concentrates) mirrors that of wettable powders—with the exception that the powder, dispersing agents, wetting agents, etc., are mixed with water before packaging. The result is a suspension requiring further dilution with water before use. The product is applied as a spray, with all the advantages of a wettable powder. The benefit of this formulation is that there is no inhalation hazard to the applicator during mixing since the powder already is suspended in water, permitting it to be poured.

Liquid flowables form a suspension in the spray tank and have the same potential problems inherent in any suspension. However, they usually do not require constant agitation during application due to the extremely small size of the suspended particles. One further problem noted with this formulation is the difficulty in removing all of the product from the container during the triple rinsing process.

8. **Microencapsulates**: Microencapsulates consist of a solid or liquid inert (containing an active ingredient) surrounded by a plastic or starch coating. The resulting capsules can be sold as dispersible granules (dry flowables), or as a liquid formulation. Encapsulation enhances applicator safety while providing timed release of the active ingredient. Liquid forms of microencapsulates are further diluted with water and applied as sprays. They form suspensions in the spray tank and have many of the same properties as liquid flowables.
9. **Emulsifiable Concentrates**: Emulsifiable concentrates consist of an oil-soluble active ingredient dissolved in an appropriate oil-based solvent to which an emulsifying agent is added. Emulsifiable concentrates are mixed with water and applied as a spray. As their name implies, they form an emulsion in the spray tank. The emulsifying agents allow oil-soluble active ingredients to be sprayed in water as a carrier. Some agitation is typically required to maintain dispersion of the oil droplets. They are not abrasive to application equipment, nor do they plug screens and strainers. Emulsifiable concentrates have several disadvantages: They present a dermal hazard; they can penetrate oily barriers like human skin; they usually have an odor problem; they can burn foliage; and they can cause the deterioration of rubber and plastic equipment parts.
10. **Solutions**: Solutions (water-soluble concentrates) consist of water-soluble active ingredients dissolved in water, for sale to the applicator for further dilution prior to field application. They will obviously form a true solution in the spray tank and require no agitation after they are thoroughly dissolved. Solutions are not abrasive to equipment and will not plug strainers and screens. There are several major herbicides with wide-scale use that are formulated as solutions. They include products containing paraquat, glyphosate, and 2,4-D. Solutions have few disadvantages; however, some that are produced as dissolved salts can be caustic to human skin.
11. **Miscellaneous Liquid Formulations** Most liquid formulations are designed to be mixed with a carrier before application. However, some products are sold ready-to-use. (RTU). This type of formulation generally will have a low concentration of active ingredient. Typically, the container also serves as the application device. Low and ultra low volume (ULV) concentrates used in specialty situations (e.g., space spraying and fogging) are frequently applied undiluted. Dermal hazards are a problem when mixing these products because of the high concentration of active ingredient. Low and ultra low volume concentrated formulations utilize special equipment to deliver the product in the form of very tiny droplets. Consequently, while they provide excellent coverage, drift potential and inhalation problems during application can be quite high.
12. **Aerosols and Fumigants**: Aerosols and fumigants are frequently confused, yet they have very different properties and uses. Aerosols really refer to a delivery system that moves the active ingredient to the target site in the form of a mist of very small

particles: solids or liquid drops. The particles can be released under pressure or produced by fog or smoke generators. Aerosols are especially useful for indoor insect control, as coverage is thorough. But it can be difficult to confine the aerosol to the target area, and there is always the danger of inhalation. Fumigants deliver the active ingredient to the target site in the form of a gas. Some fumigants are solids that turn into a gas in the presence of atmospheric moisture. Others are liquids under pressure that vaporize when the pressure is released. Fumigants can completely fill a space, and many have tremendous penetrating power. They can be used to treat objects (e.g., furniture), structures, grain, and even soil for pest insects and other vermin. Fumigants are among the most hazardous pesticide products to use due to their inhalation danger.

Different formulations

EC - Emulsifiable concentrate	SU- Ultra-low volume suspension
CG - Encapsulated granule	TB- Tablet
CS - Capsule suspension	FS - Flowable concentrate for seed treatment
DC - Dispersible concentrate	G - Granule
DP - Dispersible powder	GC - Macrogranule
EG - Émulsifiable granule	GL - Emulsifiable gel
EO - Emulsion, water in oil	GP - Flo-dust
EW - Emulsion, oil in water	GW - Water soluble gel
ES - Emulsion for seed treatment	OL - Oil miscible liquid
FG- Fine granule	OP -Oil dispersible powder WDP- Water dispersible powder
SC- Suspension concentrate	
SE- Suspo- emulsion	WG- Water dispersible granules
SG- Water soluble granule	WP- Wettable powder
SL- Soluble concentrate	WS- Water dispersible powder for slurry treatment
SP- Water soluble powder	
SS- Water soluble powder for seed treatment	WSC –Water soluble concentrate

Experiment No: 7 **Date:**

Pesticides application techniques

Objectives:

To study on different pesticide application techniques.

Different types of pesticides are used for controlling various pests. For example Insecticides are applied against insect pests, Fungicides against crop diseases, Herbicides against weeds etc. in order to protect the crop losses. But it is essential that besides choosing an appropriate pesticide for application it has to be a quality product i.e., proper quantity of pesticide active ingredient (a.i) must be ensure that the quantity is maintained in production and marketing of pesticide formulations.

Most of the pesticides are applied as sprays.

The liquid formulations of pesticide either diluted (with water, oil) or directly are applied in small drops to the crop by different types of sprayers. Usually the EC formulations, wettable powder formulations are diluted suitably with water which is a common carrier of pesticides. In some cases however, oil is used as diluent or carrier of pesticides.

On the basis of volume of spray-mix the technique of spraying is classified as:

a. High Volume Spraying ---------- 300 - 500 L/ha
b. Low Volume Spraying ----------50 - 150 L/ha
c. Ultra Low Volume Spraying ----------< 5 L/ha

Sprayers (Hydraulic energy)

A. Manually operated

- Syringes, slide pump
- Stirrup pumps
- Knap sack or shoulder-slung
- Lever operated K.S. sprayer
- Piston pump type
- Diaphragm pump type
- Compression sprayer
- Hand compression sprayer
- Conventional type
- Pressure retaining type
- Stationary type
- Foot operated sprayer
- Rocker sprayer

B. Powered operated

- High pressure sprayer (hand carried type)
- High pressure trolley/ Barrow mounted

- Tractor mounted/ trailed sprayer
- High pressure knap sack sprayer
- Air craft, aerial spraying (Fixed wing, helicopter)

❖ **SPRAYERS** (**Gaseous energy**)

A. Manually operated: Hand held type

B. Powered operated: Knap sack, motorized type; Hand/ Stretcher carried type, and Tractor mounted.

❖ **SPRAYERS** (**Centrifugal energy**)

i. Hand held battery operated ULV sprayer.
ii. Knapsack motorized type
iii. Tractor/ vehicle mounted ULV sprayer
iv. Aircraft ULV sprayer

❖ **Other Sprayers**

A. Aerosol sprayers
B. Liquefied-gas type dispensers
C. Fogging machines
D. Exhaust Nozzle Sprayer

❖ **Dusting Equipment**

A. Manually operated
   - Plunger duster
   - Bellow duster
   - Rotary duster
   - Belly mounted model
   - Shoulder-slung model

B. Powered operated 1. 1.
   - Knapsack motorized duster
   - High pressure trolley/ Barrow mounted
   - Tractor mounted/trailed duster
   - Aircraft

❖ **Granule Applicator**

A. Manually operated
   - Broad-casting tins
   - Knapsack Rotary granule

B. Powered operated
   - Knapsack motorized type
   - Tractor mounted/ trailed duster
   - Aircraft

Activities:

1. To study on operations of a sprayer machine in the field.
2. To study on operations of a duster machine in the field.

Experiment No: 8 **Date:**

Collection and preservation of disease samples from Forest nursery and plantation

Objective: To collect and preserve the tree disease samples

Collection and preservation of plant disease samples

Preservation is a technique which allows the diseases samples to maintain its original appearance for a long time. Preservation of disease materials (herbaria) on their natural substrates as dry specimens or wet specimens is essential for conducting systematic study of pathogens and important taxonomic research on various micro-organisms.

Materials required: Polythene bags, Newsprint paper, Hand saw, Trowel, Pruning shear, knife, Scissors, Hand lens, Pencil, Ink markers, Plant press, Paper bags, Envelopes, Ice box Manual

Specimens

A herbarium specimen may be a single sporocarp or a portion of it, dried culture, slide or the material on its host or substrate (e.g. leaf, stem, bark, rock, soil, paper, cloth). The following two types of preservation methods are used for diseased plant specimen:

1) Dry Preservation: It involves following steps:

a) Collection and drying: The sample should have distinctively visible symptoms. Dry the specimen in layer of blotting sheets under sunlight or in hot air oven for few days.

b) Labelling and packaging: The material should be kept in good herbarium packets. This is attached to a chart paper sheets. The two sides of packet are folded first, then bottom flap and finally top flap. The name of pathogen, host, locality, date, name of scientist who identified the specimen, should be mentioned on the label.

c) Disinfection and storage: The specimen folders are fumigated with methyl bromide vapours in fumigation chamber for 24-48 hrs before storage.

2) Wet Preservation

Washed fresh diseased specimens are put in a boiling mixture of 1 part of glacial acetic acid saturated with normal copper acetate crystals and 4 parts of water till the green colour reappears and then kept preserved in 5 per cent formalin in the glass jars.

All mounted or preserved specimens must be labeled with as much of the following information as far as possible:

1. Host (name of the diseased plant)
2. Name of the disease (the name of the organism causing the disease)

3. Place of collection

4. Date of collection

5. Name of the collector

Size of the specimen: A specimen should ideally be 25–40 cm long and up to 26 cm wide, allowing it to fit on a standard herbarium mounting sheet which measures 42 x 27 cm. This is also the approximate size of tabloid newspapers. Plant parts that are too large for a single sheet may be cut into sections pressed on a series of sheets, for example a palm or cycad frond. Long and narrow specimens such as grasses and sedges can be folded once, twice or even three times at the time of pressing. In this way a plant of up to 1.6 metres high may be pressed onto a single sheet. For very small plants, a number of individuals may be placed on each sheet.

Exercise:

1. **Prepare herbarium of disease sample with the following details:**

a. Host (name of the diseased plant)
b. Name of the disease Parasite (the name of the organism causing the disease)
c. Place of collection
d. Date of collection
e. Name of the collector

2. Collect the disease sample and preserve in the glass bottle following wet preservation protocol.

Experiment No: 9 Date:

Isolation of Fungus from infected tree species

Objectives: To isolate and identify the fungus from infected leaf samples

Isolation: a culture or subpopulation of a microorganism separated from infected samples and maintained in some sort of controlled circumstance.

Materials required: Peeled potatoes, Dextrose, Cheese cloth, Agar-agar, Sterilized distilled water, Knife, Metallic container, Streptocycline, Beakers, Measuring cylinder, Flasks, Non absorbent cotton, Muslin cloth, heating source, infected plant material, Petri plate, Razor blade, Forceps , Inoculation needle, Spirit lamp, Mercuric chloride (0.1 %) or Sodium hypochlorite (1%), Alcohol (70%).

Procedure:

I. Preparation of PDA (Potato Dextrose Agar) media (For 1 lit)

1. Boil 200 g of peeled and sliced potatoes and boiled in a little quantity of water in a metallic container and strain the solution through cheese/muslin cloth into a beaker.
2. Add 20 g of dextrose and 20g of agar-agar to the extract and make up the volume to 1000 ml.
3. Dispense the medium into conical flask ($2/5^{th}$ of the volume) or test tubes (5 ml) depending upon the purpose of study.
4. Plug the mouth of the flasks or test tubes with non-absorbent cotton and wrap them with paper and then with a twine thread or rubber band.
5. Place them in autoclave for 20mins at 121. 6ºC. or pressure cooker and sterilize.

II. Isolation of fungus from leaf tissue:

1. Surface sterilizes the isolation chamber and working table with 70% alcohol or U. V. light.
2. Disinfect your hands with 70% alcohol or any other disinfectant.
3. Arrange the sterilized petri plates near the lighted burner and pour 15ml of molten sterile PDA (temperature around 45^0 C) into each Petri plates by slightly lifting the top plate.
4. Distribute the medium well in the Petri plates by shaking gently. Keep them aside and allow the medium to solidify.
5. Arrange five Petri plates in a row near the burner.
6. Pour 0.1 % mercuric chloride solution into the first plate and sterilized distilled water into the remaining four Petri plates.
7. Select the infected host tissue showing typical symptoms and cut it into 3 to 4 mm bits using sterilized razor blade.
8. Submerge these bits in the solution of 0.1 % mercuric chloride for 1 min and agitate.
9. Using sterilized forceps, transfer these bits into sterilized water taken in a petri plate and allow them for 2 min. Then pass on these bits through 3 remaining plates containing sterilized water.

10. With the sterilized forceps, lift these bits one by one and remove the excess water by placing on the tissue paper.
11. Bring the Petri plates containing PDA near the flame one at a time and gently open the upper lid and place the bits individually. After transfer close the lid immediately. Care should be taken not to open the lid fully.
12. Incubate the inoculated Petri plates at 28º C and observe for the growth of the pathogen.

Exercise:

1. Collect the infected leaves from nursery and tree plantation of different tree species
2. Isolate and study the morphological characteristics of the fungi

Experiment No: 10 Date:

Isolation of Bacteria from infected tree species

Objectives: To isolate the bacterial plant pathogens from infected leaves

Materials required:

Beef extract, Peptone, Agar-agar, pH paper, 0.1 N NaOH, 0.1 N HCL, Beaker Conical Flask, Non absorbent cotton, infected material, Petri plate, Sterilized water blanks, NA slants, Razor blade, Forceps, Inoculation needle, Spirit lamp, Mercuric chloride (0.1 %) or Sodium hypochlorite (1%), Alcohol (70%).

Procedure:

I. **Preparation of Nutrient Agar (NA) media (for one liter):**

1. Add 3g of beef extract and 5g of peptone into a beaker containing 500 ml of distilled water. Dissolve the ingredients by stirring the suspension.
2. Add 20 grams of agar-agar into it and stir it again thoroughly. Make up the volume to 1000 ml by adding, distilled water. Heat the solution till it become Luke warm to assure proper mixing of agar-agar and the other nutrients. Test the pH of the medium using paper. If the medium found acidic add few drops of NaoH and if found alkaline add few drops of HCl and adjust the pH to neutral.
3. Dispense the medium into conical flask (2/5th of the volume) or test tubes (5 m) depending upon the purpose of study.
4. Plug the mouth of the flasks or test tubes with non-absorbent cotton and wrap them with paper and tie them with a twine thread or rubber band. Place them in autoclave or pressure cooker and sterilize.

II. Isolation of Bacteria:

1. Select the infected host tissue showing typical symptoms and cut it into small pieces using sterilized razor blade.
2. Dip them in 1 % sodium hypochlorite kept in the first Petri plates for 30 seconds.
3. Transfer these pieces to the second Petri plate and pass through third and fourth plates containing sterilized water.
4. Transfer these pieces to a test tube containing about 2 ml sterilized water and crush them well with a sterilized rod so as release the bacterial cells into the suspension.
5. Lift a loopful of this suspension and streak gently on the surface of NA in the plates using inoculation needle.
6. Incubate the inoculated plates at 28^0 C for 48h and observe for well isolated colony.

Exercise:

1. Collect the infected leaves from nursery and tree plantation of different tree species

2. Isolate and study the morphological characteristics of Bacteria

Experiment No: 11 Date:

Isolation of DNA from fungal mycelia

Objectives: To isolate the DNA from the fungal mycelia culture

Materials required: C-TAB buffer, Chloroform isoamyl alcohol, isopropanol, 70% ethanol, 1X TE buffer

C-TAB buffer composition

2 per cent (w/v) CTAB
1.4 M NaCl
20 mM EDTA
100 mM Tris- HCl (pH 8.0)
0.2 per cent (v/v) β -mercapta ethanol

Procedure

1. Grind 100-200 mg of fungal mycelia using pestle and mortar.
2. Add 1-2 ml CTAB extraction buffer and grind thoroughly.
3. Transfer 0.75 ml of grinded sap into 1.5 ml micro centrifuge tube and incubate at 60º C for 30 min in a water bath.
4. After incubation, add 750 µl of chloroform: isoamyl alcohol (24:1) and mix well to form an emulsion by inverting the tube.
5. Centrifuge the mixture at 13,000 rpm for 10 min.
6. Collect the supernatant and mix thoroughly with 300 µl of isopropanol and incubate at -20º C for at least one hour.
7. Centrifuge the contents centrifuged at 13,000 rpm for 10 min and discard the supernatant without losing pellet.
8. Wash the pellet with 500 µl of 70 per cent ethanol and centrifuge at 14,000 rpm for 5 min.
9. Remove the ethanol and dry the pellet in vacuum drier for 5 min.
10. Finally the dried pellet is resuspended in 100 µl of 1X TE buffer and stored at -20º C.
11. Quantify the DNA by using Spectrophotometer.

Exercise: Isolate the DNA from the pure culture of fungus

Experiment No: 12 **Date:**

Polymerase Chain Reaction (PCR)

Objective: To identify the fungus through molecular characterization

Introduction: polymerase chain reaction enables researchers to produce millions of copies of a specific DNA sequence in approximately two hours. This automated process bypasses the need to use bacteria for amplifying DNA. PCR is a technique widely used in molecular biology. It derives its name from one of its key components; a DNA polymerase used to amplify a piece of DNA by in vitro enzymatic replication. As PCR progresses, the DNA generated is used as a template for replication. This sets in motion to amplify a single or few copies of a piece of DNA across several orders of magnitude, generating millions or more copies of the DNA piece. PCR can be extensively modified to perform a wide array of genetic manipulations. PCR is now a common and often indispensable technique used in medical and biological research labs for a variety of applications. These include DNA cloning for sequencing, DNA-based phylogeny, or functional analysis of genes; the diagnosis of hereditary diseases; the identification of genetic fingerprints; and the detection and diagnosis of infectious diseases.

Materials required:

***Taq* polymerase:** Enzyme that extends growing DNA strand complementary to DNA template

$MgCl_2$: Provides ions needed for enzyme reaction

dNTP's: Nucleotides (Adenine, Cytosine, Guanine, Thymine) building blocks for new DNA strands

Buffer: Maintains optimal pH for enzyme

Primers: Anneal to single-stranded DNA template, provide initiation site for extension of new DNA. Forward primer: Anneals to DNA anti-sense strand; Reverse primer: Anneals to DNA sense strand

DNA template: the product of our fungal DNA

Procedure:

The PCR usually consists of a series of 30 to 35 cycles. Most commonly, PCR is carried out in three steps, often preceded by one temperature hold at the start and followed by one hold at the end.

1. **Initialization step**: This step consists of heating the reaction mixture to a temperature of 94-96°C (or 98°C if extremely thermo stable polymerases are used), which is held for 1-9 minutes. It is only required for DNA polymerases that require heat activation by hot-start PCR.
2. **Denaturation step:** This step is the first regular cycling event and consists of heating the reaction mixture to 94-98°C for 20-30 seconds. It causes melting of DNA template and primers by disrupting the hydrogen bonds between complementary bases of the two DNA strands, yielding single strands of DNA.
3. **Annealing step:** The reaction temperature is lowered to 50-65°C for 20-40 seconds allowing annealing of the primers to the single-stranded DNA template. Stable DNA-DNA hydrogen bonds are formed only formed when the primer sequence very closely

match the template sequence. The polymerase binds to the primer template hybrid and begins DNA synthesis.

4. **Extension / elongation step:** The temperature at this step depends on the DNA polymerase used; Taq polymerase has its optimum activity temperature at 75-80°C, and commonly a temperature of 72°C is set for elongation. At this step the DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand by adding dNTP's that are complementary to the template in 5' to 3' direction, condensing the 5'-phosphate group of the dNTPs with the 3'-hydroxyl group at the end of the nascent (extending) DNA strand. The extension time depends both on the DNA polymerase used and on the length of the DNA fragment to be amplified. As a rule-of-thumb, at its optimum temperature, the DNA polymerase will polymerize a thousand bases in one minute.
5. **Final elongation:** This single step is occasionally performed at a temperature of 70-74°C for 5-15 minutes after the last PCR cycle to ensure that any remaining single stranded DNA is fully extended.
6. **Final hold:** This step at 4°C for an indefinite time may be employed for short term storage of the reaction.

Exercise:

1. Perform the PCR for the isolated fungus DNA

Experiment No: 13 **Date:**

Gel electrophoresis:

Principle of electrophoresis

The fundamental driving force of electrophoresis is the voltage applied to the mixture of charges molecules in solution. The separation speed of charged molecules is directly proportional to the surrounding voltage gradient.

Objective: To visualize separated DNA fragments according to their molecular size by applying an electric current to the gel matrix.

Materials required:

- **An electrophoresis chamber and power supply**
- **Gel casting trays**, which are available in a variety of sizes and composed of UV transparent plastic. The open ends of the trays are closed with tape while the gel is being cast, then removed prior to electrophoresis.
- **Sample combs**, around which molten agarose is poured to form sample wells in the gel.
- **Electrophoresis buffer**, usually Tris-acetate- Ethylene Diamine Tetra Acetic Acid (EDTA) (TAE) or Tris-borate-EDTA (TBE).
- **Loading buffer**, which contains something dense (e.g. glycerol) to allow the sample to "fall" into the sample wells, and one or two tracking dyes, which migrate in the gel and allow visual monitoring to track the movement of molecules.
- **Ethidium bromide**, a fluorescent dye used for staining nucleic acids.
- **Transilluminator** (an ultraviolet lightbox), which is used to visualize ethidium bromide-stained DNA in gels.

Procedure:

1. Agarose powder is mixed with electrophoresis buffer to the desired concentration and then heated in a microwave oven until completely melted. Most commonly, ethidium bromide is added to the gel (final concentration 0.5 ug/ml) to facilitate visualization of DNA after electrophoresis.
2. After cooling the solution to about 60°C, it is poured into a casting tray containing a sample comb and allowed to solidify at room temperature.
3. After the gel has solidified, the comb is carefully removed without ripping bottom of the wells. The gel, still in its plastic tray, is inserted horizontally into the electrophoresis chamber and just covered with buffer.
4. Samples containing DNA mixed with loading dye are then loaded into the sample wells, the lid and power leads are placed on the apparatus, and a current is applied.
5. Transmission of electricity is confirmed by observing bubbles coming off the electrodes.
6. DNA fragments will migrate towards the positive electrode.
7. The distance that the DNA fragments migrate through the gel can be judged by visually monitoring migration of the tracking dyes.
8. Bromophenol blue and xylene cyanol dyes migrate through agarose gels at roughly the same rate as double-stranded DNA fragments of 300 and 4000 bp, respectively.

9. When adequate migration has occurred, DNA fragments are visualized on a ultraviolet transilluminator.
10. It should be noted that DNA will diffuse within the gel over time. Documentation of DNA fragment migration is accomplished shortly after cessation of electrophoresis.

NOTE:

- **Ethidium bromide is a known mutagen and should be handled carefully wearing gloves.**
- **Always wear protective eyewear when observing DNA on a transilluminator to prevent damage to the eyes from UV light.**

Exercise:

1. Run the gel, excise the amplified the DNA fragment and send for sequencing
2. Identify the fungus at species level

Experiment No: 14 **Date:**

Preparation of fungicidal solutions

Objectives: To prepare the different fungicidal solutions

Introduction: In 1882, Millardet in France (Bordeaux University) accidently observed the efficacy of the copper sulphate against the downy mildew of grapes caused by *Plasmopara viticola.* When copper sulphate was mixed with lime suspension, it effectively checked the disease incidence. The mixture of copper sulphate and lime was named as "Bouillie Bordelaise" (Bordeaux mixture).

The original formula developed by Millardet contains 5 lbs of CuSO4 + 5lbs of lime + 50 gallons of water.

The chemistry of Bordeaux mixture is complex and the suggested reaction is:

$CuSO_4 + Ca(OH)_2 = Cu(OH)_2 + CaSO_4$

The ultimate mixture contains a gelatinous precipitate of copper hydroxide and calcium sulphate, which is usually sky blue in colour. Cupric hydroxide is the active principle and is toxic to fungal spores.

Preparation of Bordeaux mixture

Materials required: One kg of copper sulphate, 1 kg of lime, 100 litres of water, containers, pH paper

Procedure

1. One kg of copper sulphate is powdered and dissolved in 50 litres of water.
2. Similarly, 1 kg of lime is powdered and dissolved in another 50 litres of water.
3. Then copper sulphate solution is slowly added to lime solution with constant stirring or alternatively, both the solutions may be poured simultaneously to a third contained and mixed well.
4. The ratio of copper sulphate to lime solution determines the pH of the mixture.
5. The mixture prepared in the above said ratio gives neutral or alkaline mixture.
6. If the quality of the used is inferior, the mixture may become acidic. If the mixture is acidic, it contains free copper which is highly phytotoxic resulting in scorching of the plants.
7. Therefore, it is highly essential to test the presence of free copper in the mixture before applied. There are several methods to test the neutrality of the mixture, which are indicated below:

(i) **Field Test**: Dip a well polished knife or a sickle in the mixture for few minutes. If reddish deposit appears on the knife/sickle, it indicates the acidic nature of the mixture.

(ii) **Litmus paper test**: The colour of blue litmus paper must not change when dipped in the mixture.

(iii) **pH paper test**: If the paper is dipped in the mixture, it should show neutral pH.

(iv) **Chemical test:** Add a few drops of the mixture into a test tube containing 5 ml of 10% potassium ferrocyanide. If red precipitate appears, it indicates the acidic nature of the mixture.

If the prepared mixture is in the acidic range, it can be brought to neutral or near alkaline condition by adding some more lime solution into the mixture.

Bordeaux mixture preparation is cumbersome and the following precautions are needed during preparation and application.

1. The solution should be prepared in earthen or wooden or plastic vessels. Avoid using metal containers for the preparation, as it is corrosive to metallic vessels.
2. Always copper sulphate solution should be added to the lime solution, reverse the addition leads to precipitation of copper and resulted suspension is least toxic.
3. Bordeaux mixture should be prepared fresh every time before spraying. In case, the mixture has to be stored for a short time or a day, jaggery can be added at the rate of 100kg/100 litres of the mixture.
4. Bordeaux mixture is used as a protective fungicide for foliage applications. The freshly prepared mixture has high tenacity. Major uses include the control of *Phytophthora*, Downy mildew, damping off, leaf diseases of several crops *etc*.

Bordeaux paste: It is prepared by mixing 1 kg of CuSO4 and 1 kg of lime in 10 litres of water. It is used as a wound dressing fungicide. It is painted after removing dead tissues or after pruning in tree crops.

Burgundy mixture: It is prepared by mixing 1 kg of CuSO4 and 1 kg of Sodium Carbonate in 100 litres of water. It is a best substitute for Bordeaux mixture. It is used in the crops sensitive to Bordeaux mixture.

Cheshnut compound: It is prepared by mixing 2 parts of copper sulphate and 11 parts of Ammonium Carbonate. It is used as a soil fungicide against diseases caused by *Pythium* and *Rhizoctonia*.

Chaubattia Paste: This is prepared by mixing 800 g of copper carbonate and 800 g of Red lead in 1 litre of linseed oil or lanolin. It is successfully used in managing pink disease, stem canker and collar rot of many trees. It is also a wound dressing fungicide.

Exercise:

1. Prepare 10 litre of Bordeaux mixture and check the efficacy.
2. Prepare 5 litre of Bordeaux paste and apply it on pruned trees as wound dresser and record the observation.

Experiment No: 15 **Date:**

Testing the *In-vitro* efficacy of fungicide

Objective: To test the efficacy of different fungicides under laboratory level

Materials required: Two common fungicides, PDA media, Petri plates, culture plate, Sterilized distilled water, Beakers, Measuring cylinder, Flasks, Non absorbent cotton, Razor blade, Forceps, Inoculation needle, Spirit lamp, Alcohol (70%).

Procedure:

- The poisoned food technique is followed to evaluate the efficacy of fungicides in inhibiting the mycelial growth of fungus.
- Isolate the fungus from infected plants on PDA media one week prior to setting up of the experiment (Refer experiment No.9).
- Prepare the PDA medium and melt it properly.
- Add the fungicidal suspension to the melted medium to obtain the required concentrations (50 ppm, 100 ppm, 200 ppm).
- Pour twenty mL of poisoned medium in each sterilized Petri plate.
- Maintain the control plate without adding any fungicide.
- Take mycelial disc of 5 mm from the periphery of one week old colony and keep at the center of the poisoned plates and incubate at 25±1° C.
- Measure the radial growth of the fungus in mm at 24 hours intervals up to eight days.

Calculate the Percent inhibition of mycelial growth by using the following formula

$$\text{Percent growth inhibition} = \frac{\text{Growth in control - Growth in treatment}}{\text{Mycelial growth in control}} \text{ X } 100$$

Exercise:

1. Evaluate the fungicides like Bavistin 50 WP, Dithane M-45, against *Alternaria*

Experiment No: 16 **Date:**

Testing the *In-vivo* efficacy of fungicide

Objective: To test the efficacy of different fungicides under field level

Material required: Fungicides**,** infected plants, Sprayer

Procedure:
Fungicides perform well under *in vitro* testing conditions is used under field condition to test the efficacy.

1. Spray the fungicides on infected plants at 15 days interval.
2. Maintain control plants.
3. Repeat the spray minimum of three times.
4. Record the disease intensity by applying 0 - 9 disease rating scale as described below.

Numerical rating	Description
0	No symptoms on leaf
1	Small, irregular brown spots covering 1% or less of the leaf area
3	Small, irregular, brown spots with concentric rings covering 1-10% of the leaf area.
5	Lesions enlarging, irregular, brown with concentric rings covering 11-25% of the leaf area.
7	Lesions coalesce to form irregular brown patches with concentric rings covering 26-50% of the leaf area. Lesions also on stem and petioles.
9	Lesions coalescing to form irregular, dark brown patches with concentric rings covering 51% or more of the leaf area. Lesions seen on the stem and petiole.

Per cent disease incidence is calculated by applying following formula.

$$\% \text{ Disease Incidence} = \frac{\text{No. of infected plant observed}}{\text{Total no. of plant observed}} \text{ X } 100$$

Per cent disease intensity (PDI) is calculated by applying following formula.

$$\% \text{ Disease Intensity} = \frac{\text{Summation of numerical ratings}}{\text{No. of leaves observed x Maximum rating}} \text{X } 100$$

Exercise:

1. Test the efficacy of fungicides on infected plants.
2. Calculate the disease incidence and disease intensity.