MATERIAL AND METHODS

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I — MATERIAL :—

There are many varieties of genus Ipomoea. but the Ipomoea carnea, Jacq is taken of the present investigation because it is a weed and easily available.

In the present investigation attempts have been made to study the effect of growth harmones (G.A., I.A.A. & Kinetin) on the two Ipomoea carnea, Jacq varieties i.e. Ipomoea carnea sub sp. carnea, Jacq and Ipomoea carnea sub sp. fistulosa, Mart ex choisy. These varieties were collected from Shivaji University, Kolhapur Campus.

II — METHODS :—

A) PHYSICAL PROPERTIES OF THE LEAVES :-

The physical properties of the leaves were studied by the method (Linacre ; 1964). The green and scenescent leaves of Ipomoea carnea, Jacq varieties were selected for the study. The average leaf area, leaf thickness and leaf weight were measured. From these average volume and density were calculated by using following formula.

Volume = Average leaf area x Average leaf thickness.

$$\text{Density} = \frac{\text{Average leaf weight}}{\text{Volume}}$$

B) a) ORGANIC CONSTITUENTS :-

1) MOISTURE PERCENTAGE :-

The green and scenescent leaves of Ipomoea carnea, Jacq plant were taken and cleaned well with distilled water, surface blotted to dry and weighed acurately. Then the leaves were dried at 80°C in an oven till constant weight obtained. The moisture percentage can be calculated by using following formula

$$\text{Moisture Percentage} = \frac{\text{Fresh wt. - Dry wt.}}{\text{Fresh wt.}} \times 100.$$

$$\text{Dry matter percentage} = \frac{\text{Dry wt.}}{\text{Fresh wt.}} \times 100$$

2) RELATIVE WATER CONTENT (R.W.C.) :-

The clean washed and surface dried green and senscent leaves of Ipomoea carnea, Jacq plant were taken and the leaf discs were prepared by punching leaf lamina and weighed acurately. Then the same leaf discs were kept in distilled water for 4 hours and again weighed acurately (turgid wt.). Later on these discs were dried at 80°C in an oven till constant weight obtained. The RWC can be calculated by the following formula.

$$\text{Relative water content} = \frac{\text{Fresh wt. - Dry wt.}}{\text{Turgid wt. - Dry wt.}} \times 100$$

3) TITRATABLE ACID NUMBER (TAN) :-

TAN was estimated by the method of Thomas and Beevers (1949). The green and senescent leaves were cut into small pieces and weighed for 1 g separately and were boiled with distilled water for half an hour, then cooled and aliquot was titrated against N/40 NaOH using phenolpthalein as an indicator. NaOH was standardised against N/40 Oxalic acid using the same indicator. Titratable acid number (TAN) represents the number of ml of decinormal NaOH required to neutralize the acid present in 100 g. of fresh tissue. It was estimated by using following formula.

$$\text{Titratable acid number (TAN)} = \frac{\text{Volume of Oxalic acid titration}}{\text{Titration reading of NaOH.}} \times \frac{\text{Total Volume of extract}}{\text{wt. of plant material in g.}} \times \frac{\text{Extract titration 'X' reading}}{\text{Volume of extract taken for titration.}} \times \frac{100}{4}$$

b) GROWTH HARMONE TREATMENT :-

1 g of growth harmones, Gibberelic acid, Indole acetic acid and Kinetin weighed accurately and dissolved in acetone and prepared 1000 ppm stock solution in distilled water. This stock solution is diluted to 100 ppm & used for treatment of matured leaves of both the sub species. These harmones are sprayed on both the surfaces of leaf. After 12 days these treated leaves are used for various investigation of organic and inorganic constituents.

4) CHLOROPHYLLS :-

The chlorophylls were estimated by the method of Arnon (1949). Chlorophylls were extracted in 80% acetone from 1 g of the plant material. The extract was filtered through Buchner funnel using Whatman No. - 1 filter paper. Residue was washed repeatedly with 80% acetone. Collecting the washings in the same filtrate, the volume of the filterate was made to 100 ml with 80% acetone. Extraction was carried out in dark and chlorophylls in mg 100 g^{-1}. Fresh tissue were calculated by using following formula.

Chlorophyll `a' = 12.7 x A 663 - 2.69 x A 645 = `X'.

Chlorophyll `b' = 22.9 x A 645 - 4.68 x A 663 = `Y'.

Total Chlorophyll = 8.02 x A 663 + 20.20 x A 645 = `Z'.

$$\text{Chlorophyll `a' or `b' or total Chlorophylls (mg 100 g.}^{-1}\text{)} = \frac{\text{X/Y/Z x Volume of extract x 100}}{\text{1000 x wt. of the plant material in g.}}$$

5) TOTAL POLYPHENOLS :-

Polyphenols were estimated by the method of Folin and Denis (1915). Polyphenols from senescent and green leaves of both varieties of Ipomoea carnea, Jacq were extracted in 80% acetone and filtered through whatman filter paper No-1 using Buchner funnel under suction. Polyphenols were extracted repeatedly from the residue. The volume of filterate was made to 50 ml. 0.5 ml of filterate was taken in a 50 ml marked Nesselor's tube. In other such tubes the different concentrations eg. 0.5, 1, 2, 3, and 4 ml of standered polyphenol solution(Tannic acid, 0.1 mg mt^{-1}) were taken. Then 10 ml of

20% Na_2CO_3 were added to each take to make the medium alkaline .2 ml of Folin - Denis reagent (100 g of sodium tunastate and 20 g of phosphomolybdic acid dissolved in 200 ml distilled water were mixed with 25% phosphoric acid. This was refluxed for 2.5 hours then cooled room temperature and diluted to 1 litre with distilled water) were than added to each Nesselor's tube and finaly the volume was made to 50 ml with distilled water.

A blank was prepared with out polyphenols. The ingredients were allowed to mix thoroughly well. After sometime the optical density(OD) of each mixture was read at 660 nm polyphenols were calculated from the calibration curve of standered tannic acid.

6) **CAROTENOIDS** :-

Carotenoids were extracted by crushing the fresh leaves in 80% acetone. Procedure is similar to that of chlorophylls described earlier and carotenoids were estimated spectrophotometrically at 480 nm by following the methods of Kirk and Allen (1965). Total carotenoids were estimated using the following formula of Liaaen-Jensen and Jensen(1971).

$$C = D \times V \times F \times \frac{10}{2500}$$

where, C = Total carotenoids in mg.

D = Optical density.

V = Total volume in ml.

F = Dilution factor.

2500 = Average extinction.

C) INORGANIC CONSTITUENTS :-

i) PREPARATION OF ACID DIGEST (EXTRACT) :-

The leaf material of random sampling was taken, cleaned well in distilled water and dried at 80°c in an oven till constant weight obtained. This oven dried material was taken for the estimation of different inorganic elements by following the methods of Toth et al (1948).

0.5 g of oven dried powdered material was transfered to a 150 ml beaker to which 20 ml concentrated HNO_3 were added. The beaker was coverd with watch glass and kept till the primary reaction subsides. It was then subjected to slow heating to dissolve solid particles completely. After cooling to room temperature 10 ml of 60% perchloric acid were added and mixed throughly. It was then heated strongly and vigorously until a clear and colourless solution reduced to about 2-3 ml. while heating the liquid was made to 100 ml. with distilled water and kept overnight. Next day it was filtered through a dry whatman filter paper No - 44 (ashless and the filterate was used for the estimation of different inorganic elements.

ii) ESTIMATION OF SODIUM, POTASSIUM, CALCIUM, MAGNESSIUM, IRON, MANGANESE, COPPER, ZINC.

These elements were analysed using the acid digest on atomic absorption spectrophotometer (Perkin-Elmer 3030 model). The readings were available in ppm. concentration which were further converted into per 100 g of dry weight.

CALCULATION :-

Inorganic constituents = 20 x Reading x dilution factor.

b) INORGANIC CONSTITUENTS IN GROWTH HARMONE TREATED LEAVES.

D) ENZYMES :-

1) PEROXIDASE :-

Peroxidase from fresh plant leaves was determined by following the method described by Maebly (1954). The enzyme was extracted by homogenizing the plant material (0.5 g.) in 10 ml ice cold water. It was then filtered through two layered chesse cloth and the filtrate was centrifuged for 15 minutes at 5000 rpm at 0 to 4°c and supernatent was used as an enzyme source. Enzyme assay mixture contained 2 ml of 0.1 m phosphate buffer (pH - 7) 1 ml of 20 mm guiacol and 0.5 ml of enzyme. The reaction was started by addition of 0.04 ml of 10 mM H_2O_2. The change in optical density (OD) due to oxidation of guiacol was recorded per minute at 470 nm on spectrophotometer with frequent stirring of the reaction mixture with glass rod. Enzyme activity is expressed an change in $\triangle OD\ min^{-1}\ g^{-1}$ fresh tissue.

2) ACID PHOSPHATASE :-

The enzyme was isolated from fresh leaves following the method of Mclachlan (1980). The enzyme was prepared by homogenizing 0.5 g of plant leaves in 10 ml of 0.1 m acetate buffer (pH-5) with a mortar and pestle. The extract was filtered through the musclin colth already moistened with acetate

buffer and the filtrate was centrifuged at full speed of 5000 rpm for 10 minutes. The supernatent was stored at 0 to 4°c and used as an enzyme source.

Enzyme assay mixture contained 3 ml of p-nitrophenyl phosphate (0.1 mg. ml^{-1}), 2 ml of 0.1 m acetate buffer (pH-5) and 1 ml of enzyme. Enzymatic reaction was initiated by the addition of enzyme and was stopped by the addition of 1.5 ml of 1.68 N NaOH. Yellow coloured complex p-nitrophenol produced as a result of reaction between enzymatic breakdown of p-nitrophenyl phosphate and NaOH was estimated spctrophotometrically at 420 nm. The enzyme activity was expressed as change in ▲OD hr^{-1} g^{-1} fresh tissue.