PRACTICAL E-MANUAL

FOR

SEMESTER III

BOTANY (HONORS) STUDENTS

Submitted by

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PAPER: BOT- HC- 3026 (PRACTICAL)

1.Test of Alkaloids : Neem , Vinca *rosea*

2.Cereals: Study of useful plants : Rice/ Bean (habit sketch ,study of paddy & grain , starch , starch grain , micro –chemical test)

CBCS (REGULAR)

PAPER: BOT-RC-3016 (PRACTICAL)

1. Determination of osmotic potential of plant cell sap by plasmolytic method.
2. Calculation of stomatal index & stomatal frequency evolution in photosynthesis.
3. To study the effect of bicarbonate concentration on O_2 evolution in photosynthesis.

Experiment no.

Aim of the experiment : Alkaloids test of Neem (*Azadirachta indica*)

Theory : *Azadirachta indica* is a common medicinal plant of India. Recent scientific studies have been revealed that it contains different chemicals like azadirachtin, nimbin, salanin which have insect repellant, antiviral, antifident properties. Neem leaves contain not uses less than 1.0% w/w of rutin.

Requirements :

i. Plant material : *Azadirachta indica*
ii. Chemicals: alcohol, ether
iii. Reagents : Wagner's reagent , Mayer's reagent and Marme's reagent
iv. Glass ware: Petridish , beaker , measuring cylinder
v. Other accessories : morter and pastle , test tube stand, filter paper, etc.

Preparation of reagents :

a) Wagner's reagent: KI (20 g) + 20ml distilled water + Cadmium chloride (10 g in 50 ml distilled water)
b) Mayer's reagent: Iodine (1.27g) +KI (2g) + 100ml distilled water
c) Marme's reagent : Mercuric chloride ($HgCl_2$) (1.36g) in 60 ml distilled of distilled water + KI 5g in 10 ml of distilled water + another 30 ml of distilled water.

Procedure :

Preparation of plant extract:

Fresh leaves were washed thoroughly in water and made a paste in morter and pastle. Then added ether in one part and in another part alcohol was because alkaloids are soluble in alcohol and ether.

The two types of plant extract were filtered and performed the following experiments:

Observation :

Test	Experiment	observation	Inference
Wagners test	3ml of plant extract + a drops Wagner's reagent	Brownish ppt	Alkaloid present
Mayers test	3ml of plant extract + a few drops of Mayer's reagent	Ppt occured	Alkaloid present

Marmes test	3ml of plant extract + a few drops of Marme's reagent	Whitish yellow ppt occured	Alkaloid present

Conclusion :

Experiment no.

Aim of the experiment: Study of Paddy Plant

(N.B. Habit sketch/morphology of rice/ paddy plan have to draw in the Practical note book before writing the chemical test of carbohydrate)

Scientific name*: Oryza sativa*

Family*: Poaceae (Gramineae). The Grass Family*

Morphological characters:

Usually annual or perennial herbs. Stems erect, ascending, creeping, terete, internode hollow, nodes swollen, creeping rhizome or stolon present. Leaves simple, alternate, consisting of sheath, blade and ligule, blade usually long, narrow and entire. In florescence is a spikelet,f lowers arranged in spike, raceme or panicle, flowers stalked or sessile. Each spikelet bears at its base two minutes bracts called empty glumes and one third bract called flowering glume or lemna. Flowers small, inconspicuous, bisexual or unisexual, zygomorphic; Androecium generally 3, sometimes 6 or reduced to 2 or 1; Gynoecium bi or tricarpellary, syncarpous, overy superior, unilocular with an ovule; fruit a caryopsis.

Parts used: *Caryopsis (Seed/Fruits)*

Chemical composition*:* The rice grain constitutes 12% water,75%-80% carbohydrate which include starch, glucose, sucrose and dextrin; only 7%protein, minerals like Ca, Zn and Fe, Na, K and fatty acids; fibre content, around 0.85%, amino acids etc.

Uses: (One can write it from any Economic Botany book)

Aim of the experiment : Carbohydrate test for Rice.

Theory : Carbohydrate are the condensation products of polyhydroxyaldehydes or polyhydroxyketons and their derivatives. They contain carbon, hydrogen and oxygen, is generally 2:1. In living world there are a large number of carbohydrate materials, which may be conveniently classified as :

a) Monosaccharides
b) Oligosaccharides
c) Polysaccharides

Requirements:

Plant material: Rice
Chemicals :
Reagents : Fehling's reagent ,Benedict's reagent , Barfoed's reagent, Trommer's reagent, Moore's reagent.
Glass ware :
Other accessories : weigh balance

Preparation of reagent :

1.Fehling's reagent : Equal volume of Fehling's A and B
Fehling's solution A : $CuSO_4.5H_2O$ (35 g in 500 ml of distilled water)
Fehling's solution A : NaOH -50g and Na-K-tartarate (Rochelle salt)-173 g in 500ml in dist. Water

2. Benedict's qualitative reagent : Dissolved sodium citrate 86.5 g and sodium carbonate. 50g in 350 ml distilled water and then dissolved copper sulphate 8,65g in 50 ml dist. water. Finally mix together and dilute up to 500ml

3.Barfoed's reagent : Dissolved 13.3 g of cupper acetate in 200ml of 1% acetic acid solution .

Test for reducing sugar:

Experiment :

Experiment	Observation	Inference
1.Fehling's Test: In a clean and dry test tube 1ml of Fehling's solution A and 1ml of Fehling's solution B are	The solution initially turns yellow and brick red. Precipitation of Cu_2O takes	Presence of reducing sugar in the supplied sample

taken, mixed thoroughly and then boiled for a minute. It is observed that the solution remains unchanged. Finally an equal volume of sample solution is added to it and boiled in a water bath for 5-10 minutes.	place in the tube containing reaction mixture.	
2.Benedict's Test : To a clean and dry test tube containing about 3ml of sample solution ,2-3 ml of Benedict's qualitative reagent is added and then boiled for 5 minutes.	The solution turns green, yellow and finally red, depending upon the amount of reducing sugar present in the sample.	Presence of reducing sugar in supplied sample.
3.Barfoed's Test: About 1ml of sample solution is added to about 3ml of Barfoed's reagent in a test tube and then boiled for 1-2 minutes and finally cooled.	Red precipitate of Cu_2O of formed at the bottom of the test tube.	Presence of reducing sugar in the sample.
4.Trommer's Test: About 1ml of 2.5% $CuSO_4$ solution and 2ml of 5% NaOH solution are added to 3-5 ml of carbohydrate solution. The mixture is boiled in a water bath for 3-5 minutes.	A blue precipitate of Cupric hydroxide is formed which dissolves to give a blue solution. A yellow or red precipitate of Cu_2O is formed in presence of reducing sugar.	Presence of reducing sugar in the supplied sample.
5.Moore's Test : to about 2ml of sample solution , an equal volume of 5% NaOH solution is added and then boiled for 2- 5 minutes in a water bath.	The solution turns yellow and then reddish-brown due to the formation of a condensation product of the sugar	The sample contains reducing sugar, particularly glucose.

Test for non - reducing sugar:

Hydrolysis of non -reducing sugar: In a 100ml capacity conical flask, about 20 ml of sugar solution is taken and 2.5ml NH_4SO_4 is added. The mixture is boiled in a water bath for 10-15 minutes, then cooled and neutralised by slow addition of Na_2CO_3 salt , till effervescence continues finally, the mixture is filtered and tests for detection of sugars(reducing type derived from no reducing type on acid hydrolysis) are made in usual way as shown previously. The complete hydrolysis is noticed by the positiveness of the reducing sugar tests.

The neutralization of hydrolysed samples can also be done by addition of 0.1 N NaOH and 0.1 N H_2SO_4 solution using 1% phenolphthalein indicator.

The following tests are perfomed with hydrolysed samples :

Experiment :

Experiment	Observation	Inference
1.Fehling's Test: In a clean and dry test tube 1ml of Fehling's solution A and 1ml of Fehling's solution B are taken ,mixed thoroughly and then boiled for a minute. It is observed that the solution remains unchanged. Finally an equal volume of sample solution is added to it and boiled in a water bath for 5-10 minutes.	The solution initially turns yellow and brick red. Precipitation of Cu_2O takes place in the tube containing reaction mixture.	Presence of reducing sugar in the supplied sample
2.Benedict's Test :To a clean and dry test tube containing about 3ml of sample solution ,2-3 ml of Benedict's qualitative reagent is added and then boiled for 5 minutes.	The solution turns green, yellow and finally red ,depending upon the amount of reducing sugar present in the sample.	Presence of reducing sugar in supplied sample.
3.Barfoed's Test: About 1ml of sample solution is added to about 3ml of Barfoed's reagent in a test tube and then boiled for 1-2 minutes and finally cooled.	Red precipitate of Cu_2O of formed at the bottom of the test tube.	Presence of reducing sugar in the sample.
4.Trommer's Test: About 1ml of 2.5% $CuSO_4$ solution and 2ml of 5% NaOH solution are added to 3-5 ml of carbohydrate solution. The mixture is boiled in a water bath for 3-5 minutes.	A blue precipitate of Cupric hydroxide is formed which dissolves to give a blue solution A yellow or red precipitate of Cu_2O is formed in presence of reducing sugar.	Presence of reducing sugar in the supplied sample.
5.Moore's Test : to about 2ml of sample solution , an equal volume of 5% NaOH solution is added and then boiled for 2- 5 minutes in a water bath.	The solution turns yellow and then reddish-brown due to the formation of a condensation product of the sugar	The sample contains reducing sugar, particularly glucose.

Experiment no.

Aim of the experiment : Alkaloids test of *Vinca rosea*.

Theory :

Requirements :

i. Plant material : *Vinca rosea*
ii. Chemicals: alcohol, ether
iii. Reagents : Wagner's reagent , Mayer's reagent and Marme's reagent
iv. Glass ware: Petridish, beaker, measuring cylinder
v. Other accessories : morter and pastle , test tube stand, filter paper, etc.

Preparation of reagents :

a. Wagner's reagent: KI (20 g)+ 20ml distilled water + Cadmium chloride (10 g in 50 ml distilled water)
b. Mayer's reagent: Iodine (1.27g) +KI(2g) + 100ml distilled water
c. Marme's reagent : Mercuric chloride (Hg Cl_2) (1.36 g) in 60 ml distilled of distilled water + KI 5g in 10 ml of distilled water + another 30 ml of distilled water.

Procedure :

Preparation of plant extract:

Fresh leaves were washed thoroughly in water and made a paste in morter and pastle. Then added ether in one part and in another part alcohol was because alkaloids are soluble in alcohol and ether.

The two types of plant extract were filtered and performed the following experiments:

Observation :

Test	Experiment	observation	Inference
1.Wagner's test: To about 3ml of sample, solution a few drops of Dragendroff's reagent are added.	3ml of plant extract + a drops Wagner's reagent	Brownish precipitate.	Alkaloid present
2.Mayer's test : To about 3 ml extract ,a few drops of Mayer's regent are added.	3ml of plant extract + a few drops of Mayer's reagent	Precipitate occurred.	Alkaloid present
3.Marme's test: To about 3 ml	3ml of plant extract + a	Whitish yellow	Alkaloid

of extract , a few drops Wagner's reagent are added.	few drops of Marme's reagent	precipitated occurred.	present

Conclusion :

Experiment no.

Aim of the experiment : Determination of Osmotic potential of plant cell by plasmolytic method.

Theory : The process of transmission of liquid of different concentrations through the semi-permeable membrane is called osmosis and the pressure exerted by liquid on the membrane is termed as osmotic pressure. When two solutions separated by semi-permeable membrane, the less concentrated solution starts diffusing through the membrane to the solution with higher concentration to equalized the strenght of the two solutions. Osmotic potential is defined as the pressure in the atmosphere which will be required in opposite direction to stop the entry of a solvent, when a concentrated solution is separated from a dilute solution by the semi-permeable membrane.

When a plant cell is placed in a hypertonic solution osmosis occurs and water comes out from the cell, as a result ,the protoplasm shrinks and moves away from the cell wall .the shrinkage of protoplasm is known as plasmolysis .At the isotonic point, the concentration of water solution and the cell sap become same. The osmotic pressure is given by –

$$OP = CRT$$

Where,

OP=Osmotic potential
C=value of isotonic point
R= Universal Gas constant
T = Absolute temperature

Where, cell cap osmotic potential is to be determined, molar solution of sucrose acts as solution of different concentration and the cell membrane acts as the semipermeable membrane which separate the sap (cell) and the molar soltution. Osmotic potential will be equal to that molar solution in which incipient plasmolysis occurs.

Requirements :

i. Plant material
ii. Chemicals: Sucrose,distilled water
iii. Glass ware: Petridish , beaker , measuring cylinder ,slides and coverslip
iv. Other accessories : Balance and weight box , blade, forcep , Microscope

Procedure :

A. Preparation of 1M sucrose solution (stock solution) : The molecular weight of sucrose is 342.2g. 1M of sucrose solution is prepared by dissolving 34.2 g of sucrose in about 70 ml distilled water and the volume is made 100ml in a volumetric flask.

B. Preparation of different molar solution :
Molar solution of different concentrations are prepared from the stock solution already made.The strength of the molar solution from 0.1 M (10 cc stock solution + 9 cc distilled water) to 1.0M (10 cc stock solution + 0cc distilled water) in serial order i.e 0.1 ,0.2 ,0.3 ,0.4 ,0.5.0.6 ,0.7 ,0.8 , 0.9 , 1.0 M .

C.P rocess:

- A few thin pieces of the thin layer of the epidermis of *Tradescantia* leaf separately peeled off and immersed in distilled water. So that all that cells were deplasmolysed.
- Now, these are taken out and placed in the different concentration of prepared solution in different petri dish and kept for at least 30 minutes.
- All the petridishes are covered well to check the evaporation.
- The strips are taken out after 30 minutes and each is mounted on slide and observed under microscope to calculate the number of plasmolysed and non -plasmolysed cells.
- The number of plasmolysed and non-plasmolysed cells are counted and recorded.
- The incipient plasmolysis condition is calculated from the graph are recorded.

Observation:
From the recorded data the table is made as follows -

No. of observ-ation	Concentration of sucrose (M)	No. of cells considered	No. of cells plasmo-lysed	No.of cells non-plasmolysed	% of plasmolysed cels	% of non-plasmolysed cells
1	00					
2	0.1					
3	0.2					
4	0.3					
5	0.4					
6	0.5					
7	0.6					
8	0.7					
9	0.8					

10	0.9					
11	1.0					

N.B.From the above recorded data one Graph have to be plotted in the graph paper.

Observation:

Result:

Calculation:

Inference

Experiment no.
Aim of the experiment: Determination of stomatal index and stomatal frequency.

Theory: The excess loss of water in form of vapour through the stomata of leaves is called transpiration. The maximum amount of (80-90%) absorbed water is transpired through stomata. The stomata are found distributed on the both the upper and lower surface of leaves. In dorsiventral leaves, their number is more on lower side. That is why in dorsiventral leaves, transpiration occurs more on lower surface than the upper surface.

Requirements :

i. Plant material
ii. Glass wares : conical flask
iii. Chemicals : veselin, clove oil
iv. Other accessories : balance and weight box, graph paper, microscope

Procedure :

- The three dorsiventral leaves are placed on a graph paper and outer margin of the leaves drawn carefully so that area of the leave can be calculated out.
- The lower and the upper surface of the first leaf is taken without Vaseline coating.
- The upper surface of the second dorsiventral leaf is coated with Vaseline and lower surface of the third leaf is also coated by vaseline to check the transpiration.
- The ends of the petioles are kept immersed in water in the conical flask. Clove oil is poured to the conical flask to check the vapourisation.
- Now the conical flask are weighted separately and the weights are noted.
- Then each of the flask is allowed to stand 1 hour in sunlight.
- The flasks are reweighted and noted down. The weight are now different from the previous weight as transpiration takes place .
- The area of the leaves are calculated from the graph paper separately.

- From the graph, the amount of water transpired per sq. cm of the leaf has been calculated.

Observation:

Leaf	Initial weight (g)	Final weight (g)	Difference in weight (g)
1.leaf with upper surface free	_	_	_
2.leaf with lower surface free			
3.leaf with both surface free			

Calculation :

The leaf with upper surface free :
The area of the leaf surface=X
Total water transpired by both surface = y

$\therefore$ In 1 cm^2 area transpired by uuper surface = y/x

Stomatal Frequency :
Stomatl frequency means the number of stomata per unit area of leaf surface. It varies from plant to plant and also depends upon the effect of environment.

Stomatal frequeny = No. of stomata present per unit area/area of the field of vision

Stomatal index: Salisbury used the term and formula for stomatal index, where, 'I' stands for stomatal index , 'S' for number of stomata and 'E' for number of epidermal cell in the same unit area –

$$I = (S /E+S) \times 100$$

Observation:

Conclusion:

Experiment no.

Aim of the experiment : To study the effect of bicarbonate concentration on O_2 evolution in photosynthesis.

Theory: The rate of photosynthesis is influenced by O_2 along with external and internal factors like light, temperature. For each factor, a minimum of which is indicates photosynthesis is just started, it is optimum when photosynthesis is highest and gradually it decreases beyond which it comes to end.

Requirements :

i. Plant material
ii. Chemicals : $KHCO_3$, distilled water
iii. Glass ware: Beaker, funnel with jet, solid glass rod
iv. Other accessories : Balance and weight box ,stopwatch , paper.

Procedure:

- A branch of fresh plant was taken in a big beaker and covered by inverted funnel. The stem of which was attached to a fine jet.
- A test tube was filled with water and inserted over the jet in a manner so that the jet is insight the tube.
- Now the jet was exposed to sunlight in case of light period experiment.
- After sometime, bubbles of gas began to emerge from the plant and collected in the test tube. Then the no. of bubbles evolved per unit time is counted.
- Then 0.2 gm of $KHCO_3$ added and stirred carefully.
- The no. of bubbles found to be greater per unit time than before.
- The concentration of the solution had been increasing by adding 0.2 g of $KHCO_3$ after each time interval and number of bubbles evolved per minutes was counted in each concentration.
- The time interval was taken as 5 minutes.

Observation:

Measurement of O_2 evovled in light

No. of observation	KHO_3 concentration	Time interval	No. of bubbles evolved	Mean of bubble evolved	Remark
1	100m g	5min			
2	200mg	5min			

3	300mg	5min			
4	400mg	5min			
5	500mg	5min			
6	600mg	5min			
7	700mg	5min			
8	800mg	5min			
9	900mg	5min			
10	1000mg	5min			

N.B. From the above Data Table one Graph have to be plotted in the graph paper

Result:

Inference: It is inferred that when other factors like temperature, light etc. remain constant, the rate of photosynthesis is increased with increase of CO_2 concentration upto a certain point. But after a certain period increase of CO_2 concentration makes no effect on photosynthesis as the other factors such as temperature, light become the limiting factor.