BGS Science Academy & Research Centre

Agalagurki, Chikkaballapura
Affiliated to Bangalore University

A MANUAL OF PRACTICAL BOTANY

III BSc. VI SEMESTER

CBCS Scheme - PAPER –VIII
Plant Physiology- III

2019-2020

DEPARTMENT OF BOTANY

Laboratory Instructions

- Do not forget to carry laboratory apron, observation book and other required accessories.
- Handle equipments, microslides, glasswares, chemicals and specimen bottles with proper care.
- Report breakages / damages to the batch incharge or laboratory assistants.
- Keep the laboratory clean and tidy.

PRACTICAL PAPER VIII

PLANT PHYSIOLOGY- III

TOTAL UNITS: 13

1. Determination of rate of photosynthesis at different wavelengths of light. **1 unit**
2. Rate of photosynthesis in different concentrations of Bicarbonate. **1 unit**
3. Evolution of oxygen during photosynthesis. **1 unit**
4. Mohl's half leaf experiment. **1 unit**
5. Evolution of heat during respiration. **1 unit**
6. Evolution of carbon dioxide during respiration. **1 unit**
7. Kuhne's fermentation vessel. **1 unit**
8. Determination of R_Q of Carbohydrates, fats and proteins. **1 unit**
9. Demonstration of phototropism, geotropism and hydrotropism. **2 unit**
10. Determination of rate of plant growth using Arc auxanometer. **1 unit**
11. Separation of photosynthetic pigments by Paper Chromatography and measurement of R_f values. **1 unit**
12. Estimation of ascorbic acid content in plant sample. **1 unit**
13. An industrial visit to study the manufacture of alcohol/ antibiotics/ enzymes. Bioinformatics/ Molecular biology lab.

PRACTICAL QUESTION PAPER VIII

PLANT PHYSIOLOGY-III

Time: 3 hours **Max marks: 35**

1. Separate the photosynthetic pigments from sample **A** by paper chromatography and measure their Rf values. **8 marks**
2. Estimate the ascorbic acid content in the sample **B**. **8 marks**
3. Set up and comment on **C**. **6 marks**
4. Identify and comment on physiological setup **D** & **E**. **2X2 ½ = 5 marks**
5. Record and submission. **5+3 = 8 marks**

SCHEME OF VALUATION

1. A. Requirement - **1** mark, principle - **2** marks, procedure and conducting the experiment - **3** marks, Rf values - **2** marks.
2. B. Requirement - **1** mark, principle - **2** marks, procedure and conducting experiment - **3** marks, result - **2** marks.
3. C. Identification - **1** mark, set up - **2** marks, comments - **2** marks, labeled diagram - **1** mark.
4. D. Identification - ½ mark, comments - **1** mark, labeled diagram - **1** mark.
   E. Identification - ½ mark, comments - **1** mark, labeled diagram - **1** mark.
5. Record and submission of field report (hand written field report only) - **5+3** marks.

PLANT PHYSIOLOGY-III

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1. Determination of rate of photosynthesis at different wavelengths of light

Aim: To determine the effect of quality of light on the rate of photosynthesis.

Principle: The rate of photosynthesis is very high in multichromatic light when we compare with monochromatic light, in the monochromatic red light the rate of photosynthesis is higher than blue light but not greater than white light or multichromatic light.

In Monochromatic light only few chlorophylls which are capable to absorb a particular wavelength of light are active in photosynthesis but other chlorophylls are in inactive state.
In multichromatic light different wavelength of light is simultaneously absorbed by different chlorophylls as a result, the net yield of photosynthesis will be high.
Oxygen is a by-product of photosynthesis. The rate of photosynthesis is measured in terms of amount of oxygen evolved in unit time. Rate of photosynthesis is influenced by different colours of light. So, it is studied under direct sunlight, red, yellow and blue lights. Its effect on the rate of the photosynthesis is studied by counting the number of oxygen bubbles evolved in unit time.

Requirements: *Hydrilla* plants, funnel, beakers, tap water, test tubes and red, blue and yellow cellophane papers.

Procedure:

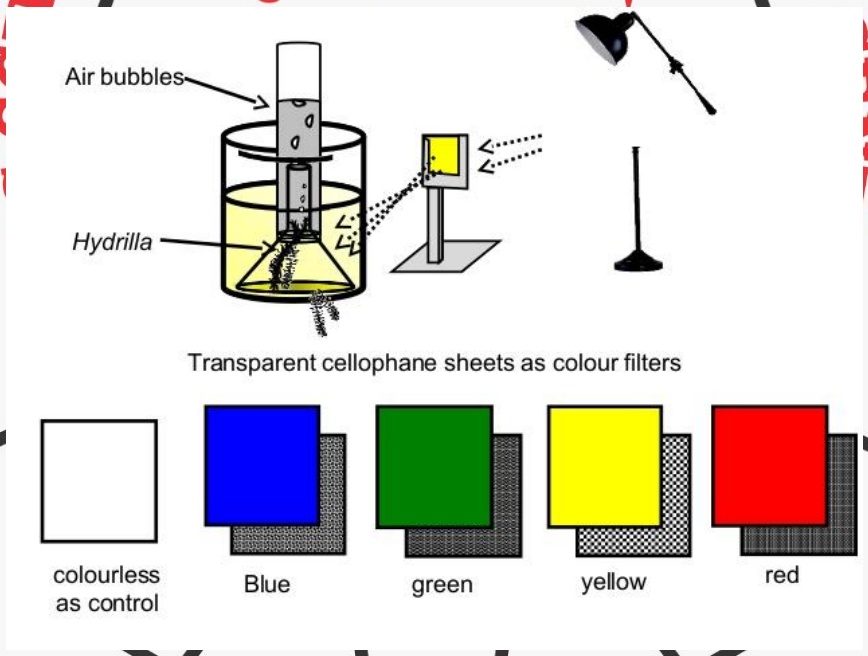
1. Take about 500 ml of tap water in the beaker.
2. Cut healthy twigs of *Hydrilla*, about 10 to 15 cm long from the growing apex, under water.
3. Place *Hydrilla* twigs of more or less same length in the funnel and place within the beaker.
4. It is better to tie up the cut shoots gently into a bundle with a thread and keep the cut ends projecting upwards, facing the stem of the funnel.
5. Test tube filled with water is inverted over the stem of the funnel.
6. Photosynthesis is measured under direct sunlight by counting the number of oxygen bubbles for every 5 minutes. Take 3 readings.
7. Cover the setup with red colour cellophane paper and keep it in the sunlight. Count the number of air bubbles for every 5 minutes. Take 3 readings.
8. Repeat the same procedure with blue and yellow cellophane papers. Tabulate the results.
9. Draw a graph by taking time in minutes in the X-axis and the number of bubbles evolved in the Y- axis.

Observation: The formation of air bubbles, at the cut end of *Hydrilla* stem. Count the air bubbles in each setup and record alternately. Under direct sunlight, production of more air bubbles is observed. In yellow light, production of air bubbles is very less compared to red light and blue light.

Inference: The amount of oxygen gas collected is more in control setup followed by red, blue, yellow and green light respectively because the chlorophylls of all types activate in photosynthesis but under red light the collected gas is more than blue because of the red light is absorbed by the

chlorophylls more than the blue and remaining colour. The rate of photosynthesis is directly depending upon the liberation of oxygen as a byproduct, simple it can be measured by its volume.

Quality of light	0-5 minutes	5-10 minutes	10-15 minutes
Sun light			
Red light			
Blue light			
Yellow light			



2. Rate of photosynthesis in different concentrations of Sodium Bicarbonate ($NaHCO_3$).

Aim: To study the effect of concentration of sodium bicarbonate on the rate of photosynthesis by bubble counting method.

Principle: During photosynthesis, oxygen is evolved and carbon dioxide is consumed. The rate of photosynthesis is measured in terms of amount of oxygen evolved in unit time. Plants can use bicarbonate as the source of Co_2, so that the rate of photosynthesis varies with concentration of bicarbonate.

Its effect on the rate of photosynthesis is studied by counting the number of bubbles of oxygen evolved in unit time. The optimum concentration of bicarbonate required for maximum photosynthetic rate is found out.

Requirements: *Hydrilla* plants, beakers, test tubes, measuring cylinder, funnel, glass rod, boiled and cooled water, sodium bicarbonate salt and graph sheet.

Procedure:

1. Take two beakers of 250 ml capacity and fill them with pre- cooled boiled water.
2. But several healthy actively growing twigs of *Hydrilla*, about 10 to 15 cm long from the growing apex, underwater.
3. Please 4 or 5 weeks of the same length in each beaker and cover them with a glass funnel under water.
4. It is better to tie up the cut shoots gently into a bundle with a thread and keep the cut ends projecting upwards, facing the stem of the funnel.
5. Over the stem of the funnel in each case invert a test tube completely filled with water without any air bubble.
6. Keep one of the setups as control, i.e., do not add sodium bicarbonate to the Water in the beaker in this case. But exposed it to light and observe for the evolution of bubbles of oxygen.
7. In the second set up, add a known quantity of salt of sodium bicarbonate to the Water in the beaker at a regular interval until saturated solution of sodium bicarbonate is formed in the beaker.
8. Initially add 0.5 gram of $NaHCo_3$ to the water and stir well with a glass rod until it gets dissolved completely. Expose the setup to light. Count the number of bubbles evolved for every 5 minutes up to 15 minutes. Then calculate the average number of bubbles evolved per minute. While counting, discard the very small bubbles. Select only the relatively bigger bubbles of more or less of uniform size for counting.
9. Now increase the quantity of salt as given in the table and observe the effect of this on the rate of photosynthesis by counting the number of bubbles evolved per minute.

10. Repeat the procedure by gradually increasing the concentration of salt in water at every step 0.5, 1.0, 1.5 and 2.0 grams. Then study the effect of the varied concentration of sodium bicarbonate on the rate of photosynthesis.
11. Record the observations.
12. Plot the obtained data on a graph sheet with the concentration of $NaHCO_3$ in X-axis and number of oxygen bubbles evolved per minute in the Y-axis.

Observation:

The rate of photosynthesis is at its maximum at _____ grams of sodium bicarbonate.

Concentration of Sodium Bicarbonate (In gm)	No. of oxygen bubbles evolved after		
	5 minutes	10 minutes	15 minutes
0.5			
1.0			
1.5			
2.0			

Inference:

The graph shows that the concentration of carbon dioxide plays an important role in photosynthesis. The increase in sodium bicarbonate to some extent increases the rate of photosynthesis, but beyond the optimum level, the concentration of carbon dioxide is toxic to the plants. So it reduces the rate of photosynthesis.

Plants use carbon dioxide in the form of bicarbonates. The rate of photosynthesis is at its maximum at _____ grams of sodium bicarbonate.

3. Evolution of Oxygen during photosynthesis.

Aim: To demonstrate that oxygen is liberated during photosynthesis.

Requirements: *Hydrilla* plants, beaker funnel, test tube, water and glowing splinter.

Procedure: Fill up the 3/4 of the beaker with water. Cut healthy *Hydrilla* plants and place them under inverted funnel placed in the beaker. The cut end of the plant should face the tube of the funnel. Carefully invert the test tube filled with water over the stem of the funnel as indicated in the figure. Keep the whole set up in the sunlight.

Observation: As photosynthesis occurs in the leaves of the H*ydrilla* plant, gas bubbles are released from the cut ends of the twigs. The bubbles are collected at the apex of the inverted test tube.

When water in the test tube is completely replaced by gas, it is carefully removed and a glowing splinter is introduced into the test tube. Now the splinter catches fire and burns.

Inference: From the above experiment, it is clear that the gas collected in the test tube is Oxygen.

The oxygen (end product) is released from H*ydrilla* plant as a result of photosynthesis.

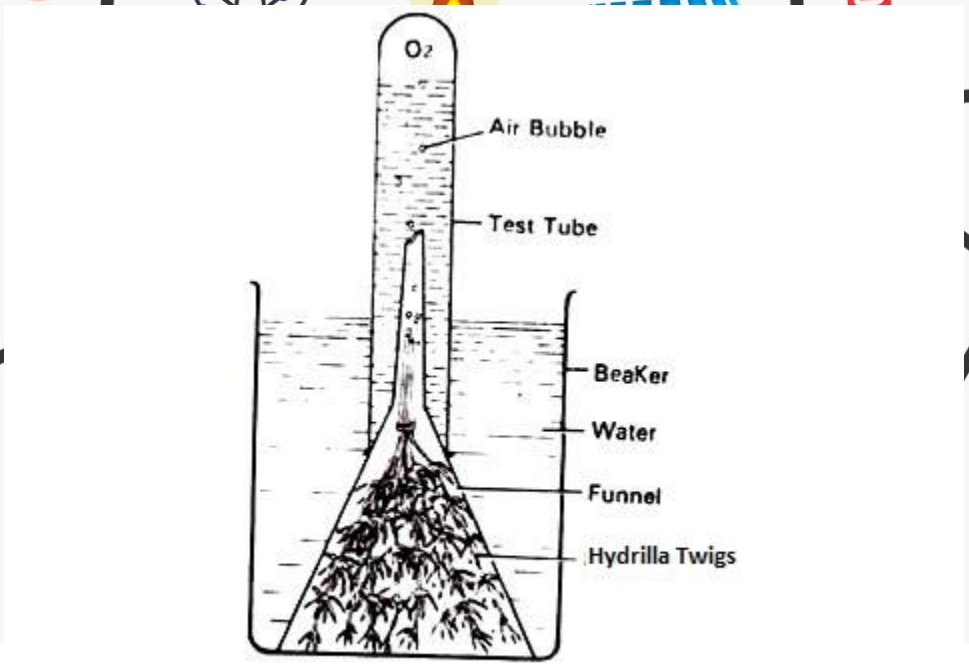


Fig. 37. Demonstration of evolution of oxygen during photosynthesis.

4. Mohl's half leaf experiment.

Aim: To demonstrate that carbon dioxide is essential for photosynthesis.

Requirements: A potted plant, wide mouthed bottle with one holed rubber cork, KOH solution, alcohol, vaseline and Iodine solution.

Procedure:

1. Take a wide mouthed bottle fitted with the split cork.
2. Pour small quantity of KOH solution into the bottle which is laid horizontally.
3. Inert starch free green leaf into the bottle through the split cork in such a way that half of the leaf blade will be inside the bottle and the other half outside.
4. See that the part of the leaf inside the bottle does not touch the KOH solution.
5. The leaf should be attached to the plant or if the leaf is detached the petiole of the leaf should be kept dipping in water in a beaker.
6. Smear the edges of the split cork with vaseline to make the apparatus airtight and keep it exposed to sunlight for a few hours.
7. Take out the leaf in the evening and do the test for starch.

Starch Test: Put the leaf in boiling water for about 5 minutes to soften and kill the tissues. Then transfer it to a beaker containing 90% warm alcohol. When the leaf is completely decolourised i.e., when all chlorophyll has been extracted, place the bleached leaf in iodine solution for a few minutes. Note the colour change.

Observation: The lower part of the leaf which remained outside the bottle becomes blue, indicating the presence of starch. The upper part of the leaf which remain inside the bottle does not become blue, indicating the absence of starch.

Inference: The lower part of the leaf has utilised the carbon dioxide of the atmosphere and synthesized starch by photosynthesis. The KOH solution inside the bottle has absorbed the carbon dioxide in the air inside the bottle. Hence the upper part of the leaf could not prepare starch by photosynthesis because of lack of carbon dioxide. This experiment shows that carbon dioxide is essential for photosynthesis.

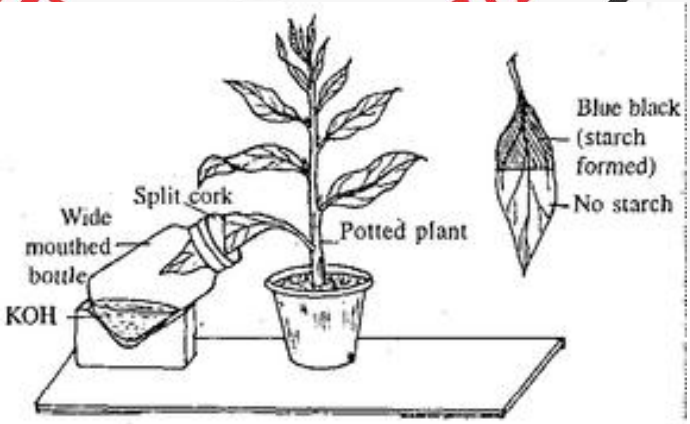


Fig. 5.2.6.5 : Mohl's half-leaf experiment to demonstrate that CO_2 is necessary for photosynthesis

5. Evolution of Heat during respiration.

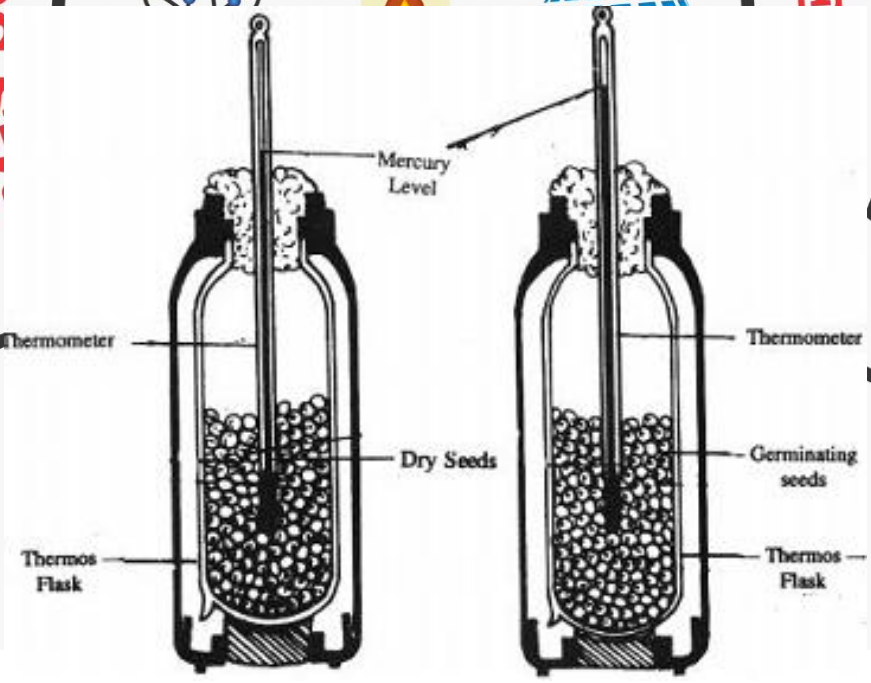
Aim: To demonstrate that some energy is released in the form of heat during respiration.

Principle: Respiration is a biological oxidation where oxygen is utilized and carbon dioxide is released the liberation of energy. During respiration, the complex organic materials are broken down to simple substances like carbon dioxide and water with the generation of energy. This energy is utilized in various metabolic activities and some amount is lost as heat.

Materials required: Two thermo flasks, two thermometers, germinating and dry seeds and two corks or cotton plugs with a bore for fixing the thermometer.

Procedure: one of the thermos flasks is half filled with germinating seeds and the other thermo flask is half filled with an ungerminating or dry seeds. Both the flasks are tightly closed with a cork fitted with the thermometer. The temperatures in the two thermo flasks are recorded. The setup is kept undisturbed for few hours and the temperatures in both the flasks are recorded.

Result: The thermometer of thermos flask containing germinating seeds records a marked rise in temperature because during respiration some amount of energy is released in the form of heat. The flask containing an germinating seeds do not show any change in the temperature. It has revealed that some amount of heat is released from the germinating seeds during respiration.



A
Thermos Flask with Dry seeds (Ungerminated)

B
Thermos Flask with Germinating seeds

6. Evolution of carbon dioxide during respiration

Aim: To demonstrate the liberation of carbon dioxide during aerobic respiration.

Materials: A bottle or conical flask, two holed cork, bent tube, reservoir with tube and stop cock, beakers, stand, water, lime water, germinating seeds, KOH solution.

Procedure:

- Germinating seeds are placed in a bottle.
- It is closed by a cork through which a glass tube is inserted, bent twice at right angles. The free lower end is allowed to dip in water.
- Through another hole is inserted a tube holding a water reservoir with a stop clock.
- The water seal (water in beaker) and stop clock of the reservoir are closed. The seeds are allowed to respire for some time.
- Water seal is then replaced by beaker containing lime water. Stop clock of the reservoir is opened and air inside the bottle is driven out.

Result: The air bubbles come out through the bent tube into the beaker containing lime water which turns milky.

Conclusion: The turning milky lime water indicates the presence of carbon dioxide. This gas is apparently released during germination of soaked seeds.

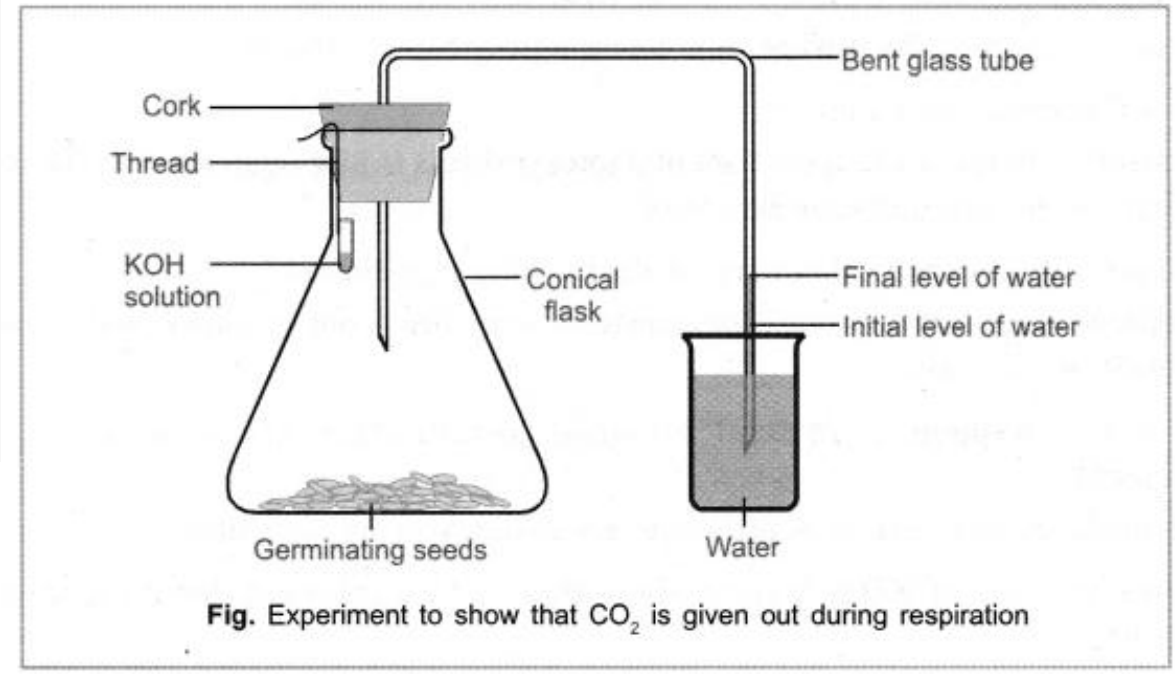


Fig. Experiment to show that CO_2 is given out during respiration

7. Kuhne's fermentation vessel.

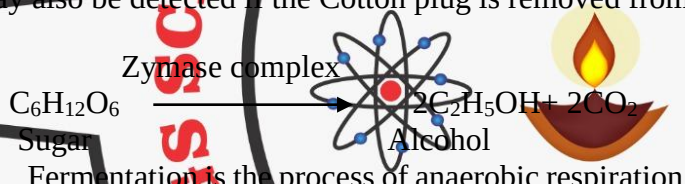
Aim: To demonstrate fermentation by yeasts.

Requirements: Kuhne's fermentation tube, 10% glucose solution, baker's yeast and cotton plug.

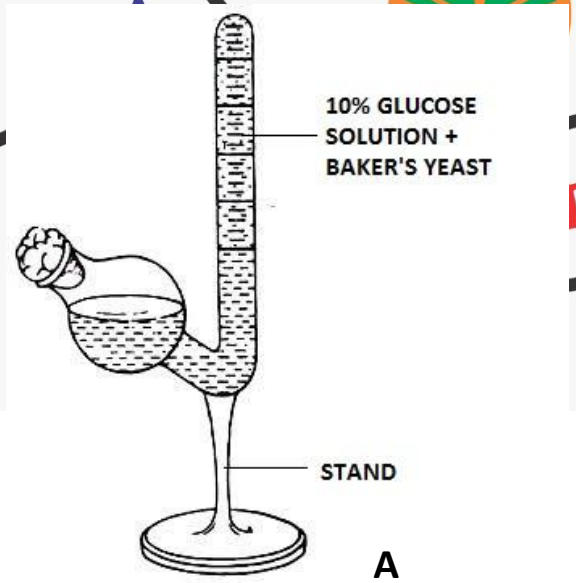
Procedure: Kuhne's fermentation tube consists of a vertical and slightly oblique tube connected with a side bulb. The vertical tube is closed at the upper end. Fill the Kuhne's fermentation tube with 10% glucose solution. Add a little of baker's yeast into it and shake well. See that the glucose solution is completely filled up the upright arm, and about half of the side bulb. Close the mouth of a side bulb with cotton plug. Keep the Kuhne's fermentation tube in warm dark place (temperature about 30^0C).

Observation: A gas starts accumulating in the upper end of the vertical tube. At the same time, the level of sugar solution falls down in the tube.

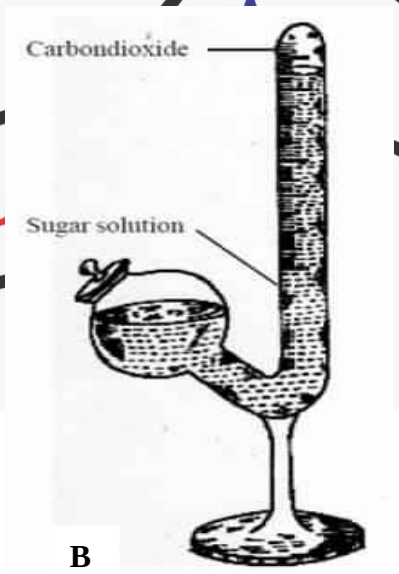
Inference: The yeast cells respire anaerobically and cause fermentation of the glucose solution. During the process carbon dioxide is liberated and the gas gets collected at the apex of the tube replacing the glucose solution. So, the level of the solution falls down in the tube. The gas on testing with KOH (potassium hydroxide) is found to be carbon dioxide. A faint smell of alcohol may also be detected if the Cotton plug is removed from the mouth of the bulb.



Fermentation is the process of anaerobic respiration. The enzymes secreted by the living yeast cells the (zymase) acts upon the substrate(sugar solution) in the absence of Oxygen and bring about the incomplete oxidation of sugar into alcohol and carbon dioxide.



A



B

Kuhne's fermentation vessel

8. Determination of R.Q of carbohydrates, fats and proteins.

Aim: To determine R.Q (respiratory quotient) of different kinds of germinating seeds using Ganong's respirometer.

Principle: R. Q is respiratory quotient. It refers to the ratio between the volume of carbon dioxide evolved and volume of oxygen absorbed during respiration.

$$R.\ Q = \frac{\text{Volume of } CO_2 \text{ evolved}}{\text{Volume of } O_2 \text{ absorbed}}$$

1. R. Q is > 1, when CO2 is more than O2 used in the process of respiration. In this case the brine level in the respirometer tube will fall down. The R. Q is calculated by the formula

$$R.\ Q = \frac{V_2}{V_2 - V_1}$$

Where,

V_2 = Volume of CO_2 evolved as measured by adding KOH in unit time.

V_1 = Volume of excess of CO_2 released over the amount of O_2 consumed.

2. R. Q is < 1, when CO2 is evolved is less than O2 utilises in respiration. Here the brine level will rise in the tube of the respirometer. The R. Q is calculated by the formula.

$$R.\ Q = \frac{V_2}{V_1 + V_2}$$

Where,

V_2= Volume of CO_2 evolved as measured by the addition of KOH in unit time.

V_1= volume of excess of O_2 absorbed over the amount of CO_2 released.

3. R. Q is = 1, when the volume of CO2 evolved is equal to the volume of O2 absorbed in respiration. Here the level of brine in the manometer will remain stationary.

R. Q is unity when the respiratory substrate is fat or protein. It is more than one when the substrate is some organic material rich in oxygen.

In this experiment, the volume of CO_2 evolved in respiration is directly measured. But, the amount of O_2 utilized in the process is determined in directly from the fall or rise in the level of the brine solution in the respirometer tube. Instead of Mercury, brine solution is used to set up the experiment as CO_2 does not dissolve in the saturated solution of NaCl.

Requirements: Ganong's respirometer, Germinating seeds such as wheat, rice (carbohydrate), castor or groundnut (fat), pea or green gram (protein), flower buds, saturated solution of NaCl (brine), KOH, cotton and stand.

Description of Apparatus: Ganong's Respirometer consists of a glass-bulb connected with a graduated glass tube. The neck of the bulb bears a hole. The lower part of the glass stopper which fits in the neck of bulb also bears a similar hole. The graduated tube is connected with a leveling glass tube filled with brine solution by means of a rubber-tube. Whole of the apparatus is fixed in a stand.

Procedure:

1. Pour the brine solution through the levelling tube; so, it rises into the graduated tube into a definite level.
2. See that the level in both tubes remains the same initially by adjusting the levelling tube.
3. Note the initial level of brine solution in the graduated tube.
4. Take the plant material in the bulb of the respirometer and closet with the stopper.
5. Rotate the stopper in such a way that its hole is in the line with the hole in the neck of the bulb. Now the air inside the bulb communicates with the atmospheric air. Hence, it is the atmospheric pressure.
6. After few minutes, again rotate the stopper slightly so that the two holes move apart. That's the connection with the outside air is cut off.
7. Keep the setup in darkness for a definite period to allow the respiration of the seeds to go on in the closed chamber.
8. At the end of the experimental period, note the change in the level of brine solution in the graduated tube. The level may remain same or rise or fall depending on the materials taken for the experiment and the substrate being oxidized.
9. Note the difference between the first and second readings, if there is rise or fall in the level of brine solution. Let this be the volume of oxygen absorbed, V_1 c.c.
10. Then introduce a small crystal of KOH, to find out the actual amount of carbon dioxide released in the process of respiration.
11. Observe the rise of the salt solution in the graduated tube after introduction of KOH. Note the reading again. Find out the difference between the second and third readings. this will give the actual amount of carbon dioxide evolved in the respiration during the experimental period. Let this be V_2 c.c.
12. Record the data obtained.

Observation:

There is a rise in the level of brine solution in the graduated tube.

Volume of carbon dioxide evolved in unit time =

Volume of oxygen absorbed in unit time =

Result:

R. Q of the given material =

Inference: For the given material R. Q is _____. This indicates that being a proteinaceous seed, lesser volume of carbon dioxide has been produced compared to the volume of oxygen absorbed. After introducing KOH, there is a rise in the level of brine solution due to absorption of carbon dioxide by KOH.

Conclusion:

No change in the level of saline solution indicated the amount of O_2 consumed is equivalent to the amount of CO_2 released. There will be no change in the volume of air in the bulb. Hence it does not press down nor suck the saline solution from the graduated tube. Thus the RQ is equivalent to 1 or unity. RQ is calculated as follows:

RQ = Amount of CO_2 evolved / Amount of O_2 consumed

In case of carbohydrates (Wheat, rice) such as sugars volume of CO_2 is equivalent to volume of O_2 consumed

$C_6H_{12}O_6 + 6O_2 + 6H_2O = 6CO_2 + H_2O$

RQ = Amount of CO_2 evolved / Amount of O2 consumed = 6/6 = 1 or unity

When fats are respiratory substrates (castor, groundnut, mustard) the level of saline solution in the graduated tube will rise. Fats have less oxygen and hence require more oxygen and for oxidation. O_2 consumed in respiration is more than the CO_2 evolved then RQ is less than unity.

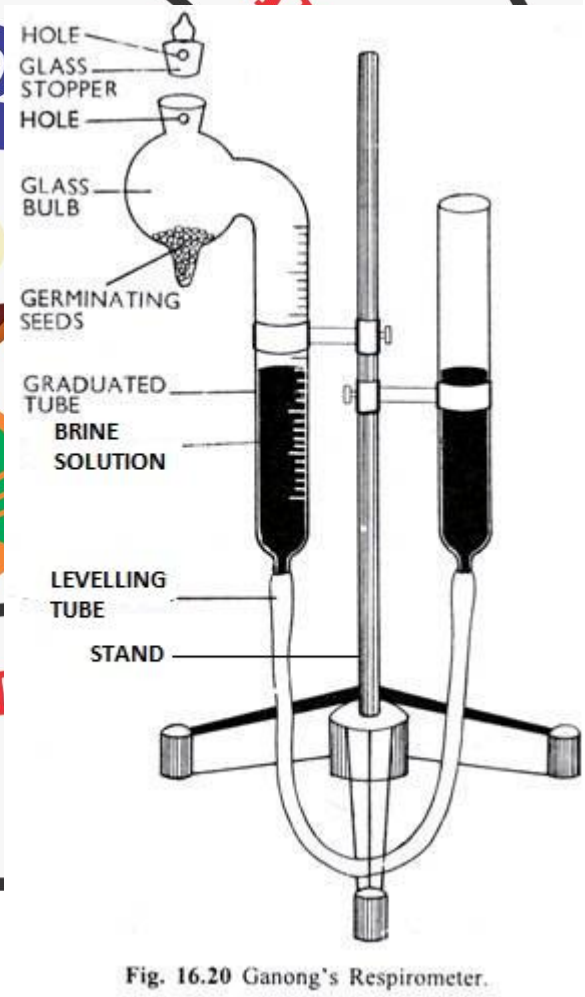


Fig. 16.20 Ganong's Respirometer.

9. (i) Demonstration of Geotropism.

Aim: To prove geotropic movement in plants using clinostat.

Principle: The curvature movement of plant organs in response to the force of gravity is called geotropism. It has a marked effect on direction of growth of plant organs.

Materials required: Clinostat and potted plant (7 days old).

Procedure: Clinostat is an instrument by which the effect of lateral light and the force of gravity on an organ of plant (root or stem) can be eliminated. A potted plant maybe fixed in the clinostat at in any position (vertical, horizontal or at an angle) handmade to rotate by clockwork mechanism in the clinostat. The plant can be rotated 2 to 4 times in an hour. The direction of growth of plant is observed.

Inference: The root grows towards Gravity. Hence the root is positively geotropic. The stem grows away from the gravitational force. Hence, the stem is negatively geotropic.

When the clinostat is not rotated and the plant is kept horizontally, the stem curves upwards and root curves downwards. This is because the stem and root receives gravity on the lower side only.

When the clinostat is rotated and the plant is kept horizontally, the root and stem grow horizontally only. They never bend downwards or upwards. This is because; the effect of gravity is uniform all around the stem.

The building growth is caused by auxin, a growth hormone. The stem and root tips synthesize auxin in response to gravity. The bending is due to unequal distribution of auxin. In the root tip, the concentration of auxin is more on the upper side and less on the lower side. This results in more growth on the upper side and less growth on the lower side, resulting in positive geotropism.

In the stem tip, the concentration of auxin is more in the lower side and less on the upper side. This results in more growth on the lower side and hence the stem bends upward.

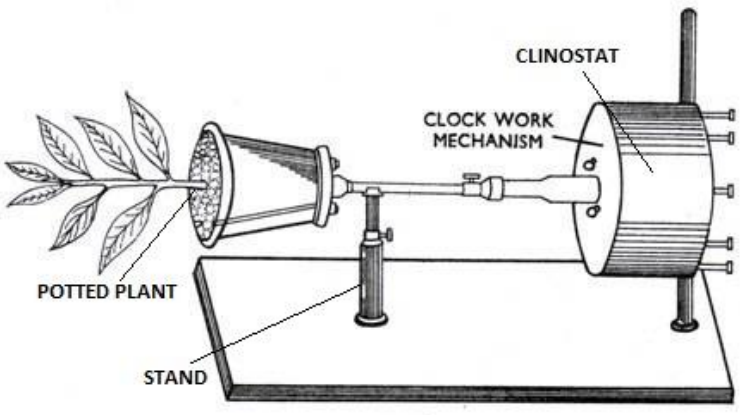


Fig. 21.4. Clinostat.

9 (ii). Demonstration of Phototropism.

Aim: To prove the phototropic movement in plants.

Principle: The curvature growth movement of plant parts in response to light is called phototropism. Some plant organs grow towards the light, while others grow away from it. The cause of phototropic curvature is explained on the basis of plant growth substances.

Materials required: Phototrophic chamber and 7 days old seedling in a pot.

Procedure: 7 days old Seedling in a pot is placed in the phototrophic chamber. The plant is watered daily. After one week the phototrophic chamber is open and the growth of the plant is observed.

Observation: The plant grows towards the open window.

Inference: The plant tends to grow towards the open window of the chamber i.e., towards source of light. The bending of plant parts in response to light is called phototropism. Phototropism is a curvature movement. It is caused by growth and hence it is a growth movement. As it is stimulated by an external factor (light), it is called a paratonic moment. As the plant is growing towards light it is called positive phototropism.

The phototrophic curvature is due to synthesis of auxin, a growth hormone. The stem tip synthesizes this hormone. The higher concentration of auxin on the shaded side leads to greater growth on this side and the stem bends towards light.

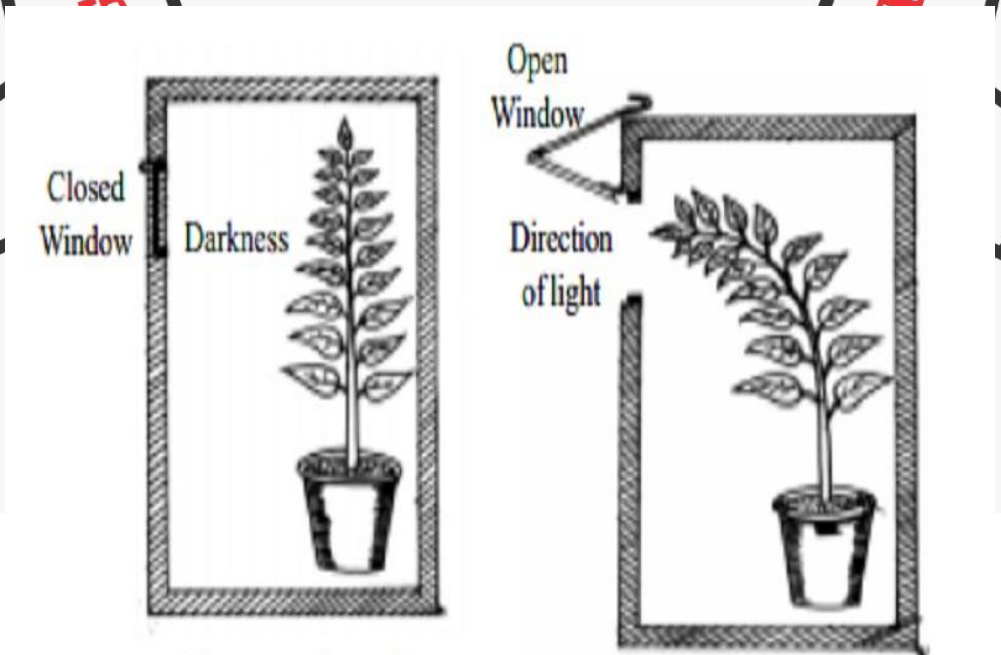


Fig. Experiment to demonstrate phototropism

9 (iii). Demonstration of Hydrotropism.

Aim: To prove the hydrotropic movement in seeds.

Principle: The bending moment of an organ in response to stimulus of moisture is known as hydrotropism. Roots show a tendency to grow towards the source of moisture or water.

Materials required: Conical flask or wide mouthed bottle, perforated funnel, saw dust, filter paper, seeds (cereals) and water.

Procedure: A filter paper is placed on the perforated funnel. The filter paper is kept moist. The dry saw dust is piled on the same and the soaked seeds are arranged in a circle. Few drops of water is added to the seeds to help their germination. The perforated funnel is placed on a wide mouthed bottle containing water. After a week the direction of the movement of radicle of the germinated seeds are observed.

Inference: the radicles of the germinating seeds, instead of growing vertically downwards in response to the force of gravity, pass out through the pores towards the moist filter paper and grow upwards alongside the paper into the moist sawdust.

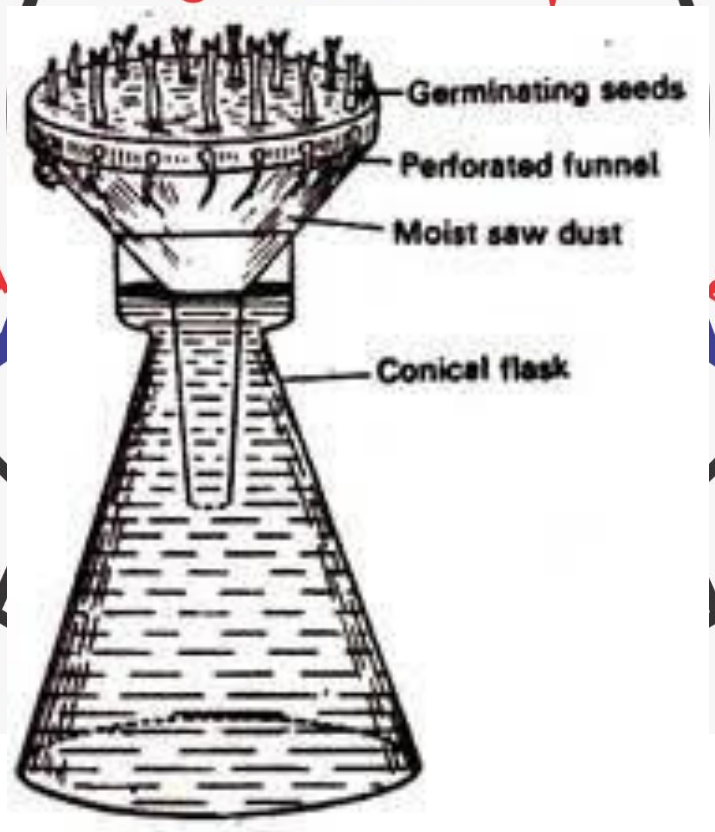


Fig. 43.

10. Determination of rate of plant growth using Arc Auxanometer.

Aim: To determine the rate of plant growth using Arc auxanometer.

Principle: Using the instrument a small increase in length can be magnified many times. From the total known magnification, the actual length attained by the plant in a specified time can be calculated.

Materials required: Arc auxanometer, small potted plant, thread and small weight.

Procedure: Arc auxanometer has a movable lever fixed to a pulley. A thread is passed over the pulley with one end tied to the growing point of the plant and the other end carrying a weight to keep the thread stretched. As the stem increases in length, the pulley slowly rotates and the pointer moves down on the graduated arc. The growth in length of the plant is recorded on a magnified scale for 1 hour. The actual length of the stem is calculated from the record using the formula given below.

Growth rate = X/90×1/24

X = The distance travelled by the lever in mm.

90= Scale's magnification.

24= Hours.

Result: If the pointer moved 35 mm/hr in the scale, then growth of the plant in 24 hrs is given by

35/90 × 1/24

= 0.39 × 0.042

= 0.02 mm/day

Therefore, the experimental plant growth is 0.02 mm per day during experimentation.

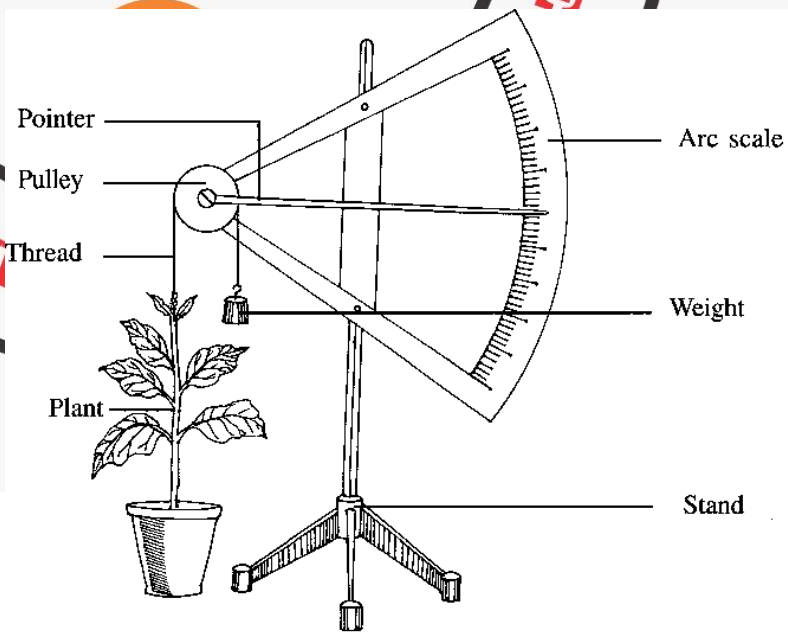


Fig. 2.16 Lever Auxanometer

11. Separation of photosynthetic pigments by Paper Chromatography and measurement of R_f values.

Aim: To separate the photosynthetic pigments from the green plant tissues by paper chromatography.

Principle: The technique of chromatography is used for separating and identifying substances present in a mixture. The substances get separated because of their different affinities for a stationary phase and their differential solubility in a moving phase. In paper chromatography, the hydrated cellulose molecules of the paper constitute the stationary phase and the mobile phase is a liquid. The separation of compounds in a mixture depends on the partition, between the mobile and stationary phases.

The rate of movement of different substances in the mixture is determined by the partition coefficients. Hence some compounds will move faster while others move at low rates and get separated. The compounds are identified by their ***Rf values***.

Rf value (resolution front) is the ratio between the distance travelled by organic compound (d) and the distance travelled by the solvent (D) from the point of origin, Rf= d/D. The values thus obtained are compared with standard values.

Requirements: Leaves of *Tecoma*, a piece of carrot, a pinch of $CaCo_3$, acetone, petroleum ether, solvent mixture (9:1 ratio petroleum ether and acetone) Whatman filter paper number 1, mortar and pestle, beaker measuring jar, Chromatographic jar or Chamber, split cork, micropipette and muslin cloth, Hair drier.

Procedure:

1. Cut the *Tecoma* leaves and carrot into small pieces. Grind thoroughly until the tissue become homogenized along with a smallvolume of acetone in mortar and pestle. Add few sand particles or a pinch of $CaCo_3$ to facilitate grinding.
2. Decant the liquid part into test tube by filtering with cotton cloth (centrifuge for 5 minutes at 300 rpm). Collect the supernatant and use it as a source of pigments.
3. Take a Chromatographic jar containing 5 ml of solvent mixture. Close it tightly with the split cork.
4. Take a long strip of Whatman paper No- 1 (2×20cm). Apply 3 to 10 drops of the concentrated extract in the form of a spot, about 3 cm away from the base of the strip, using fine micropipette. The spot must be within 1 cm in diameter. Care should be taken to avoid over spreading of the extract.
5. Allow the spot to dry for some time.
6. Place the strip inside the chromatographic jar containing the solvent mixture (9:1 ratio petroleum ether and acetone) in such a way that the spotted side of the paper is just

immersed for 0.5 cm in the solvent. Care should be taken in such a way that the loaded spot should not be dipped in the solvent.

7. Close the chamber with the split cork. Make the setup air tight and another solvent to run from the basal end of the paper strip almost to the top. Support the test tube on a stand vertically. Leave it undisturbed for an hour or until the solvent reaches the line marked at the top.
8. After the pigments have got separated clearly in the form of distinctly coloured bands on the paper, take out the strip and immediately mark the place of various colours and identify the pigments and calculate Rf value for each pigment.
9. To find out the value of Rf for each pigment, measure the distance travelled by it from the loaded spot and also the distance travelled by the solvent.
10. Then record the colour of each pigment band in the tabular form as shown below.
11. Compare the Rf values obtained with standard values for the particular solvent used, to identify the pigments.

Sl. No	Name of the pigment	Colour of the pigment
1	Chlorophyll b	Light green
2	Chlorophyll a	Dark green
3	Xanthophyll	Light yellow
4	Carotene	Orange yellow

Observation: The filter paper here acts as the ***absorbent.*** The solvent mixture is the ***developer.*** The solvent moves only in one direction that is upwards. Hence, this method is called ascending unidimensional paper chromatography.

The distance travelled by the solvent '**D**' = 10.5 cm.

The distance travelled by the pigment ='d'

Distance travelled by the pigment

Rf = $\frac{\text{Distance travelled by the solvent (D)}}{\text{Distance travelled by the solvent (D)}}$

Result:

Rf value of Chlorophyll b =

Rf value of Chlorophyll a =

Rf value of Xanthophyll =

Rf value of Carotene =

Inference: There are 4 bands appeared on the paper, namely chlorophyll b, at the bottom in the light green colour, chlorophyll a in dark green colour, xanthophyll in light yellow colour and carotene as Orange Yellow band at the top.

Carotene moves faster comparing to other pigments due to high solubility and partition coefficient.

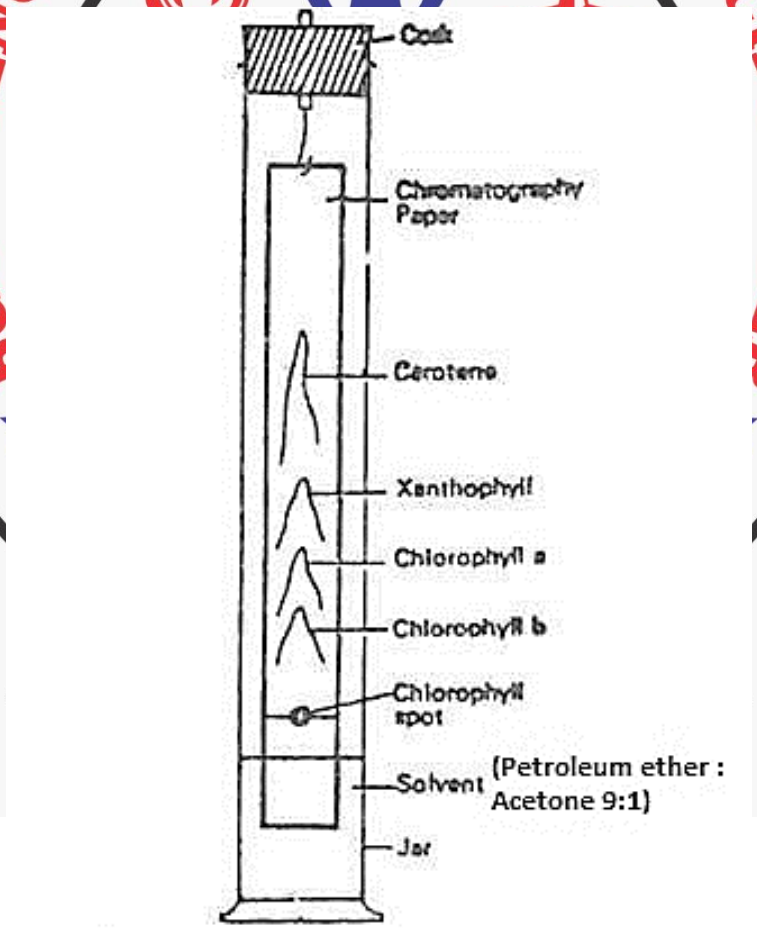


Fig. 41. Demonstration of paper chromatography.

12. Estimation of ascorbic acid content in plant sample.

Aim: To estimate the ascorbic acid content in a given plant sample.

Principle: Ascorbic acid popularly known as vitamin-C is regularly present in many common fruits like lemon, sweet lime, orange, chillies, guava etc. The quantity of ascorbic acid can vary with species, variety, age of the fruit and the plant and many other factors. Estimation of some can be done using its reaction with a dye called dichlorophenol indophenol (DCPIP).

An aqueous solution of DCPIP is blue in colour, but it turns into pink in acidic medium such as a solution containing of metaphosphoric acid or a mixture of oxalic acid and acetic acid. But this conversion does not take place as long as ascorbic acid is present in the medium. This technique/principle is used in a titration experiment in order to find out the amount of ascorbic acid in a plant extract.

Materials required: Freshly collected plant material (fruit of guava, gooseberry, capsicum or lemon) standard vitamin C tablet (such as celin tab 500mg), acid mixture aqueous solution of DCPIP, conical flask (100ml capacity), beakers, pipettes, burettes and stand.

Preparation of reagents:

Aqueous solution of DCPIP: 26 mg of DCPIP and 21 mg of sodium bicarbonate are dissolved in a small quantity of distilled water and the volume is then made up to 100 ml, this solution has to be taken in the burette.

Acid mixture: 1g of oxalic acid is dissolved in 100 ml of glacial acetic acid and the solution is then made up to 1 litre.

Standard ascorbic acid solution: One fresh tablet of Vitamin C is dissolved in 40 ml of oxalic acid or acetic acid mixture and then the result solution is made up to volume of 500 ml, thus 1 ml of the solution would contain 1 mg of ascorbic acid.

Plant extract: 5 gram of freshly collected plant material is taken in 40 ml of oxalic acid or acidic acid and ground in a mortar and pestle if necessary filtration is done to remove solid suspensions, the filtrate is then made upto a volume of 100 ml.

Procedure: In the first phase of experiment 1 ml of standard ascorbic acid solution is mixed with 5 ml of oxalic acid Acetic Acid mixture and the solution is titrated against DCPIP solution.

In the Second Phase 1 ml of plant extract is mixed with 5 ml of oxalic acid or Acetic Acid mixture and the solution is titrated against DCPIP solution. The end point in both cases is the retention of Pale pink colour at least for 30 seconds.

Observation of end points: the blue colour of the DCPIP turns to pink as soon as it enter the solution in the conical flask but it quickly vanishes as long as ascorbic acid is available in the

solution. When all ascorbic acid is exhausted, the pink colour stays for not less than 30 seconds, image base titration distributed in order to get concurrent values.

SL. NO	I	II	III
FINAL READING			
INITIAL READING			
VOLUME OF DCPIP CONSUMED			

Calculations:

Phase I

Volume of DCPIP required for neutralizing 1mg of ascorbic acid present in 1ml of the standard ascorbic acid solution: Final reading – initial reading = Va

Phase II

Volume of DCPIP required for neutralizing the unknown quantity of ascorbic acid present in 1ml of plant extract: Final reading – initial reading = Vp

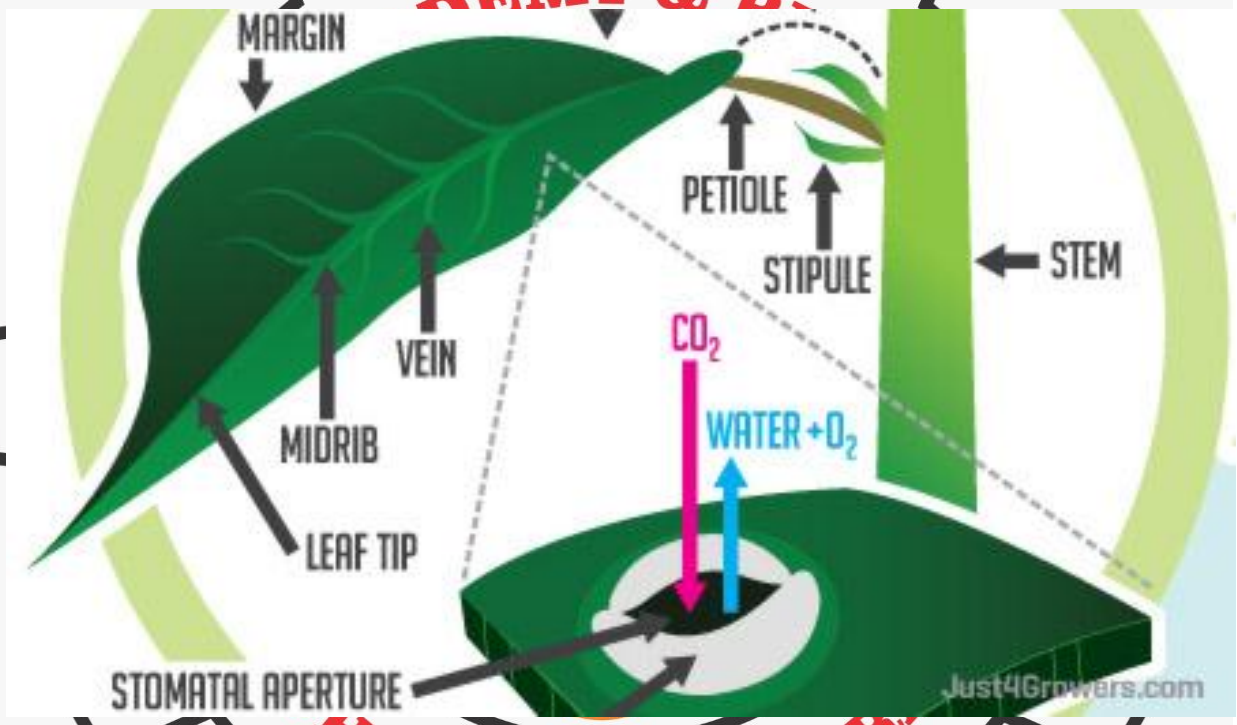
Therefore, quantity of ascorbic acid present in 1ml of plant extract = Vp/Va=Vx

Therefore, quantity of ascorbic acid present in 5gm of plant material = Vx ×100

Results:

Amount of ascorbic acid present in the given sample:

1. Lemon sample = _____
2. Orange/Chilly sample = _____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