# MATERIAL METHOD

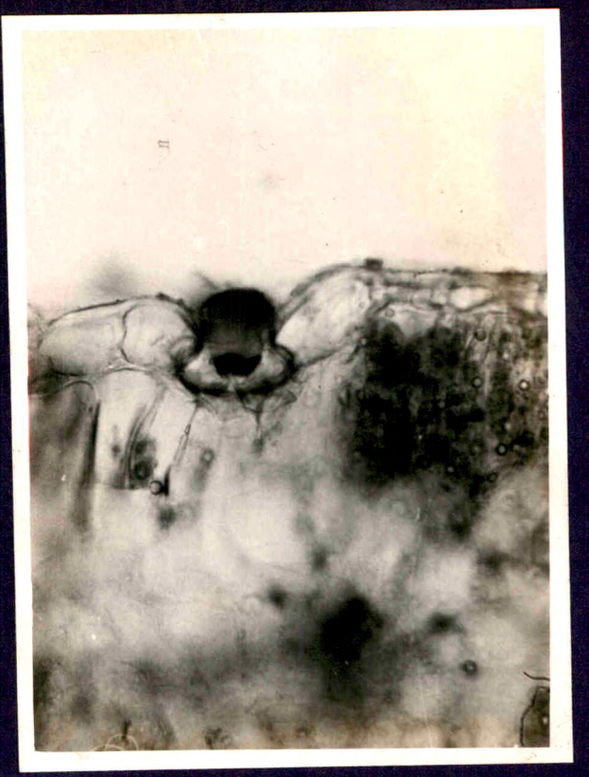
About the Plant Phyla nodiflora (L) Michaux.

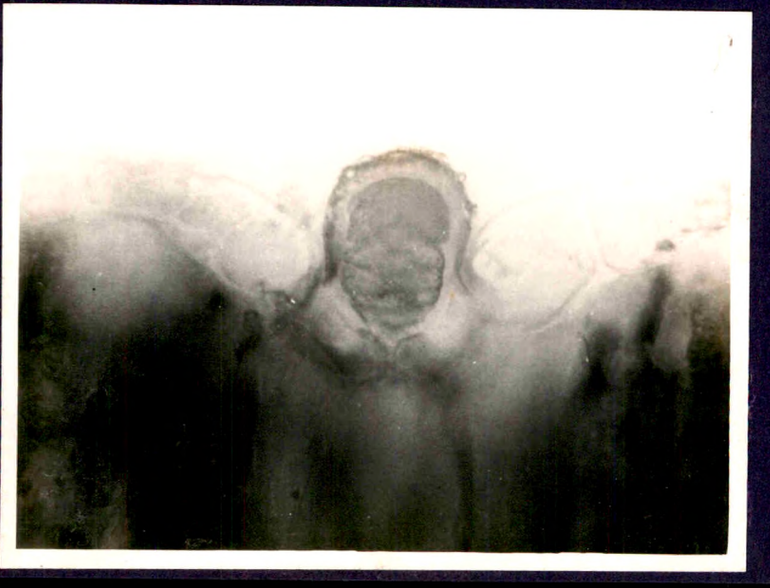
A creeping perennial herb, stems rooting at the nodes much branched, subquadrangular more or less clothed with appressed modified white hairs, subtimes nearly glabrous. Leaves opposite subsessile or 1/4 by in. spathulate luneate at the base, rounded at the apex, deeply and sharply serrate in the upper part, appressedly hairy on both sides with nedifixed while hairs. Flowers sessible, densely packed in long pedunculate axillary heads which are at first globose, afterwards elongate, and becoming spicate and ablong in fruit, peduncles 2.3 in. long, usually from the axil of one only of each pair of leaves, bracts 1/10 in. long, broadly elliptic or abovate with a somewhat cuneate base, mucronate glabrous. Calyx 1/12 in. long membranous, deeply 2 labbed, compressed, pubescent on the back with basifixed hairs closely concerning the fruit, the 2 accuminate lobes projecting beyond it. Corolla 1/10 - 1/8 in. long white or pale pink, pushed off as a calyptra by the ripening fruit, 2 lipped, upper lip erect bifid, lower lip - 3 lobed, the middle lobe is the largest. Fruit 1/16 m. long globose, oblong dry, spitting in two 1 seeded plano-convex glabrous pyrenes, common in grassy and sandy places throughout the Bombay Presidency, Deccan - Woodrow, Poona Cooke, Bijapur Woodrow, Gujarat Woodrow, Porbandar cooke, Sind cooke, Woodrow- Distrib. throughout India, Ceylon, Africa and most tropical and sub-tropical regions.

Plate 1 A :

Photographs of Lippia (Phyla) nodiflora indicating habit of the plant.


4 5 6 7 8 9 10 1 2 3 4 5 6 7 8 9





A. Collection of Plants and Raising Them :

The plants were collected after a brief survey of the locality. The Phyla nodiflora plants were collected from the field of black cotton soil. The entire plants were collected and raised into the garden both in small plots and in earthern pots. The plants were allowed to grow for fifteen days with regular watering, when they started rooting from the internodes, six sets of pots in replication were kept aside for giving the sodium chloride (NaCl) treatment.

B. Method of Giving NaCl Treatment :

The method followed is essentially as per U.S.D.A. Hand Book No.60. However, one molar stock solution each of NaCl and $CaCl_2$ were prepared and mixed. It was diluted to 50, 100, 150, 200, 250 and 300 mM. Each of the above dilutions were given to six pots with different concentration of NaCl to the established potted plants with holding a pair of pots in control. The soil was checked periodically to maintain the concentration of salt at root zone constant. If the concentration increased they were given tap water. After the doses of salt were set the morphological responses of plants such as leaf morphology, stomatal frequency etc. were recorded. Periodically leaf wash was taken to qualify the amount of salt exuded in 12 hours under light.

C. Sand Culture Technique :

The six earthen pots were taken and filled with clean acid washed white sand. The sand was washed several times by water. The plants were

raised in the sand with regular feeding with Hoaglands solution. The plants were given Hoaglands solution regularly twice or thrice a day. After establishing in culture pots these plants were treated with different dilutions of NaCl solution in the same way as described earlier. The leaf wash was taken and titrated against silver nitrate solution to determine NaCl exuded. The leaf area was recorded and measured by using graph paper. The root morphology was studied by recording in sand culture.

D. Method of Culturing in Liquid :

The Phyla nodiflora were raised in the coloured bottles in Hoagland solution. Bottles of 500 ml capacity with (amber coloured) wide mouth were selected for the study. The entire plant with roots were held suspended at the mouth of bottles filled with Hoagland solution with the help of cotton and the plants were allowed to establish in this condition for fifteen days. Effects on the morphological characters were recorded. After establishing in the liquid culture these plants were given NaCl solution treatment. The NaCl sodium chloride of increasing concentration such as 50, 100, 150, 200, 250 and 300 mM were given through the culture solution by gradually increasing the NaCl concentration till the requisite concentration is reached. Each of the treatment was given in replication. The plants were kept under close observation for their response till they established. The leaf wash was taken after two days and titrated against the standard $AgNO_3$ solution and salt exudatesd were quantified.

E. Determination of Osmotic Potential :

The osdmotic potential of cell sap of leaf was determined by the method of Janardhan et.al. (1975) The 1 gm leaf tissue was homogenized, filtered through the cheese cloth and volume was made upto 25 ml. by adding distilled water and electrical conductivity of cell sap was measured with the help of conductivity bridge (ELICO Model CM 82 T). The electrical conductivity of cell sap was 1.437 mmhos/cm at room temperature.

The determination of electrical conductivity of soil extract was carried out, the soil extract for this purpose was prepared by the following method: Soil and distilled water were taken in proportion of 1:5. It was stirred vigorously for 30 minutes and allowed the soil particals to settle down. The supernatant water is used as extract.

Another method is the preparation of saturated paste extraction. The 250 gm of soil is taken in a dish and distilled water (glass distilled) was added. With the help of glass rod, fine paste was prepared. Care was taken not to exceed just adequate quantity of water. It was allowed to stand for one hour and filtered by applying vacuum pressure.

The soil extract was taken and the electrical conductivity was measured by conductivity bridge (ELICO Model CM 82 T).

The E.C. of soil extract was measured of all the six sets of pots which received NaCl treatment.

F. Measurement of Stomatal Index :

Stomatal index is the percentage of ratio of number of stomata per unit area to number of epidermal cells per $mm^2$. This ratio for a given species is constant. Therefore, it serves as a characteristic feature of the species.

The epidermal impressions of both sides of leaf were taken with the help of Step's colourless nail paint. This peel was kept on slide and observed under microscope. A window of 1 $mm^2$ graph paper was kept above the cover slip of preparation (slide). The stomata and epidermal cells were counted. Similarly epidermal cells and gland cells were also counted. Such three replicates were taken.

The calculations were carried out by the formula -

$$\text{Stomatal index} = \frac{S}{S + E} \times 100$$

where S = No. of stomata per unit area
E = No. of epidermal cells.

In case of salt gland index the values for stomatal S was replaced by salt gland

$$\text{Slat gland index} = \frac{Sg}{Sg + E} \times 100$$

where Sg = No. of salt glands.

G. Method of Studying Anatomical Picularity :

The anatomical observations of plants were carried out. The hand sections of root, stem and leaf were taken. The internal structure of plants were examined immediately after the collection. These studies were carried

out in plants raised under sand culture as well as liquid culture in Hoagland. The slow differentiation of aerenchyama was closely examined under microscope.

H. Method ofAssaying Enzyme :

Enzyme cellusose :

1,4(1,3., 1,4.)-P-1-Glucan 4-glucanohydrolase EC 3:2.1.4, Hydrolyses ß-1, 4-Glucan links in cellulose. Method followed is of Bergmayer. Method of Enzymatic Analysis. The reaction is as under.

$$\text{Carboxymethyle cellulose} \xrightarrow[H_2O]{\text{Cellulase}} \text{Carboxymethyl oligosaccharides}$$

Carbonymethyl oligosaccharides + 3,5 Dinitrosalicylic acid - Red colour.

Extraction method :

1 gm of plant organ root and stem was taken and macerated in 10 ml distilled water, filtered through muslin cloth. Filterate is centrifuged at 3000 rpm for 10 minutes. The supernatant is taken for assay.

Assay method :

1 ml of extract + 1 ml of distilled water + 1 ml of Acetate bufer + 1 ml of carboxymethyle Cellulose - incubation for 15 minutes at $37^{o}C$ - 1 ml of Reagent i.e. Dinitrosalicylic acid - Heat in water bath at $100^{o}C$ for 10 minutes - Red absorbance against a blank in the sample was added after the addition of colours reagent and obtain glucose content as reducing equivalent from a glucose standard curve.

The above procedure was used to study enzymatic activity in root, stem of ( Lippia ) Phyla nodiflora.

Cellulase enzyme activity was also calculated by the method used by Kawase (1974). This method given us the reaction of enzyme on cellulose and the change in viscocity of carboxymethyle cellulose is studied. The flow time of cellulose from pipette is measured. The flow of cellulose with enzyme is faster than the pipette of blank enzyme cellulose.

The Assay :

Material or plant organ sections + 5 ml. 0.02 ml. K phosphate buffer at pH 6.1, containing 1 m NaCl macerated Extract centrifuged at 3000 rpm for 20 minutes. 2 ml of supernatant solution + 2 ml. of 1.5 % carboxy methyle cellulose incubated at 25$^{o}$C for 5 h in water bath. Change in the viscocity could be determined by flow times of CM cellulose in verticle 1 ml. pipettes. Cellulose activity and flow time of CM cellulose was repeated for three times. The time taken by CMC flow in verticle pipette was 50 sec., 45 sec., 40 sec. while the time taken by CM cellulose without plant material was 43.16 minutes.