MATERIAL AND METHODS

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STUDY AREA

An ecophysiological study of Ipomoea carnea spp. fistulosa was done on the basis of distribution. This was worked out from August 1994 to August 1995. The present investigation was undertaken in two districts of Western Maharashtra viz. Satara and Kolhapur. Satara is situated between 16° 15' and 18° 10' North latitude and 73°45' and 15° 00' East longitude having latitude of 3049 feet to 3502 feet. The average rainfall is between 1723 to 1826 mm. The soil type is red and black.

The site from Satara district is Dahiwadi which belongs to drought region. It is 65 km. east to Satara city. Rainfall is scanty and soil type is black.

Kolhapur is situated near 16° 42' 5" North latitude and 74° 14' East longitude having an altitude of 650 m MSL. The average rainfall is between 1800-1900 mm. The soil type is red. From Kolhapur district, two sites were selected as Kolhapur and Ichalkaranji. Ichalkaranji is 28 Km. east to Kolhapur. Due to textile industries and other activities soil, water and air pollution is remarkable at this site.

Per site two habitats were selected i.e. of two ecological extremes viz. arid and aquatic habitat. The aquatic habitat of Dahiwadi site is situated near canal of well in which water remains forever while arid habitat was selected

STUDY SITES

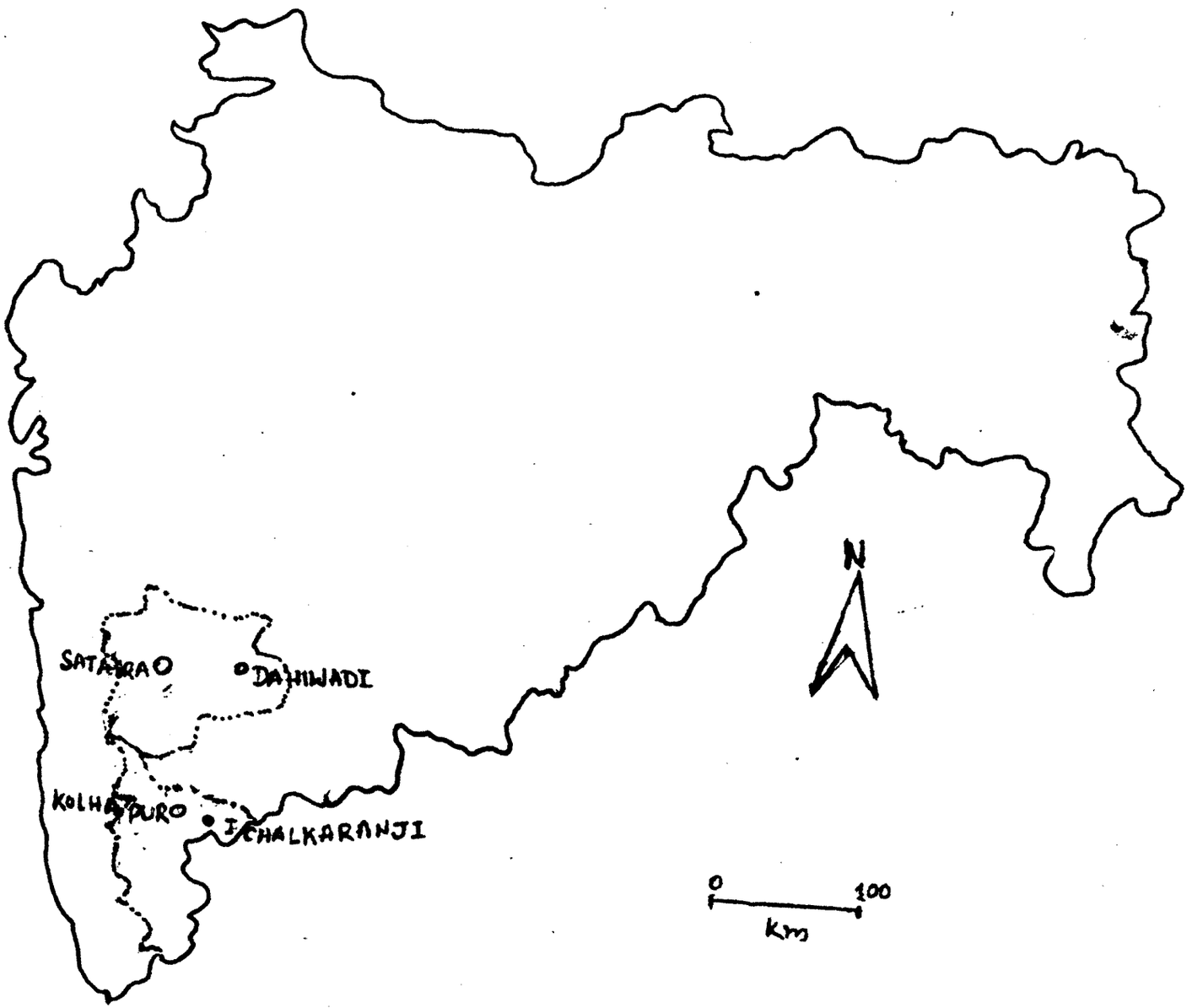

Plate - 2

A - *Ipomoea carnea* spp. *fistulosa* in arid habitat

B - *Ipomoea carnea* spp. *fistulosa* from aquatic habitat

PLATE-2

from non-irrigated area. Aquatic habitat of Kolhapur was selected along the bank of the Panchganga river. This study site is situated near sugarcane crop field, where water remains forever. Arid habitat site selection was done in the campus of Shivaji University. Aquatic habitat of Ichalkaranji was selected from the stream of industrial polluted water which is continuous throughout year. The arid habitat was selected out side the city which gets only rain water.

To study distribution of Ipomoea carnea spp. fistulosa Mart ex. Choicy. Observations were made in above mentioned sites and habitats.

Seed germination experiments were set under laboratory condition. To test viability of seeds old seed lots were used. (Collected in November 1987 from Dahiwadi). Fresh seeds were collected during August 1994 from the same locality were also tested. All seeds were surface sterilized with 0.1% $HgCl_2$ and washed thoroughly with distilled water. Four sets of 20 seeds each were placed for germination. 1st of control was kept in distilled water. 2nd set was treated with hot water (50°C), 3rd set was treated with 50% H_2SO_4 and 4th set was arranged using scarified seeds with the help of polish paper. All sets were kept for incubation. Observations were recorded periodically with the interval of 24 hours. The germination procedure was carried out by standard petriplate method.

For growth analysis of Ipomoea carnea spp. fistulosa, cuttings from various habitat were collected and brought to the laboratory in polythene bags. Selection of cuttings were made on the basis of size and age of the plants. Cuttings were 18 to 20 cm in length and 2.8 to 3.2 cm. in girth. 20 cuttings of each site were grown in polythene bags having 20 x 25 cm. The cuttings were irrigated on alternate days. Observations were recorded for different phases of growth i.e. initiation, vegetative and reproductive phase with the interval of 15 days, observations were recorded for hight and girth of cuttings and number of leaves per cutting in replicates. Comparative growth performance of cuttings from various sites was studied under laboratory condition.

For phenological records different phases were observed from each site periodically. Phenophases were also noted from the plants which were grown under laboratory condition.

In leaf behaviour studies stomatal density and leaf area were studied from each site and habitat. The present study was carried out throughout the year.

The observations on stomatal features were made with isolated epidermal peelings from both the surfaces of leaves at a fixed node as far as possible. The measurements of

number of stomata size made with microscope fitted with a precalibrated occular and micrometer. The stomatal index (SI) was determined according to Salisburg (1927).

$$SI = \frac{S}{S + E} \times 100$$

Where 'S' is the number of stomata per mm^2 and 'E' is the number of epidermal cells per mm^2. Same observations were made in case of cuttings grown under laboratory condition. Stomatal resistance, transpiration rate, leaf temperature were measured with the help of steady state porometer (LI Cor. in model LI - 1600, of USA).

The leaf samples of Ipomoea carnea spp. fistulosa were collected during morning hours fully mature leaves were chosen for the analysis from different sites (representing varytng leaves of environmental parameters). The leaf samples were analysed for total chlorophyll, relative water content, ascorbic acid and pH. The chlorophyll estimation was done according to Arnon (1949). Calculations were done using formulae.

Relative water content (RWC) was measured according to Singh (1977) as per the following formula.

$$\text{Relative water content (\%)} = \frac{F - D}{T - D} \times 100$$

Where F = Fresh weight of leaves

D = dry weight of leaves

T = turgid weight of leaves

The leaves were washed before dipping into water for 24 hours and then weighed (F). The weight of turgid (T) leaves was recorded. Then the leaves were dried at 80°C for 24 hours and the dry weight was (D) noted.

Ascorbic acid content was estimated according to Sadashivam and Balsubsramnian (1987) 0.59 leaf sample was homogenised in 4% oxalic acid solution. Then centrifuged for 15 min and the supernatant was used as the source of ascorbic acid. 5 ml working standard solution was taken in conical flask. 10 ml of oxalic acid was added and titrated against dye (DCPIP) (Vl ml). End point was appearance of pink colour which persists for a few minutes.

The titration for supernantant give (V2 ml). Amount of Ascorbic acid mg/100g, sample was calculated by using formula :-

$$= \frac{0.5 \text{ mg}}{Vl} \times \frac{V2}{5 \text{ ml}} \times \frac{100}{\text{wt of sample}} \times 100$$

Leaf extract pH :

For this 5 gm of fresh leaves were washed and homogenised with 25 ml double distilled water. The pH of the homogenate was measured with a pH meter.

Air pollution tolerance index (APTI) give an empirical value representing the tolerance level of a plant species to air pollution. This was calculated by the formula according to Singh and Rao (1983).

$$APTI = \frac{A\ (T + P) + R}{10}$$

where A = ascorbic acid content of leaf in mg/gm fresh weight

T = total chlorophyll of leaf in mg/gm

P = pH of leaf extract

R = RWC of leaf

The entire sum is divided by 10 to obtain a small manageable two replicates figures were maintained for each sample.

To study anatomical characters, plant parts like stem, root and leaves were collected from the sample sites, observations of transversa section of leaf, stem and root are based on thin hand cut sections. Sections were studied under microscope using camera lucide.

Plant and soil samples were subjected to analysis of physical and chemical parameters.

Methods used for analysis are described below in brief :

To study photosynthetic pigments plant material from different sites were brought to the laboratory in air tight polythene bags. Estimation of chlorophylls and carotenoids was done during rainy, winter and summer season. Estimation of chlorophylls and carotinoids were also carried out from the samples of cuttings which were grown under laboratory conditions.

Chlorophylls were estimated following the method of Arnon (1949). Chlorophylls were extracted in 80% chilled acetone from 0.5 g of fresh plant material in dark. These extracts were filtered through Whatman No.1 filter paper using Buchner's funnel. Residues were washed repeatadly with 80% acetone collecting the washings in the same filtrate. The volume of filtrates were made to 50 ml with 80% acetone. The absorbance was read at 663 and 645 nm for chlorophylls a and b respectively.

Chlorophylls (mg g^{-1} fresh tissue) were calculated using the following formulae.

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 chlorophylls = (8.02 x 1 663) + (20.2 x A 645) ... Z

$$\text{Chlorophyll a or Chlorophyll b or Chlorophyll a + b} = \frac{\text{X/Y/Z X volume of extract x 100}}{\text{1000 x Weight of material}}$$

Carotenoids were estimated by reading the absorbance at 480 nm (Kirk and Allen 1965). Total Carotenoids were estimated using the formula of Liasen - Jensen and Jensen (1971).

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

Where C = Total carotenoids in mgs
D = Optimal density
V = Total volume in ml
F = dilution factor and
2500 = average extinction.

Electrical conductivity was measured by preparing 1:5 aqueous solution using field conductivity meter Elico model PE - 133.

pH of aqueous extract was measured by pH meter Model LI-10T

To estimate inorganic constituents from plant material sampling was done (leaves, stem and root) from different sample sites. For the preparation of acid diguest, leaves, stem and roots were oven dried at 60°C. Oven dry material was used for wet digestion. An acid digest was prepared following the method of Toth et al., (1948). Five hundred mgs of oven dried powdered material was transferred to 150 ml capacity beaker to which 20 ml concentrated HNO_3 was added. The beaker was covered with watch glass and was kept till the primary reaction subsided. It was then heated slowly to dissolve solid particles. After cooling to

room temperature 10 ml of perchloric acid (60%) was added to it and mixed thoroughly. It was then heated strongly until a clear and colourless solution (about 2-3 ml) was obtained while heating the liquid was not allowed to dry. It was then cooled and transferred quantitatively to 100 ml. capacity volumetric flask, diluted to 100 ml with distilled water and kept overnight. Next day it was filtered through dry Whatman No. 44 (ashless) filter paper and the filtrate was used as the source of different inorganic constituents.

Minerals were estimated by using atomic absorption spectro photometer. Parkin - Elmer 3030 model.

By using formula, 0.02 x reading x d.f. g/100g of mineral elements were calculated.

The method followed for nitrogen estimation was developed by Hawk et al., (1948), 0.5 gm of plant material was accurately weighed and carefully transferred to clean dry kjeldhal's flask. 8 ml concentrated H_2SO_4 (1:1) and pinch of microsalt and few glass beats were added to avoid bumping. The flask was kept inclined and plant material was digested till yellow colour was obtained. Flask was cooled, 15-20 ml distilled water was added. The amount of plant was filtered through Whatman No.1 filter paper in 100 ml volumetric flask. The flask was washed repeatedly with distilled water and washings also filtered. The volume of filtrate was made 100 ml with distilled water.

For estimation of nitrogen clean dry Nessler's tubes were taken and properly labelled. Different reagents and plant extract was accurately taken in test tubes. These tubes were kept for 20 minutes for colour development and absorbance was read at 520 nm in the spectrophotometer.

A graph of absorbance against standard nitrogen concentrations was plotted and with help of graph, amount of total nitrogen in plant material was determined.

For the estimation of phosphorus the method of Sekin et al., (1965) was followed. Phosphorus react with Molybdate Vanadate reagent to give Yellow colour complex. By estimating colorometrically the intensity of the colour development and by compairing it with the colour intensity of known standard phosphorus content was estimated.

By taking 1 ml of acid digest in a test tube 2 ml 2N. HNO_3 was added followed by 1 ml of molybdate vanadate reagent. Volume was made to 10 ml with distilled water. The ingradients were mixed well and allow to react for 20 minutes. After 20 minutes colour intensity was measured at 420 nm using a reaction blank containing no phosphorus.

Standard curve for phosphorus was preapred using various concentrations of phosphorus (0.025, 0.05, 0.1, 0.2, 0.4 mg phosphorus) and standard KH_2PO_4 solution containing 0.025 mg phosphorus/ml.

With the help of standard curve amount of phosphorus in the plant material was calculated.

Energy content was studied by titrimetric method (cal/g) Karzinkin and Tarkovaskya (1964) oven dry material was used for wet digestion.

10 mg oven dry powdered material was taken into round bottom flask to which 3 ml of 5% potasium iodate and 20 ml concentrated H_2SO_4 were added. This flask was heated on water bath to reflux for one hour than it was cooled to room temperature. 60 ml distilled water was added in it and heated until the colour and smell of iodine disappears. Total volume was made 250 ml with distilled water and cooled to room temperature. 50 ml was taken out and 2 ml 10% potasium iodide was added and immediately kept in dark for 10 minutes. Then 2-3 drops of starch was added as an indicator and titrated against 0.1 N $Na_2S_2O_3$ till blue colour disappears.

With the help of titration reading and formula energy content was calculated.

Formula :

$$\text{Energy content Cal g}^{-1}\ \text{dry wt} = \frac{(B-A) \times 3.38 \times 0.1857 \times 3.567}{0.01}$$

Where A = ml of titrant used for sample /
B = ml of titrant used for blank./

Soil samples were collected from different sample sites and used for analysis viz. E.C., pH, organic matter content and water holding capacity.

For E.C. and pH (1:5) aqueous solution was prepared. Electrical conductivity was measured by using field conductivity meter Elico model PE - 133.

pH of soil solution was measured by pH meter model LI - 10 T.

To find out organic matter content weighed quantity of soil along with crucible and lid kept in Muffle furnace (Expo. industrial corporation Sr. No. 220) at temperature 550°C for two hours. Difference in initial and final weight was calculated.

To find out water holding capacity weighed quantity of soil samples were inserted in tin box with holes at bottom along with bloting paper. These boxes were kept in tray containing water. After complete moistening of soil boxes were removed drained then weighed. The difference in initial and final weight of tin box along with soil was calculated.

Allelopathic effects of this species on crops were also studied under laboratory condition. For this purpose seeds of wheat (Triticum aestivum L.) HD 2189, Jowar (Sorghum vulgare pers) Local Shalu, Rice (Oryza sativa L.) Indrayani

and Kidney bean (Phaseolus aconitifolium Jacq) Local were obtained from seed lots of farmers from Dahiwadi. Late aged corolla, senescent leaves and roots of Ipomoea carnea spp. fistulosa Mart ex, choicy were collected from the Shivaji University campus and dried in oven. These dried plant parts were finely powdered with the help of grinder. This powder was used for preparation of extract.

Accurately weighed powder of the senescent leaves, corolla and roots were taken (0.5 g, 1.0 g and 1.5 g). These quantities were mixed thoroughly into 100 ml sterilized distilled water separately in volumetric flask and allowed to settled for two hours. The solutions were then filtered through double layered muslin cloth. The filtrates were used as extract of 0.5%, 1.0% and 1.5% for the allelopathic studies. The extraction was done according to Nelson et al., (1960).

Seeds of cereals and legume crops were first treated with surface sterilent (0.1% $HgCl_2$). Then washed with distilled water. Four sets of 20 seeds each were arranged for each crop. The germination procedure was carried out by standard petriplate method. Petriplates were incubated for 96 hours in case of Jowar, wheat and Kidney bean while seeds of rice were incubated for 120 hours.