AIM:
To study the different effects of antibiotics on microorganisms.

THEORY:
In simple terms antibiotics are the organic secretions produced by microorganisms, which in low concentrations are antagonistic to the growth of other microorganisms (mostly pathogens). These have proved very beneficial in combating several bacterial diseases in man and animals. These are commonly obtained from actinomycetes and some eubacteria. Streptomycin, aureomycin, teramycin, chloromycetin, erythromycin, neomycin etc. are some of the important antibiotics.

Soil is a natural medium that harbours several types of microorganisms. These microorganisms can be grown on culture media. We can study the effect of different types of antibiotics on the growth of microorganisms growing in culture medium. This is an important subject, due to this reason the study of effect of antibiotics on microorganisms has been taken for the present project.

MATERIALS REQUIRED:
Syringe, agar, beef, peptone, pipettes, NaCl, Sodium bicarbonate, distilled water, five different types of antibiotics (such as penicillin, streptomycin, aureomycin, terramycin and chloromycetin), potato, garden soil, oven sterilized petridishes, flasks, beakers, dextrose, glass marker pen.

METHOD:
A. Preparation of culture medium
1. Potato Dextrose Agar (PDA) Medium:
   (i) First of all take 200 g of peeled potato chips.
   (ii) Then boil them with 500 ml of water in a beaker for 15 minutes.
   (iii) Squeeze the potato pulp thus obtained through a muslin cloth and keep it in a flask.
   (iv) Now take 20 g of agar in a beaker and warm it with 500 ml of water.
   (v) Mix both the solutions of potato and agar and add 20 g dextrose to it. In this way you can prepare one litre of PDA medium.
   (vi) Autoclave the medium at 15 pounds pressure for 15 minutes.

   Figure 1.1: Culture medium in petridishes for culturing soil microorganisms

2. Meat Extract Agar Medium:
   (i) Weigh 3 g beef extract, 10 g peptone, 5g NaCl and mix these in 1 litre of distilled water.
(ii) Now heat the mixture to 65°C stirring until the material are completely dissolved.
(iii) Filter the mixture through filter paper and by adding a bit of sodium bicarbonate adjust the pH to 7.2 to 7.6.
(iv) Now, add 20 g agar to the broth and autoclave the medium at 15 pounds pressure for 15 minutes.

B. Effect of antibiotics on soil microorganisms

1. First of all take 2 g of soil and dissolve it in 10 ml of water in a beaker and let the soil particle settle down.
2. Take 6 oven sterilized petridishes and pour 1 ml of soil suspension in each of the plates. Pour 1 ml of the five antibiotics separately in five petridishes by using syringe, and mark them with marker pen. Leave the sixth petridish without antibiotics. The sixth petridish will serve as control.
3. Pour PDA or meat extract agar in each of the petridishes and mix the suspension by rotating the petridishes. Leave the petridishes undisturbed at a warm place for a week.

**OBSERVATION:**
Colonies of microorganisms appear on the culture medium. Count the colonies in each petridish and present your observations in the following table:

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Antibiotics</th>
<th>No. of colonies in a petridish</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Penicillin</td>
<td>........</td>
</tr>
<tr>
<td>2.</td>
<td>Streptomycin</td>
<td>........</td>
</tr>
<tr>
<td>3.</td>
<td>Terramycin</td>
<td>........</td>
</tr>
<tr>
<td>4.</td>
<td>Chloromycetin</td>
<td>........</td>
</tr>
<tr>
<td>5.</td>
<td>Control</td>
<td>........</td>
</tr>
</tbody>
</table>

We can assess the effect of different antibiotics on the microorganisms by counting the number and size of the colonies growing in the petridishes.

**PRECAUTIONS:**
1. Proper kind of stains should be used for different types of microorganisms.
2. Do not expose the culture of the petridish to the atmosphere.
3. Before use sterilise the petridishes properly in the oven.

**Project - 2**

**AIM:**
To estimate the amount of fats present in different dry fruits.

**THEORY:**
Fats and oils belong to class called lipids. Lipids are the biomolecules which are insoluble in water but soluble in organic solvents of low polarity like other and chloroform.

There are three types of lipids:
1. **Phospholipids:** These are main constituents of cell membranes, and derivatives of glycerols in which two of hydroxyl groups are esterified with fatty acid but the third ‘OH’ is esterified with phosphoric acid.
Lecithin is an example of phospholipid which is present in eggs. Each phospholipid molecule has a polar hydrophilic (water attracting) phosphate group and non-polar hydrophobic (water repelling) fatty acid chain and so phospholipids have good emulsifying and membrane forming properties.

2. **Waxes**: These are esters of long chain fatty acids with long chain monohydric alcohols.

3. **Triglycerides (oils and fats)**: These are the simplest and most abundant lipids. Triglycerides constitute the main forms in which fat is stored in plant and animal cells. These are trimesters of glycerols with unbranched long chain fatty acids. Fatty acids may be saturated or unsaturated.
   - Saturated fatty acids do not contain double or triple bonds but contain only C — C single covalent onds. Some examples of saturated fatty acids are Lauric acid (\(C_{11}H_{23}COOH\)) and stearic acid (\(C_{17}H_{35}COOH\)).
   - Unsaturated fatty acids are those which contain one or more double or triple bonds in between their carbon atoms, e.g., Oleic acid (\(C_{17}H_{33}COOH\)), Linolic acid (\(C_{17}H_{31}COOH\)), etc.

Fats are triglycerides which are stable and solid at room temperature on the other hand those which are unstable and liquid at room temperature are called oils.

**Hydrogenation of oils**: It means addition of hydrogen across double or triple bonds in presence of catalyst like nickel converts oils into fats.

**Significance of fats**
1. **Absorption of vitamins**: Fats are required in metabolism of fat soluble vitamins like A, D, E and K.
2. **As fuel**: Fats are rich source of energy. 1 g of fat gives 9 kcal of energy on oxidation.
3. **Storage**: Fats are stored in adipose tissue to protect internal organs from shock and jerks.
4. **Component of cell organelles**: Fats are components of cytoplasm and membranes.

**Source of fat**
1. **Animal sources**: Milk, butter, cheese, egg yolk, meat, fish, etc.
2. **Plant sources**: Mustard seeds, groundnut, coconut, linseed, dry fruits, etc.

**MATERIALS REQUIRED:**
Funnels, beakers, conical flask, filter papers, test tubes, stirring rod, carbon tetrachloride (CCl\(_4\)), heater/spirit lamp, different types of dry fruits (like coconut, walnut, almond, cashew nut and groundnut).

**METHOD:**
1. First of all grind 10 g of dry fruits in a pestle and mortar into fine powder.
2. Heat each sample with sufficient amount of carbon tetrachloride over a water bath for 5 minutes.
3. Now filter the mixture and separate the solid part.
4. Weigh the solid part. Assume that sample contains negligible amount of volatile substances. Subtract the weight of solid part from initial amount of dry fruit taken and this is the amount of fat and oil present in dry fruits.
5. Compute % weight of fats in each dry fruit.

**OBSERVATION:**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of the dry fruit</th>
<th>Initial wt. (g) ( (B) )</th>
<th>Wt. of solid part (g) ( (X) )</th>
<th>Wt. of fat (g) ( A = B - X )</th>
<th>% of fat ( \frac{A}{B} \times 100 )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**CONCLUSION:**

It is concluded from the experiment that ________ contains maximum fat (__________). Therefore the people having high cholesterol level should avoid these and prefer _________ which have minimum % of fat (__________).

**PRECAUTIONS:**

1. During heating hold the test tubes with test tube holder.
2. Weighing should be done properly.

### Project - 3

**AIM:**

To study pollen structure and calculate pollen viability.

**THEORY:**

Microspores or pollen grains are male reproductive bodies of seed bearing plants. They are produced in sac like structure known as microsporangium. Commonly the pollen grains are globular in outline, though several other shapes are also found. A pollen grain is single celled in the beginning but it becomes 2 celled at the time of liberation. It has two layered wall. The outer is known as exine and the inner is termed intine. Intine is plecto-cellulosic in nature. Exine is made up of a highly resistants fatty substance called sporopollenium. The exine provides a characteristic sculpturing or designs over the surface of pollen grain. It helps in identification of the species to which a pollen grain belongs. Palynology is the branch of study of pollen grains. The insect pollinated grains have a yellowish sticky and oily covering over the exine called pollenkitt. The exine is thin or absent at certain places. These areas are known as germ pores. A mature pollen grain has two cells, a large cell called vegetative cell or tube cell and a small cell called generative cell. The vegetative
cell produces pollen tube, while the generative cell divides into two male gametes after moving into the pollen tube.

Pollen viability means the ability of a pollen grain to germinate and produce male gametes. Some pollen grain may not be viable either due to some abnormality or lack of stored food in them. Since pollen grains of different species are variously sculptured (designed) and the study their viability is an interesting subject. Due to this reason, this topic has been selected for the present project work.

**MATERIALS REQUIRED:**

Reagent bottle, cavity slides, beakers, measuring cylinder, sucrose, boric acid, magnesium sulphate, microscope, flowers of different plant species, potassium nitrate, plain slides coverslips.

**METHOD:**

**A. Pollen structure**

1. First of all take a clean slide and put a drop of glycerine on it.
2. Dust a few pollen grains from the anther of a flower in the glycerine drop.
3. Now place a coverslip and observe the slide under low power and then under high power of the microscope.
4. Observe the structure of the pollen grain carefully and draw its diagram.
5. In the similar manner study the structures of pollen grains of flowers of different plant species.

**B. Pollen viability**

1. Firstly prepare a nutrient solution by dissolving 10 g. sucrose, 10 g. Boric acid, 10 mg KNO$_3$, 10 mg MgSO$_4$ in 100 ml of distilled water.
2. Stock this solution in a reagent bottle. This solution acts as a nutrient for the developing pollen grains.

![Diagram of pollen grain](image)

**Figure 3.1: A mature pollen grain of an angiosperm**

![Diagram of sculpturing](image)

**Figure 3.2: Common types of sculpturing in pollen grains**
3. Take a few drops of this solution on a clean cavity slide and dust pollen grains from mature anther of the flowers over this solution.
4. Now observe the slide under dissecting or compound microscope after 5 minutes and then regularly after every minute.
5. Perform experiment with different types of flowers in the similar manner and record the germination of pollen grains of each species.

**OBSERVATION:**

1. The pollen grains of different plant species show different types of sculpturing on the exine.
2. The pattern and distribution of germ/pores is also vary in the pollen grains of different types of flowers.
3. The rate of germination and viability of pollen grains of different species also differ very much. Record the observation in the following table.

![Figure 3.3: Germination of pollen grain and formation of male gametophyte in an angiosperm](image)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of flower</th>
<th>Time taken in germination of pollen grain</th>
<th>Number of viable pollen grains</th>
<th>Number of non-viable pollen grains</th>
<th>Percent viability</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**CONCLUSION:**

Viable pollen grains germinate in the nutrient medium but the non-viable pollen grains do not germinate. The percentage of viability vary in different flowers.
**Project - 4**

**AIM:**
To study essentiality of minerals and deficiency symptoms of essential minerals on plant growth.

**THEORY:**
Minerals are essential for normal growth and development of plants. Macronutrients are the minerals which are needed in large amounts. For example, C, H, O, N, S, P, K, Ca, Mg, and Fe. Minerals which are needed in small amounts are known as micronutrients. For example, Mn, Zn, B, Cu, and Mo.

Hydroponics = Soil less cultures = To raise the plant in solution cultures without using soil. Hydroponics is useful (a) in areas with thin, infertile and dry soils and (b) to find out the essentiality and deficiency symptoms of various minerals.

Criteria for essentiality of an element is:
1. Deficiency of the element produces deficiency symptoms. Deficiency symptoms or hunger signs include chlorosis, stunted growth, necrosis, premature leaf fall, malformed leaves.
2. It is required for growth and development and it is directly involved in plant metabolism.
3. It cannot be replaced by any other element.

Essentiality of minerals can be determined by growing plants in:
1. Sand culture, i.e., well washed, heated pure quartz sand, mixed with culture solution containing specific salts.
2. Knop’s and sach’s culture solution which contain essential element salts in specific ratio dissolved in distilled water.

**MATERIALS REQUIRED:**
Culture solutions, gram or maize seedlings, sterilized bottles, weighing balance.

**METHOD:**
1. First of all prepare the Sach’s culture solution by dissolving the following in 1000 ml water.

<table>
<thead>
<tr>
<th>Normal solution</th>
<th>– N solution</th>
<th>– Ca solution</th>
<th>– Fe solution</th>
<th>– Cl solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>KNO₃ – 1 g</td>
<td>–</td>
<td>1 g</td>
<td>1 g</td>
<td>1 g</td>
</tr>
<tr>
<td>Calcium phosphate – 0.5 g</td>
<td>0.5 g</td>
<td>–</td>
<td>0.5 g</td>
<td>0.5 g</td>
</tr>
<tr>
<td>Magnesium sulphate – 0.5 g</td>
<td>0.5 g</td>
<td>0.5 g</td>
<td>0.5 g</td>
<td>0.5 g</td>
</tr>
<tr>
<td>Calcium sulphate – 0.25 g</td>
<td>0.25 g</td>
<td>–</td>
<td>0.25 g</td>
<td>0.25 g</td>
</tr>
<tr>
<td>Sodium chloride – 0.25 g</td>
<td>0.25 g</td>
<td>0.25 g</td>
<td>0.25 g</td>
<td>–</td>
</tr>
<tr>
<td>Ferrous sulphate – 0.10 g</td>
<td>0.10 g</td>
<td>0.10 g</td>
<td>–</td>
<td>0.10 g</td>
</tr>
</tbody>
</table>

2. Grow a few gram seeds on cotton in petriplate till they become seedlings.
3. Now take 5 sterilized broad mouthed bottles A, B, C, D, E. Fill bottle A with normal culture solution, B with (–N) solution, C with (–Ca) solution, D with (–Fe) solution and E with (–Cl) solution. Cover them with split cork lid.
4. Put one seedling in each bottle through split cork. Provide normal light, temperature and oxygen to let them grow.
5. Record your observations after every two days.

**OBSERVATION:**

Bottle A shows maximum seedling growth on the other hand in other bottles some or the other deficiency symptoms are observed.

**CONCLUSION:**

1. Bottle A shows normal seedling growth.
2. Bottle B seedling shows deficiency symptoms for nitrogen like chlorosis starting from older leaves, stunted growth.
3. Bottle C seedling shows deficiency symptoms for calcium like necrosis, stunted growth of seedling and curling of leaves.
4. Bottle D seedling shows deficiency symptoms for iron like interveinal chlorosis and reduced growth.
5. Bottle E seedling shows deficiency symptoms for chlorine, i.e. leaf wilting, chlorosis and necrosis, swollen root tips.

**PRECAUTIONS:**

1. Make the culture solution carefully and accurately.
2. Provide proper conditions like oxygen, temperature and light to the seedlings.
3. Make observations carefully.

---

**Project - 5**

**AIM:**

To estimate water content in various plants adapted to mesophytic, xerophytic and aquatic conditions.

**THEORY:**

Water makes one of the ecological factor on the earth. Living organisms contain more than 80% water of their fresh weight. Living organisms shows various morphological, anatomical and physiological adaptations, depending on the availability of water. Depending on availability of water plants are classified into three types:

1. Mesophytes
2. Hydrophytes
3. Xerophytes
1. **Mesophytes**: These are the plants which live in moderate conditions of light, temperature, water and oxygen. For example: most of the cultivated plants like apple, guava, litchi, lime, orange. Mesophytic plants have the following adaptations:
   (i) Well-developed root system and root hairs.
   (ii) Stem have well-developed mechanical and vascular tissue.
   (iii) Stems is solid and freely branched.
   (iv) Leaves are large and broad.
   (v) Stomata are present on both the surfaces of leaves.

2. **Hydrophytes**: These are the plants which grow in watery places or places which remain wet throughout the year. For example, Hydrilla, Vallisneria, Pistia, Lotus. Hydrophytes have the following adaptations:
   (i) Poorly developed root system.
   (ii) Cuticle and stomata are absent.
   (iii) Leaves are thin and ribbon-like.
   (iv) Root hairs and root cap are absent.
   (v) Tissues have air spaces to keep plants afloat.

3. **Xerophytes**: These plants grow in deserts or dry places where there is scarcity of water. For example, Cactus, Agave, Asparagus, Euphorbia. Xerophytes plants have the following adaptations:
   (i) Numerous root hairs.
   (ii) Extensive root system, deep in the soil.
   (iii) Leaves coated with cuticle to check loss of water.
   (iv) Stem becomes thick, fleshy for conserving water.
   (v) Stomata few, sunken in pits or covered with fine hairs.
   (vi) Leaves are small, reduced, scale - like.

As water is indispensable for the life of plants so whenever a tissue is subjected to high temperature and it losses water and gets dried so by calculating wet and dry weights of the tissues, amount of water present in tissues can be calculated.

**MATERIALS REQUIRED:**
Oven or spirit lamp, filter paper, weighing balance, petri dish or crucible and various plants.

**METHOD:**
1. First of all clean 10 g of plant by using filter paper and kept in oven at 60° C for 4 hours till they become dry.
2. Now weigh the dried plant.
3. Again keep in over by using for 2 hrs and weigh.
4. Repeat till you get constant weight.
5. Repeat the process for different kinds of plants.
6. Compute the percentage of water by using the formula.

\[
\text{Percentage of water} = \frac{\text{Initial weight (i.e., weight of fresh tissue)} - \text{Final weight (i.e. weight of dry tissue)}}{\text{Initial weight}} \times 100
\]
Biology Lab Manual - XII

Project - 6

AIM:
To study dispersal of seeds by various agencies.

THEORY:
Dispersal on dissemination means the process of scattering of fruits and seeds to distant places away from their parent. It provides the new plants better chances of obtaining water, nutrients, light and space thereby enabling them to have a better start in life. The fruits and seeds develop many devices for better dispersal through different agencies.

Wind (anemochory), water (hydrochory) and animals including man (zoochory) are the principal agencies that aid in the dispersal of fruits and seeds. Besides, some plants show self dispersal by explosive mechanism (autochory). The dispersal through the agency of animals is considered as the best and most successful method.

OBSERVATION:

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of plant</th>
<th>Initial weight (i.e. Wt. of fresh tissue) A</th>
<th>Final weight (i.e. Wt. of dried tissue) B</th>
<th>Water content in the tissue A – B</th>
<th>% of water = ( \frac{A - B}{A} \times 100 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xerophytes</td>
<td>1.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mesophytes</td>
<td>1.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydrophytes</td>
<td>1.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1% of water = \( \frac{\text{Wt. of fresh tissue} - \text{Wt. of dry tissue}}{\text{Wt. of fresh tissue}} \times 100 \)

CONCLUSION:
The following plants __________, __________ show maximum water content whereas __________, __________ plants show minimum water content.

PRECAUTIONS:
1. Equal amounts of tissue should be taken initially.
2. The tissue should be dried in oven and weighed again till you get constant weight, i.e. all the water has been lost.
3. Weighing should be done accurately.
4. Care should be taken so as not to burn the tissue.

Project - 6

AIM:
To study dispersal of seeds by various agencies.

THEORY:
Dispersal on dissemination means the process of scattering of fruits and seeds to distant places away from their parent. It provides the new plants better chances of obtaining water, nutrients, light and space thereby enabling them to have a better start in life. The fruits and seeds develop many devices for better dispersal through different agencies. Wind (anemochory), water (hydrochory) and animals including man (zoochory) are the principal agencies that aid in the dispersal of fruits and seeds. Besides, some plants show self dispersal by explosive mechanism (autochory). The dispersal through the agency of animals is considered as the best and most successful method.
MATERIALS REQUIRED:
Hand lens, knife, forceps, different types of seeds and fruits, petridishes.

METHOD:
1. First of all collect different types of seeds. Observe their features carefully, and classify them according to their mode of dispersal.
2. In the similar manner cut open different types of fruits. Note down the features of their seeds and classify them according to their mode of dispersal.
3. Record your observation in the form of a table given below.

OBSERVATION:

Table: Characteristics of Some Common Seeds and Fruits and their Mode of Dispersal

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Plant</th>
<th>Characteristics of seeds / fruits</th>
<th>Mode of dispersal / External agency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Impatiens (Balsam)</td>
<td>Capsular fruit, mature fruit burst open with force when touched and seeds are shot several feet away.</td>
<td>Autochory/Explosive mechanism.</td>
</tr>
<tr>
<td>2.</td>
<td>Viola (Pansy)</td>
<td>Fruit capsule, tension develops in the drying wall of the carpels causing the fruit to burst open along the line of dehiscence.</td>
<td>Autochory/Explosive mechanism.</td>
</tr>
<tr>
<td>3.</td>
<td>Acer (Maple), Hopea, Tecoma, Haloptelia (Chilbil), Shorea (Sal), Elm, Moringa, Jacaranda, Cinchoria, Albizia (siris) Dalbergia (Shisham) etc.</td>
<td>Thin, wing like expansions are present in seeds/fruits, wings provide large surface area to the wind for their dispersal.</td>
<td>Anemochory/Dispersal by wind.</td>
</tr>
<tr>
<td>4.</td>
<td>Chenopodium (Bathua), Polygonum</td>
<td>Seed/Fruit very light, float on the surface of water.</td>
<td>Hydrochory/Dispersal by water.</td>
</tr>
<tr>
<td>5.</td>
<td>Calotropis (Ak), Alstonia, Bombax (Sembal)</td>
<td>Seeds have hairy outgrowth to provide large surface area to the wind.</td>
<td>Anemochory/Dispersal by wind.</td>
</tr>
<tr>
<td>6.</td>
<td>Dandilion, Sonchus</td>
<td>Fruit single seeded cypsela with a tuft of hair (pappus) at upper end. Pappus open out like a parachute.</td>
<td>Anemochory/Dispersal by wind.</td>
</tr>
<tr>
<td>8.</td>
<td>Boerhravia, Plumbago, Cleome</td>
<td>Fruits are sticky and can stick to the body of animals.</td>
<td>Zoochory/Dispersal by animals.</td>
</tr>
<tr>
<td>9.</td>
<td>Loranthus, Viscum, Cordia (Lasoora)</td>
<td>Seeds are sticky, seed stick to the beak of the birds.</td>
<td>Zoochory/Dispersal by birds.</td>
</tr>
<tr>
<td>10.</td>
<td>Achyranthus (Phuthkanda) Xanthium, Tribules, Medicago (Maina), Martynia (Tiger’s claw)</td>
<td>Fruits/Seeds have barbs, hooks, or spines to get attach with the fur of animals.</td>
<td>Zoochory/Dispersal by animals.</td>
</tr>
<tr>
<td>11.</td>
<td>Ficus (Banyan and Pipal tree)</td>
<td>Fruits edible, chiefly eaten by birds, seeds released unharmed with faeces.</td>
<td>Zoochory/Dispersal by birds.</td>
</tr>
</tbody>
</table>
12. Crop plants (Cereals, pulses, spices etc.)

| Fruits/Seeds have economic importance. | Zoochory/Dispersal by man. |

**Figure 6.1:** Fruits and seeds dispersed by wind

**Figure 6.2:** Lotus fruit dispersed by water

**Figure 6.3:** Fruits dispersed by animals

**Project - 7**

**AIM:**
To study the effect of avenue trees on temperature under canopy and outside.

**THEORY:**
Avenue trees are the trees which are grown on road side. They provide cool air and shade to the passengers and also keep the air fresh, prevent soil erosion and reduce noise, air and thermal pollution. Also the greenery of the avenue tree make the area attractive and appealing. These types of trees are also beneficial for animals as different types of animals get food and shelter from avenue trees.
Cassia fistula, Dalbergia sissoo (shisham), Azadiraeta indica (Neem), Acacia arebica (kikar), Albizia labback (siris) Eugenia jamblana (jamun). Eucalyptus etc. are some of the common avenue trees cultivated in our country. The trees transpire and impart shade, thus lowers the temperature.

**MATERIALS REQUIRED:**
Thermometers, an area with avenue trees, water, pen, beakers, record notebook.

**METHOD:**
1. Firstly find out an area with sufficient avenue trees and area without trees.
2. Take three beakers 3/4 filled with water. Put one thermometer in each of them.
3. Place one beaker under the avenue tree, second beaker near the avenue tree in the open and third beaker in the area without trees.
4. Now record the temperatures of the trees areas in the morning, noon and evening and record the data in the observation table.

**OBSERVATION:**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Time</th>
<th>Temperature under avenue tree</th>
<th>Temperature near avenue tree</th>
<th>Temperature in open area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Morning</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Noon</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Evening</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**CONCLUSION:**
The area which is without trees has maximum temperature. The area nearby avenue trees shows comparatively low temperature while the area under avenue tree exhibit the lowest temperature. The low temperature under trees is due to shade and the lose water vapours through transpiration.

**Project - 8**

**AIM:**
To study the action of various antiseptic soaps on growth of bacteria.

**THEORY:**
Culture medium refers to the solid or liquid preparation containing nutrients for the growth of microbes, animal cells or plant cells.
Culture means collection of microbial cells growing on in a medium. The different types of mediums are given below:

1. **Selective medium:** In this medium one or more substances are added that inhibit growth of all but one or few organisms. e.g., Addition of penicillin to culture medium to select organisms resistant to penicillin.
2. **Liquid medium:** This medium is useful in measuring population growth. It is placed in test tubes plugged by cotton, wool or metal cap. Few cells are inoculated and cells grown are spread throughout
3. **Enrichment medium:** In this medium substances are added to meet the requirement of certain microbes in preference to other.

4. **Solid medium:** It is suitable for bacteria and fungi. We can prepare it by mixing liquid nutrient solution with gelling agent called agar at conc. of 1 - 2%, and microbes can be added to surface of agar.

**Soaps:** These are the mixture of sodium and potassium salts of long chain fatty acids present in oils and fats having cleaning properties. Soap has large non-ionic hydrocarbon group and ionic group COONa. Sodium stearate, sodium palmitate are examples of some soaps. Saponification is the process of making soaps by hydrolysis of fats and oils with alkalis.

**Cleansing Action of soap**

When we add soap to dirty clothes/hands in water, the dirty particles and infecting agents attach themselves to hydrocarbon part of soap molecule. Ionic part of the soap molecule remains attached to water in this way the dirty particles attached to soap molecule are washed away in water and the hand or clothes are cleaned. Soaps make the dirt particles and infectious agents soluble in water so they are washed away with water.

**Antibiotics** are the substances produced by microbes to kill other microbes.

Sir Alexander Fleming discovered the first antibiotic penicillin while he working on Staphylococcus. Antibiotics are mainly of two types:

**ANTIBIOTICS**

- **Broad Spectrum Antibiotics**
  - These are effective against large range of organisms, both Gram +ve and Gram –ve.
  - For example, Tetracycline, Chloramphenicol

- **Narrow Spectrum Antibiotics**
  - These are effective against some specific microbes and not all microbes. For example, Penicillin, Gentamicin.

**Action of antibiotic:**

Antibiotic kill microbes as they-

1. Prevent cell wall formation
2. Disrupt cell membrane.

**MATERIALS REQUIRED:**

- Laminar flow cabinet, refrigerator, glassware, freezer (-20°C), pH meter, spirit lamp, magnetic stirrer with hot plate, balance, autoclave, plasticware, cotton plugs, water distillation unit, test tubes, peptone, tryptone, NaCl, NaOH, yeast extract, agar, ethanol, spirit, different kinds of soaps.

**METHOD:**

1. **Media Preparation:**
   
   (i) Take 500 ml distilled water + 10 g peptone + 5 g yeast extract + 5 g NaCl + 1N NaOH (10 ml). Dissolve
the contents by using a magnetic stirrer.

(ii) By adding distilled water make the volume to 1 litre.

(iii) Adjust the pH to 7 using 1N NaOH or 1N HCl solution according to the requirement.

2. **Divide the solution into two parts:**

```
SOLUTION

Flask A
Flask B (liquid medium)
```

Add 7.05 g agar to this solution and mix.

↓

Divide into 20 test tubes, 10-15 ml in each tube and plug with cotton wool.

↓

Autoclave at 121°C and 151 lbs for 20 minutes.

↓

3. Cool the medium and transfer it to petri dishes (25-30 ml medium/petri dish). Allow it to solidify. Now ask 8 students to wash hands with different kinds of soaps.

4. **Inoculation:** Take thumb prints of students on the medium in petriplates as sources of bacteria and seal the petriplates by using paraffin wax and label the petriplates with the names of the soaps used by the person for disinfection.

5. Leave it for 24 hrs. inside the laminar air flow. Record the number of colonies.

6. **Shifting bacterial colonies to Antibiotic Resistant Medium.**

   (i) The liquid nutrient medium is autoclaved and two different antibiotics like Gentamicin and Ampicillin are added to two flasks in 100 mg/ml amounts.

   (ii) Pour this medium in petriplates.

   (iii) Scrape few bacterial colonies from prepared plates with spreader ; spread to antibiotic resistant medium. Leave for 24 hrs. and note down the colonies on Day 1, Day 2 and Day 3 to find out whether the colonies were resistant to Gentamicin or Ampicillin medium.

**CONCLUSION:**

__________ soap was found to be most effective disinfectant as the petriplate with the thumb print from person using this soap showed no. or minimal number of bacterial colony growth.

**PRECAUTIONS:**

1. Prepare the medium carefully.
2. Take readings carefully.
3. Seal the petriplates properly in order to avoid contamination from outside.
**AIM:**
To do a comparative study of the chlorophyll content in five different species of plants.

**THEORY:**
Chlorophylls are green photosynthetic pigments. These are found in chloroplasts. Besides chlorophylls, carotenoid pigments (i.e. carotenes and xanthophylls) are also present in the chloroplast. These pigments are located in thylakoid membranes in the chloroplasts.

There are at least seven types of chlorophylls. These are known as chlorophyll a, b, c, d, e, and bacteriochlorophyll and bacteriviridin. Out of these chlorophyll a and b occur in the chloroplasts of higher plants. Chlorophyll a which is known as primary photosynthetic pigments convert light energy into chemical energy. The other pigments absorb light energy of different wavelengths and hand over the energy to chlorophyll a through electron spin resonance, due to this reason they are known as accessory pigments.

Photosynthetic pigments absorb light of specific wavelength in the visible region. We can study about the chlorophyll content of a plant by measuring the optical density (OD) of the chlorophyll extract of the plant by using a spectrophotometer or calorimeter.

The present project deals with the comparative study of the chlorophyll content in five different species of plants.

**MATERIALS REQUIRED:**
Methyl alcohol, fresh leaves of spinach, mustard, radish, coriander and clover mortar, filter paper, colorimeter, pestle, ether, petroleum ether, potassium hydroxide, distilled water, test-tubes, separating funnel, acetone, beakers.

**METHOD:**
1. First of all take 100 gms of fresh spinach leaves in a Pestle and Morter.
2. Crush them with 40 ml of 80% acetone. Now add a pinch of $\text{CaCO}_3$ and again crush.
3. Filter the extract on a Buchner filter.
4. The deep green coloured filtrate is known as acetone extract which contains chlorophylls and carotenoids. Separate the chloroplast pigments with the help of chart given under:
5. In the similar manner separate out chlorophyll a and chlorophyll b from other types of leaves.
6. Measure the chlorophyll contents of different types of leaves by using colorimeter by comparing the intensity of light absorbed (optical density) of the extract of known amount of chlorophyll content.
**OBSERVATION:**

Record your observation in the following table:

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Leaves</th>
<th>Chlorophyll a</th>
<th>Chlorophyll b</th>
<th>Total chlorophyll contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Clover (Methi)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

First of all take 40 ml acetone extract in a separating funnel. Add 60 ml of petroleum ether to it shake the funnel gently.

Pour 75 ml water and again shake the funnel. Two layers will be separated.

Discard the lower acetone-water layer. Add some more water in the separating funnel and discard the lower layer.

Take upper layer i.e. Petroleum ether layer. Add 40 ml of 92% methyl alcohol into it. Shake the funnel and allow the two layers to separate.

Upper layer (Petroleum ether layer)

Gently add 15 ml of 30% methyl alcohol–KOH solution

Add 30 ml of water and shake the funnel. The two layers will separate.

Upper layer (Blue green coloured methanolic KOH layer)

Lower layer (Orange coloured petroleum ether layer)

Chlorophyll a

Carotene

Lower layer (Methyl alcohol layer)

Take lower layer in a separate funnel (50 ml). Gently add 50 ml of ethyl ether into it. Shake the funnel.

Add 5 ml of water to wash the mixture. Wash many times (each time with 5 ml) and allow the two layers to separate.

Upper layer (Ethyl ether layer)

Lower layer (Methyl alcohol layer)

Add 15 ml of 30% Methyl alcohol KOH solution. Shake the funnel and add 30 ml of water. Again shake the mixture and allow the two layers to separate.

Upper layer (Olive green methanolic KOH layer)

Lower layer (Yellow coloured ether layer)

Chlorophyll b

Xanthophyll
**AIM:**
To study the effect of CO$_2$ concentration, light and temperature on the rate of photosynthesis using Hydrilla plant.

**THEORY:**
The process of making food by green plants in presence of sunlight and CO$_2$ is known as photosynthesis. Oxygen is also released during this process. Food and oxygen are the two basic needs of living organisms. The equations for photosynthesis can be represented as:

$$6\text{CO}_2 + 12\text{H}_2\text{O} \xrightarrow{\text{Sunlight}} \text{C}_6\text{H}_{12}\text{O}_6 + 6\text{H}_2\text{O} + 6\text{O}_2$$

Some environmental factors that affect photosynthesis are given below:

1. **Light:** It is the basic requirement for photosynthesis. Quality i.e., intensity and quantity i.e., duration of light affect rate of photosynthesis. Red and blue regions of the spectrum are essential for photosynthesis but maximum rate of photosynthesis is higher in alternating light. The rate of photosynthesis is higher in alternating light and dark periods than in continuous light.

2. **Temperature:** It is the another important factor which affect photosynthesis. Optimum temperature for photosynthesis is 35°C; average temperature being between 25° - 40°C and beyond 40°C. The rate of photosynthesis decreases because photosynthesis depends on enzymes and as enzymes are proteinaceou by nature they get denatured at high temperatures.

**CONCLUSION:**
Different species of plants have different amount of chlorophyll. This amount depends upon various factors such as genetic constitution, exposure to light, age of the plant etc.

**PRECAUTIONS:**
1. Take only the leafy part of the plant for extraction of chlorophyll.
2. Always wash the mortar and pestle before using for next plant.
3. Volume of the chlorophyll should be same for all the samples.

**Project - 10**

**CONCLUSION:**
Different species of plants have different amount of chlorophyll. This amount depends upon various factors such as genetic constitution, exposure to light, age of the plant etc.

**PRECAUTIONS:**
1. Take only the leafy part of the plant for extraction of chlorophyll.
2. Always wash the mortar and pestle before using for next plant.
3. Volume of the chlorophyll should be same for all the samples.

**AIM:**
To study the effect of CO$_2$ concentration, light and temperature on the rate of photosynthesis using Hydrilla plant.

**THEORY:**
The process of making food by green plants in presence of sunlight and CO$_2$ is known as photosynthesis. Oxygen is also released during this process. Food and oxygen are the two basic needs of living organisms. The equations for photosynthesis can be represented as:

$$6\text{CO}_2 + 12\text{H}_2\text{O} \xrightarrow{\text{Sunlight}} \text{C}_6\text{H}_{12}\text{O}_6 + 6\text{H}_2\text{O} + 6\text{O}_2$$

Some environmental factors that affect photosynthesis are given below:

1. **Light:** It is the basic requirement for photosynthesis. Quality i.e., intensity and quantity i.e., duration of light affect rate of photosynthesis. Red and blue regions of the spectrum are essential for photosynthesis but maximum rate of photosynthesis is higher in alternating light. The rate of photosynthesis is higher in alternating light and dark periods than in continuous light.

2. **Temperature:** It is the another important factor which affect photosynthesis. Optimum temperature for photosynthesis is 35°C; average temperature being between 25° - 40°C and beyond 40°C. The rate of photosynthesis decreases because photosynthesis depends on enzymes and as enzymes are proteinaceou by nature they get denatured at high temperatures.
3. **Concentration of CO\(_2\):** Conc. of CO\(_2\) in atmosphere is 0.03%. Increase in conc. of CO\(_2\) upto 3% increase rate of photosynthesis but beyond 3% conc., produces toxic effects on plants lowering the rate of reaction.

**MATERIALS REQUIRED:**

Beakers, funnels, wooden box, hydrilla plants, 0.5%, 1%, 2% and 5% solutions of sodium bicarbonate, test tubes, bulbs of different wattage, red, blue, green and yellow glass papers or butter papers.

**METHOD:**

1. First of all fix few Hydrilla plants in the inverted funnels in such a manner that all the cut ends of the twigs face upward; kept in beaker filled with water.
2. Now put a test tube, filled with water, inverted on the funnel.
3. For different conditions similar set-ups are to be made - 8 in number.

**For studying effect of CO\(_2\) conc.**

1. Firstly prepare 0.1%, 0.5%, 2% and 5% solutions of sodium bicarbonate (as source of CO\(_2\)). In the beaker instead of water, add sodium bicarbonate solutions.
2. Wait for 15 minutes for the plants to adjust to these conditions.
3. Count the number of air bubbles evolved from these set-ups containing different conc. of NaHCO\(_3\), in each minute for 3 minutes.

**For studying effect of light intensity:**

Put the experimental set-ups with water in beakers at three different places:

1. In a black wooden box fixed with bulb of 40 w.
2. In a black wooden box fixed with bulb of 100 w.
3. In a black wooden box fixed with bulb of 60 w.
4. Set-up is kept in dark.

   Note down the number of air bubbles evolved from the cut ends of Hydrilla plant in a minute for 3 minutes.

**To study the effect of light quality or wavelength of light:**

Place set-ups in different conditions.

1. In dark, i.e., in a black wooden box.
2. Wrap red glass paper over a bell jar and keep the set-up in bell jar and illuminate with a bulb.
3. Wrap green glass paper over a bell jar and keep the set-up in bell jar.
4. Wrap blue glass paper over a bell jar and keep one set-up in the jar.
5. Wrap yellow glass paper over a bell jar and keep one set-up in the jar.

   Count the no. of air bubbles evolved from the twigs in a minute. Take three readings.

**To study the effect of temperature**

1. Keep the experimental set-up at 5°C by adding ice cold water to beaker.
2. Keep the set-up at 30°C by keeping it at room temperature.
3. Keep the set-up at 80°C by adding boiling water to the beaker.
   - Wait for 30 minutes for the plants to adjust to those conditions.
   - Count the no. of air bubbles in minutes. Take three readings.
OBSERVATION:

**Effect of Light Quality**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Types of set-up</th>
<th>No. of air bubbles evolved in a minute</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>1.</td>
<td>Red light</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Blue light</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Green light</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Yellow light</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Effect of quantity of light</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>40 W</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>60 W</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>100 W</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Dark</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Effect of CO&lt;sub&gt;2&lt;/sub&gt; conc.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>0.1 % NaHCO&lt;sub&gt;3&lt;/sub&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>0.5 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>2%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Effect of temperature</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>5° C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>30° C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>80° C</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CONCLUSION:

- 2% conc. of NaHCO<sub>3</sub> shows the maximum rate of photosynthesis and after that it goes on decreasing due to the toxic effects of CO<sub>2</sub>.
- Red light is most effective for photosynthesis.
- 30°C is the best/optimum temperature for photosynthesis.

PRECAUTIONS:

1. Equal amount of water should be taken in all beakers.
2. Give 15 minutes time to the plants to adjust to the given condition.
3. Take equal no. of twigs in each set-up.

Project - 11

AIM:

To study metamorphosis of frog’s tadpole through specimens.

THEORY:

Frog is a common amphibious animal. It is commonly found in or near water of ponds, lakes, ditches, well etc. It lives both in water and on land. The frog breeds during rainy season. Frogs always move to water during this period. The male frogs produce high pitched croaking sound to attract the female frog for mating.
During the mating the male frog clasps a female behind her arms with his forelimbs having amplexusory pads. The pair stays in this sexual embrace known as amplexus until the female discharges the eggs. The male frog also shed its sperm’s immediately over the eggs. Fertilization of the eggs by sperms occurs at once in water. The eggs are covered with jelly which on coming on contact with water, swells and binds the eggs into a coherent mass which is known as frog spawn. A tadpole larva hatches from each egg in about 12 hours. The tadpole is about 7 mm long and is fish like with a tail for swimming and three pair of feathery gills for breathing. The tadpole begins to undergo many changes in which some of its organs are lost and others undergo modifications in order to form the organs of adult. All these change which transform a tadpole into a young frog are termed as metamorphosis.

**MATERIALS REQUIRED:**

Preserved specimen of different stages of frog’s tadpole, pen, notebook.

**METHOD:**

Observe the different stages of development of frog’s tadpole and record the changes in it:
1. **Fresh hatched tadpole:** The freshly hatched larva is limbless, mouthless and eyeless. It is creature of dark colour with a prominent head and a small compressed tail. It measures about 7 mm in length. On the head rudimentary eyes, olfactory pits and mouth are present. Behind the mouth the cement gland and a pair of suckers is present. On the trunk V-shaped myotomes are present. At the junction of the head with the trunk visceral arches that carry blunt external gills are present. Coacal aperture is situated at the posterior end of the trunk.

2. **Fully developed tadpole:** The larva gets transformed into a fully developed larva which swims actively in water. Its body is similar to that of a fish. The body is divided into a head and a tail provided with dorsal and ventral fins. The head bears mouth with horny jaw at the anterio-ventral end. On the lateral sides of the head four pairs of transverse slits are present which are known as a branchial clefts. Rudimentary external gills are also present. A fold of skin is present on either side which is known as operculum. It covers all the branchial clefts. The rudimentary gills covered under the operculum, gradually disappear in due course of time.

3. **Young frog:** The following changes occur when a fully developed tadpole develops into a young frog. Tail disappears, gills are replaced by lungs. Hind limbs increases in length and fore limbs develop. Mouth widens and horny teeth disappear. The body becomes pigmented. It become carnivorous and become a full frog.

---

**Project - 12**

**AIM:**
To compare the rate of transpiration in different plant species.

**THEORY:**
Plants absorb a large quantity of water from soil by root hairs. Only a part of this water is retained in the plant body which is used for the building up processes, while a greater part of it in lost in form of water vapour. The loss of water vapour from the internal tissues of living plants through aerial parts like leaves, green shoot, etc is termed as transpiration. This process takes place under the influence of sunlight, regulated to some extent by the protoplasm. Water vapour escapes into atmosphere either through stomata or through thin cuticle. The former is known as stomatal transpiration and the latter is known as cuticular transpiration.

Stomatal transpiration is many times in excess of cuticular transpiration. At night the stomata are closed, thus checking the rate of transpiration. The lower surface has large no. of stomata in dorsiventral leaves as in dicot plants and thus transpires water more vigorously than the upper surface whereas isobilateral leaves, as in monocot plants, transpiration is more or less equal from the two surfaces.

Guard cells regulate transpiration by partially or completely opening the stomata or by closing it altogether.

**MATERIALS REQUIRED:**
Eosin dye, different plant species, Ganong’s potometer, water, beaker.

**METHOD:**
1. First of all fill the Ganong’s potometer with water and fix a branch (cut under water) air-tight to the wide end of the apparatus through a cork.
2. Distal end of apparatus is dipped into water contained in a beaker. Water in the beaker may be coloured with eosin.

3. The coloured water is seen to enter the tube as transpiration goes on. Then remove the end of the tube from the beaker for a while and allow air to enter it. Dip it into water again.

4. Repeat the set-up with different plant species.

5. Record the time that the bubble takes to cover the journey from one end of the graduation to the other.

**OBSERVATION:**

1. An air bubble formed at distal end of tube rises and slowly travels through the horizontal arm of the potometer as a result of suction due to transpiration.

2. Time taken by the bubble to cover the journey from one end of graduation to the other varies from plant to plant. The duration of time depends upon various factors such as orientation of leaves, leaf surface area, number of stomata, age of leaves and environmental conditions also.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of plant spp.</th>
<th>Time taken by air bubble to travel from one to other</th>
<th>Average time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1&lt;sup&gt;st&lt;/sup&gt; reading</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; reading</td>
</tr>
</tbody>
</table>

**Figure 12.1: Ganong's potometer**
CONCLUSION:
1. __________ plant showed maximum rate of transpiration
2. __________ plant showed minimum rate of transpiration.

PRECAUTIONS:
1. By opening the stopcock the bubble may be pushed back and experiment restarted.
2. All plant twigs should be of equal size with equal no. of leaves.

Project - 13

AIM:
To study dug resistance in bacteria using antibiotics.

THEORY:
Antibiotics have proved very useful in combating several bacterial diseases in man and animals. These are
the organic secretions produced by microorganisms, which in low concentrations are antagonistic to the
growth of other microorganisms (mostly pathogens).
If we use a particular type of antibiotic drug repeatedly against a disease it may prove less effective. On
account of this, it is often said that the bacteria has developed resistance against that particular antibiotics.
Interestingly, bacteria do not develop immunity or resistance but a few strains of bacteria resistant to that
particular antibiotic evolves from the existing population of the bacteria. The bacterial population may be
having some mutant bacteria which can withstand the action of the antibiotic. The administered antibiotic kills
at bacteria except resistant. These resistant strain grow and multiply even in the presence of antibiotic and
did not respond to the treatment.
The study of the presence of some resistant strains in a bacterial population has been taken for this project.

MATERIALS REQUIRED:
Transfer loops, sterilised culture tubes with media, starch, distilled water, flask, beaker, penicillin, aureomycin,
hay, alcohol, Agar, sterilised petridishes, forcep, burner.

METHOD:
1. Add 8 gms of agar powder and 2 gms of starch to 200 ml of distilled water in a flask. Put a few pieces
   of dry hay into the medium and cover the flask with an inverted beaker. Now boil the medium for 5
   minutes and then cool the medium to room temperature. Place the flask in a warm place. Within 2-3
   days, formation of scum of cloudy suspension appear on the medium which indicates the growth of
   Bacillus subtilis.
2. Take culture tubes with agar medium and to melt agar heat the test tubes in warm water. Now cool
each test tube so that you can hold it in your hand and the agar remains liquid. Remove the cotton
plug and pass the mouth of the test tube through the burner flame twice. Flame the transfer loop after
diping it in alcohol. Let it cool. Pick up a loop full of bacterial culture from the flask and transfer it to the
warm agar in the culture tube. Flame the loop and the mouth of the culture tube and replace the cotton
plug. To mix the bacteria well with agar roll the culture tube of warm agar between palms. Transfer the
bacteria as quickly as possible.

3. Take sterilised petridishes, remove the cotton plug and flame the mouth of the culture tube. Lift the cover of the petridish at an angle of 45°. Pour the medium of the culture tube into the bottom half of the dish quickly. Remove the culture tube and replace the cover of the petridish. To distribute the medium evenly move the covered petridish along the table top in a figure eight pattern. Allow the agar to cool. Prepare two petridishes and label them A and B.

4. By dissolving the powdered drugs in distilled water prepare penicillin and aureomycin solutions. Cut a few discs of filter paper of 1 cm diameter. Soak a disc in each of the penicillin and aureomycin solutions. Dip the forceps in alcohol and pass the forceps tip quickly over the burner flame. Use the sterilized forceps to put penicillin and aureomycin soaked discs at two distant sites of petridish A. Consider petridish B as control. To allow the bacteria to grow keep both the petridishes undisturbed in a warm place or in an incubator observe the petridishes for several days.
OBSERVATION:
The area around the antibiotic discs in the petridish will be clear. Colonies of bacteria will be observed in other areas. Observe the clear area in each petridish for few more days. Very small colonies may appear in the clear areas. These are the colonies of resistant strains of the bacteria.

CONCLUSION:
As antibiotic drugs killed most of the bacterial strain, the area appeared clear. However, a few strains which were resistant in the bacterial population survived and produced colonies later. This shows the resistant strain to antibiotics were present in the bacterial population.

Project - 14

AIM:
To separate different amino acids from a mixture using paper chromatography.

THEORY:
Chromatography means differential migration of the components of a mixture to be separated depending upon its size, density, charge, molecular weight and affinity. It can also be defined as “when a solute or substance is dissolved in two immiscible solvents or solution, it will distribute itself in two phases — with higher conc. in one and lesser in the other”. This is also known as partition chromatography.

Paper chromatography was discovered by Martin and Synge in 1941. It is based on the principle of partition chromatography. Organic solvent moves up on paper by capillary action due to the fibrous nature of paper. Aqueous component of solvent binds to cellulose of paper and thus forms a stationary get like phase with it. Organic component of the solvent (butanol) continues migrating, formatting the mobile phase.

Rates of migration of amino acids being separated is determined by their relative solubilities in the polar stationary phase according to its partition coefficient which means—

\[
\text{Partition coefficient (}K_p\text{)} = \frac{\text{Conc. in stationary phase}}{\text{Conc. in mobile phase}}
\]

So molecules are separated based on their polarities with non-polar molecules moving faster than polar ones.

Amino acids more soluble in butanol move faster as compared to amino acids more soluble in water. Amino acid like glycine with smallest side chain gets absorbed or separated fast near the base.

After development or the resultant of chromatography technique chromatography paper is called chromatogram.

\[
R_f \text{ value (Retention front or Resolution front)} = \frac{\text{Distance moved by solute}}{\text{Distance moved by solvent}}
\]

As different amino acids have different speeds so \(R_f\) value also varies for different amino acids.

MATERIALS REQUIRED:
Measuring cylinder, beaker, aluminium foil, distilled water, automizer, hair drier, oven, separatory funnel,
mixture of amino acids, amino acids aspartic acid or glutamic acid, like glycine, stapler, chromatographic chamber, lysine, leucine, proline, butanol, glacial acetic acid, clamp stand, ninhydrin (0.3% W/V in acetone).

METHOD:

1. First of all prepare the solvent by adding acetic acid, water and butanol in ratio 4: 1: 1 (= Butanol: Acetic acid: water).
2. Now take the solution in chromatographic jar and cover the jar with aluminium foil to saturate the jar with vapours of organic solvent.
3. According to size of the jar; take a piece of whatmann no. 1 paper draw a line 2 cm from below and mark 7 points on it and labelled according to abbreviations of various amino acids.
4. Mixture of amino acids and 6 pure amino acids are taken with their separate micro capillaries and loaded on their respective spots.
5. 12 —15 loadings are done with sufficient drying between 2 loadings.
6. Hang the chromatography paper into the chromatographic jar.
7. After \( \frac{1}{2} \) hours, chromatogram was taken out and dried.
8. After making solvent level ninhydrin is sprayed on it.
9. Chromatogram is kept in oven at 100°C for 10 minutes for colour to develop.

OBSERVATION:
Calculate \( K_p \) for different amino acids:

\[
K_p = \frac{\text{Distance moved by the solute}}{\text{Distance moved by the solvent}}
\]

CONCLUSION:
The given amino acid mixture consists of \__________, \__________, \__________, amino acids.

PRECAUTIONS:

1. Paper should be loaded at same point each time.
2. Remember paper thoroughly after each loading.
3. Solvent level should not touch the loading spots.
4. Once the paper is suspended, it should not be disturbed.
5. Paper should not touch the walls of the jar.
6. Ninhydrin should be prepared fresh, i.e., 0.3% in acetone.
7. Don’t touch the chromatography paper with hands but with forceps and it should be kept in folds of black paper.
Q. 1. Define microorganisms.
Ans. Microorganisms are tiny microscopic organisms present practically everywhere.

Q. 2. What do you mean by sterilisation with reference to microbiology?
Ans. The process of making an article free from germs is known as sterilisation.

Q. 3. What are soil microorganisms?
Ans. The microorganisms occurring in the soil are called soil microorganisms.

Q. 4. Define antibiotics.
Ans. Antibiotics are organic secretions of certain microorganisms, which are capable of killing certain other microorganisms.

Q. 5. What type of microorganisms are present in the soil?
Ans. The main types of microorganisms found in the soil are bacteria, cyanobacteria, protozoans and fungi.

Ans. Pollen grains are microspores (male spores) produced in anther.

Q. 7. What is palynology?
Ans. Palynology is the study of pollen grains.

Q. 8. What is exine composed of and what is its importance?
Ans. Exine is composed of a hard and resistant material called sporopollenin. It helps the pollen grain to cope with environmental hazards.

Q. 9. Which part of the plant produce pollen grains?
Ans. Pollen grains are produced in pollen sacs (microsporangia) in the anther.

Q. 10. How does palynology related to taxonomy?
Ans. Palynology helps in the identification of a species, as the pollen grains of each species have characteristic type of exine.

Q. 11. Name the two layers of the wall of a pollen grain.
Ans. The two layers of a pollen grain are thin intine and thick exine.

Q. 12. Why is it necessary for plants to disperse their fruits and seeds to distant places?
Ans. Dispersal of fruits and seeds avoid competition among the plants emerging out of the seeds and it also help in the propagation of the species to distant places.

Q. 13. Write some special features of seeds dispersed by wind.
Ans. Seeds are usually light in weight, may possess wings, hairs or balloon like appendages which help the seeds to float in the air.

Q. 14. What is the role of animals in the dispersal of seeds?
Ans. Many seeds are sticky or possess spines on their surface to stick to the fur or animals and are carried to distant places. Seeds of several plants are eaten by animals and are passed out with their excreta at new places.

Q. 15. Are some plants able to disperse their seeds without an external agency?
Ans. Yes, some plants disperse their seeds by explosive mechanism.
Q. 16. Name the external agencies that help in dispersal of seeds.
Ans. Wind, water and animals are the external agencies that help in dispersal of seeds.

Q. 17. Define avenue trees.
Ans. The trees, which are planted along the road sides.

Q. 18. How are avenue trees useful to us?
Ans. Avenue trees purify air by releasing out oxygen, and also absorb certain air pollutants. They also cool down temperature due to transpiration, and prevent soil erosion.

Q. 19. Name a few common avenue trees.
Ans. Cassia fistula, Albizia labbeck, polyalthea, Neem, Jamun etc.

Q. 20. What type of plants should be planted as avenue trees?
Ans. Avenue trees should have dense foliage, evergreen with deep root system. They should not have hanging roots and thorns.

Q. 21. What role does chlorophyll play in photosynthesis?
Ans. Chlorophyll plays a very important role in chlorophyll. It traps solar energy and transforms the same into chemical energy contained in organic molecules.

Q. 22. Why do plants growing in diffused light have dark green leaves?
Ans. The plants growing in diffused light have dark green leaves due to the presence of more chlorophyll contents in order to trap maximum available light.

Q. 23. Name the other photosynthetic pigments found in the chloroplasts.
Ans. The other photosynthetic pigments found in chloroplasts are carotenoid pigments.

Q. 24. Name the types of carotenoid pigments found in chloroplasts.
Ans. Carotenes and xanthophylls.

Q. 25. Define colorimeter.
Ans. Colorimeter is an optical instrument. It is used to measure the optical density or intensity of colour of a solution.

Q. 26. How is colorimeter used to measure the chlorophyll contents of a plant?
Ans. Colorimeter measures the optical density (intensity of colour) of chlorophyll extract. Higher optical density indicate higher amount of chlorophyll contents in the plant.

Q. 27. What is spawn? Where it is found?
Ans. Spawn is the cluster of eggs of frog. It is found in ditches, ponds, lakes and other stagnant water during rainy season.

Q. 28. What is metamorphosis?
Ans. It is the process of changes that occur in the larva (tadpole in case of a frog) of an animal to change it into an adult animal.

Q. 29. Define tadpole.
Ans. Tadpole is a larval stage of frog which resembles to fish.

Q. 30. How is tadpole is adapted to aquatic life?
Ans. Tadpole has stream line body with tail, swims with fins, respires through gills, feeds upon aquatic vegetation and excretes ammonia as nitrogenous waste.

Q. 31. Why frog has hind limbs longer than fore-limbs?
Ans. Long hind limbs help the frog to take a long leap.
Q. 32. Which hormone controls metamorphosis?
Ans. Thyroxine controls metamorphosis.

Q. 33. What is the food of tadpole?
Ans. Tadpole is herbivorous animal and it feeds on leave of aquatic plants.

Q. 34. Why don’t you see frog during summers and winters?
Ans. Frog is a cold-blooded animal therefore it undergoes aestivation i.e. summer sleep during the summer months, and during the winter months it undergoes hibernation i.e. winter sleep.

Q. 35. Define a cold-blooded animal.
Ans. A cold-blooded animal changes its body temperature with the changes in the temperature of the surroundings.

Q. 36. How is frog useful for human beings?
Ans. Frog is used in various scientific studies. It eats up a number of crop pests. Its legs form a delicious food, eaten in many countries.

Q. 37. Define antibiotics.
Ans. The organic secretions of microorganisms which kill or inhibit the growth of some other microorganisms are known as antibiotics.

Q. 38. What will happen if the agar tube is not sterilized?
Ans. Some other micro-organisms may develop, thereby spoiling the culture.

Q. 39. Why do a few bacterial colonies appeared in the clear area, when lethal antibiotic was present in the area?
Ans. The antibiotic killed all bacteria except a few strains which were resistant to the antibiotic. These strains multiplied and formed colonies in the clear areas in the petridish later.

Q. 40. Why does a clear area appear in the petridish around the antibiotic drug?
Ans. A clear area appear in the petridish around the antibiotic drug as antibiotic drug prevents the bacterial growth.

Q. 41. Why is a culture medium is required for the growth of microorganisms?
Ans. Microorganisms cannot prepare their own food. Therefore to culture them an artificial medium is required.