

Aim 9

To Estimate the level of Photosynthetic Pigments in the Plant material

Introduction

A process in which centrifuge is used for the sedimentation of the heterogeneous mixture is termed as Centrifugation. This is generally used for the separation of two immiscible liquids. The angular velocity is used to specify the rate of centrifugation and is measured by RPM (Resolution per minute).

The measurement of transmittance or reflection of solution transparency and opaque solids can be done by using a spectrophotometer. The most common spectrophotometer are used in UV and Visible regions of the spectrum.

Spectrophotometer works on the principle based on Beer-Lambert law. A linear relationship between absorbance and concentration of absorbing substance is observed.

Requirements

Beakers, test tubes, centrifuge tube, measuring cylinder, spectrophotometer, centrifuge, CaCO_3 , 80% acetone, weight, leaves of the plant, pestle and mortar, blotting paper.

Procedure

1. Collect and wash the leaf sample.
2. Weigh 2 g of the sample.
3. Mash the leaf sample with 15 ml of 80 % acetone in mortar and pestle along with a pinch of CaCO_3 .
4. Take the extract in a test tube, centrifuge at 5000 rpm for 10 min.

5. Take the supernatant and raised the volume up to 10 ml by 80% acetone.
6. Observe the absorbance in spectrophotometer at different wavelength 645 nm, 663 nm, 510 nm, 480 nm.
7. Use formula given for the calculation of the amount of chl a, chl b and carotenoid.

Formula Used

$$\text{Chl a} = (12.3 A_{663} - 0.86 A_{645} \times V) / (\alpha \times 1000 \times w)$$

$$\text{Chl b} = (19.3 A_{645} - 3.6 A_{663} \times V) / (\alpha \times 1000 \times w)$$

$$\text{Carotenoids} = (7.6 A_{480} - 1.49 A_{510} \times V) / (\alpha \times 1000 \times w)$$

Where, W = Weight of leaf in µg or mg.

V = Volume of leaf extract in ml

A = Absorption at different wavelength

α = Coefficient (=1)

Observation Table

S.No.	Wavelength			
	A ₆₆₃	A ₆₄₅	A ₄₈₀	A ₅₁₀
1.				
2.				

Precautions

1. Leaf should be weighed accurately.
2. Grind the sample properly.
3. Carefully handle the supernatant.
4. Acetone should be added in the proper amount.