APPENDIX

PREPARATION OF REAGENTS

The reagents for various biochemical determinations were prepared according to the following methods:

1. Reagents for determination of chlorophyll content:
   (i) **80% acetone:**
       80% mL of acetone was mixed in 20 mL of DDW

2. Reagents for NRA estimation:
   (i) **Isopropanol (5%):**
       5 mL isopropanol was mixed in 95 mL DDW.
   (ii) **Naphthylethylenediamine dihydrochloride (NED-HCL) solution (0.02%):**
        20 mg naphthylethylenediamine dihydrochloride was dissolved in sufficient DDW and final volume was maintained upto 100 mL with DDW.
   (iii) **Phosphate buffer (0.1M) for pH 7:**
        (a) 13.6 g potassium dihydrogen orthophosphate was dissolved in sufficient DDW and final volume was made upto 1000 mL with DDW.
        (b) 17.42 g dipotassium hydrogen orthophosphate was dissolved in sufficient DDW and final volume was made upto 1000 mL with DDW.
        160 mL of solution (a) and 840 mL of solution (b) was mixed for getting phosphate buffer pH 7.5.
   (iv) **Potassium nitrate solution (0.02 M):**
        2.02 g potassium nitrate was dissolved in sufficient DDW and final volume was made upto 1000 mL with DDW.
   (v) **Sulphanilamide solution (1%):**
        1 g sulphanilamide was dissolved in 3 N hydrochloric acid and final volume was made upto 100 mL with 3 N hydrochloric acid.

3. Reagents for CA estimation:
   (i) **0.2 M aqueous cystein solution**
       48 g of cystein was dissolved in 1000 mL.
(ii) **Phosphate buffer of pH 6.8:**
5.365 g of Na$_2$HPO$_4$ 2.78 g of NaH$_2$PO$_4$.2H$_2$O were dissolved separately in 100 mL of DDW. The above solution of Na$_2$HPO$_4$ and NaH$_2$PO$_4$.2H$_2$O were mixed in the ratio of 49.51.

(iii) **Sodium bicarbonate solution (0.2M) in 0.2 M sodium hydroxide solution:**
16.8 g sodium bicarbonate was dissolved in sodium hydroxide solution (0.8 g NaOH/L) and final volume was maintained upto 1000 mL with sodium hydroxide solution.

(iv) **0.002% bromothymol blue:**
0.002 g of bromothymol blue was dissolved in sufficient DDW and final volume was made upto 100 mL, by using DDW.

(v) **0.05 N Hydrochloric acid:**
4.3 mL pure hydrochloric acid was mixed with 5.7 mL DDW.

(vi) **Methyl Red:**
A pinch of methyl red was dissolved in sufficient ethanol and final volume was made 100 mL using ethanol.

4. **Reagents for leaf-NPK estimation:**

(i) **Molybdic acid reagent (2.5%):**
6.25 g of ammonium molybdate was dissolved in 175 mL distilled water to which 75 mL of 10 N-sulphuric acid was added.

(ii) **1-amio-2-naphthole-4-sulphuric acid:**
0.5 g 1-amio-2-naphthole-4-sulphuric acid was dissolved in 195 mL of 15 % sodium bisulphide solution to which 5 mL of 20 % sodium sulphate solution was added. The solution was kept in amber coloured bottle.

(iii) **Sodium hydroxide solution (2.5 N):**
100 g NaOH dissolved in sufficient DDW and final volume was maintained up to 1000 mL with DDW.

(iv) **Sodium silicate solution (10%):**
10 g sodium silicate dissolved in sufficient DDW and final volume was maintained up to 100 mL with DDW.