PATHOPHYSIOLOGY

A) MATERIALS :

The plants selected for the present study are Arachis hypogaea L., Ricinus communis L. and Cassia sophera L. which are economically important.

The material was collected from farmer's fields where crops (Arachis hypogaea L., Ricinus communis L.) were cultivated. Material of Cassia sophera was collected from Shivaji University Campus, Kolhapur. Only healthy and infected leaves of almost same age were selected for study of physiological changes.

Every time the leaves were washed with distilled water to remove any dust and other particles then blotted to dry. The organic constituents such as chlorophylls, polyphenol, carbohydrate, protein, TAN etc. were estimated by using fresh material along with the enzyme study viz. peroxidase, catalase, Acid phosphatase and IAA-oxidase. Some inorganic constituents like Na^{+}, K^{+}, Ca^{+}, P^{+5}, Fe^{+3}, Mg^{+2} with proline were estimated by using the oven dried material.

B) METHODS :

1) Titratable acid number (TAN) :

TAN was estimated by Thomas and Beever's (1949) method. The plant material that is leaves of both healthy and infected

plants were first washed with distilled water and then blotted to dry. 5 g of each sample was accurately weighed and boiled for half an hour in boiling distilled water. Finally it was filtered through the double layered musline cloth and the volume of filtrate so obtained was recorded. 10 ml of this extract was titrated against N/40 NaOH, using phenolphthalein as an indicator. N/40 NaOH was standardized with N/40 oxalic acid using phenolphthalein as an indicator.

TAN gives the number of decinormal NaOH required to neutralize the acids present in 100 g of fresh tissue and was calculated by the following formula.

$$TAN = \frac{\text{Ml.of oxalic acid taken for titration}}{\text{Burette reading for 10 ml. oxalic acid}} \times \frac{\text{Extract reading}}{\text{ml.of extract taken for titration}} \times \frac{\text{Volume of extract}}{\text{wt.of plant material}} \times \frac{100}{4}$$

2) Polyphenols :

Total polyphenols were estimated by the method of Folin and Denis (1915). The leaves of both healthy and infected plants were first washed thoroughly with distilled water and then blotted to dry. 0.5 g of leaves were accurately weighed and transferred to morter. It was then thoroughly homogenized with pestle and polyphenols were extracted in 80% acetone (about 40 ml.). It was then filtered through Buchner's funnel using Whatman No.1 filter

paper. The residue was washed thoroughly with 80% acetone and finally volume was made to 100 ml. with 80% acetone.

For estimation of total polyphenols 1 ml extract of both healthy and infected leaves was taken seperately in 50 ml marked Nessclor's tubes. For standard curve 0.5, 1, 2 and 3 ml. of tannic acid (0.1 mg/ml) was taken in other tubes. Blank was without tannic acid. 10 ml 20% sodium carbonate (Na_2CO_3) solution was added to each test tube, to make the solution alkaline. Finally 2 ml. Folin-Denis reagent was added to each test tube and the volume was made to 50 ml. with distilled water. The ingradients were allowed to mix thoroughly over night. Finally the optical density of each mixture was read at 660 nm in spectrophotometer. Total polyphenols were calculated from the calibrated curve of standard tannic acid.

3) Chlorophylls :

The chlorophylls were estimated by the method of Arnon (1949). Healthy and infected leaves were washed thoroughly with distilled water and blotted to dry. 0.5 g of the leaves were accurately weighed and homogenized in mortor with pestle. Chlorophylls were estimated in 80% acetone (about 40 ml.), then the extract was filtered through Buchner's funnel using Whatman No.1 filter paper. The

residue was washed thoroughly with 80% acetone collecting the washings in the same container. Finally the volume was made to 100 ml with 80% acetone. All the operations were done in dim light and at 4°C. Optical density of the filtrate was read at 663 nm for chlorophyll 'a' and 645 nm for chlorophyll 'b'. Chlorophylls (mg/100 g fresh tissue) were calculated by using the formulae.

1) Chlorophyll 'a' = (12.7 x A 663 - 2.69 x A 645) = X

2) Chlorophyll 'b' = (22.9 x A 645 - 4.68 x A 663) = Y.

3) Chlorophyll 'a+b'= (8.02 x A 663 + 20.20 x A 645) = Z.

4) $$\frac{\text{Chlorophyll a/b/a+b}}{\text{mg/100 g fresh tissue}} = \frac{\text{X/Y/Z x Vol.of extract x 100}}{\text{1000 x wt. of plant material g}}$$

5) $$\text{Chlorophyll a:b ratio} = \frac{\text{mg of chlorophyll a/100 g}}{\text{mg of chlorophyll b/100 g}}$$

4) Carbohydrates :

Reducing, Non-reducing and total sugars :

The reducing sugars were estimated by Somogyi Nelson's (1944) method. Healthy and infected leaves were washed thoroughly with distilled water and blotted to dry. 2 g of leaves were accurately weighed and homogenized in mortor with pestle. Sugars were estimated in 30-40 ml 80% alcohol. Then the extract was filtered through Buchner's funnel using Whatman No.1 filter paper. The residue was washed thoroughly with 80% alcohol. Filtrate was condensed to one to two ml.

over boiling water bath using evaporating dishes. 1 g each of lead acetate and potassium oxalate with 40-50 ml. distilled water was added. Mixed thoroughly and kept for 5-10 minutes. Again it was filtered and now extract was saved and its volume was accurately noted. The residue over the filter paper during first filtration was kept for starch estimation.

Filtrate was directly used to estimate reducing sugars.

Reducing sugars :

To estimate reducing sugars 0.2 ml. extract was used and for standard curve, glucose (0.1 mg/ml) was used (0.1, 0.2, 0.4, 0.8 ml.). 0.2 ml. each extract was taken in clean test tubes and 1 ml. alkaline copper tartarate solution was added to it. It was then boiled over boiling water both for 10 min. After cooling to room temperature 1 ml. Nelson's Arsenomolybdate reagent was added. Finally the volume was made 10 ml. with distilled water, and optical density was read at 560 nm.

Total sugars :

20 ml. of above extract was taken in 150 ml. conical flask. To this 2-3 ml concentrated HCl was added. Then cotton plug was fixed to conical flask and hydrolysed in autoclave at 15 lbs. for 15 min. Due to autoclaving the non-reducing sugars (mainly sucrose) was hydrolysed and