APPENDIX XXIII

ESTIMATION OF CHLOROPHYLLS

(Yoshida et al., 1971)

Principle

Chlorophyll pigments present in the sample are extracted using acetone and the green colour of the extract is read at 645 nm and 663 nm.

Procedure

One gram of fresh leaves were used for the assay of chlorophyll. Cut one gram fresh leaves into small bits and homogenized in a mortar and pestle with enough 80 per cent acetone. Decanted and filtered the supernatant. Repeated the extraction till the residue becomes colourless with 80 per cent acetone. Pool the filtrates and make up the volume to 100 ml in a volumetric flask with acetone. Measured the absorbance at 663 nm and at 645 nm.
Calculation

Total chlorophyll (mg/g) = \[ \frac{20.2 A_{645} + 8.02 A_{663}}{a \times 1000 \times w} \times v \]

Chlorophyll a (mg/g) = \[ \frac{12.7 A_{663} - 2.69 A_{645}}{a \times 1000 \times w} \times v \]

Chlorophyll b (mg/g) = \[ \frac{22.9 A_{645} - 4.68 A_{663}}{a \times 1000 \times w} \times v \]

Where,

a = length of path light in the cell (usually 1 cm)
v = volume of the extract in ml
w = weight of the sample in g