Chapter IV

MATERIAL AND METHODS

Materials and Methods:

Literature Survey:

A critical survey of concerned literature has been made on Convolvulaceae of World in general and India in particularly. Detailed information on Convolvulaceae of Maharashtra, their distribution, status, diagnostic features etc. was collected through referring systemic account of the family in various Districts, State, National floras and Research publications.

Field Work:

On basis of literature and herbarium information several field trips of short and long duration were undertaken to various localities in South-Western Maharashtra. The different places like Ajara, Akole, Amba, Amboli, Bhandardara, Bhudargad, Chandgad, Choukul, Dajipur, Ganapatipule, Junnar, Kolhapur, Malwan, Nivati-fort, Phonda, Pune, Radhanagari, Sangamner, Satara and Sindhudurg was visited frequently during the present study.

Critical field observations on each member collected have been made in the field itself. The members have been photographed in their natural habitats with Nikon coolpix 4500 and Sony cybershot camera.

Taxonomic study:

1. **Study of general morphology:**

During field visits observations were made on life form, distribution, present field status, ecology, phenology and variations of species. Different kinds of herbs, shrubs, climber, woody climber and twinners were studied. Detailed records of field observation were made. Notes were made on species in their localities of occurrences.

Palynological study:

While visiting different places of Maharashtra; the pollen grains of each species were collected. They are preserved in glacial acetic acid in small glass viols. For further process Erdtmans (1971) acetolysis method was used.

Acetolysis:

1. The fixed pollen grains were washed with distilled water to remove traces of acetic acid and centrifuged. The same procedure was followed for 3-4 times.
2. The pollens in the tubes were treated with 5ml mixture of acetic acid and concentrated sulphuric acid in the ratio 9:1(acetolysis solution) to remove the protoplasmic contents.
3. The tubes were placed with acetolysis mixture in a water bath at 70^0C-100^0C for 5-10 minutes.
4. After this the tubes were removed from water bath and cooled at room temperature. The color of acetolysis solution was changed yellowish brown to dark brown. Centrifuge and decant off the acetolysis mixture.
5. Again pollen grains were washed with distilled water to remove traces of acetolysis solution and centrifuged. The same procedure was followed for 3-4 times.
6. Then the pollen grains were passed through different alcoholic grades i.e. 30% to 60% to 90% to absolute alcohol and finally fixed them in absolute alcohol for larger time.

Acetolysis brings about significant changes in almost all aspects of pollen morphology it destroys the protoplasmic contents providing thereby a correct picture of exine stratification and ornamentation.

Scanning electron microscopy (SEM):

1. The different samples of pollen grains preserved in absolute alcohol were poured in cavity blocks. So that the alcohol was evaporated and pollens remained in the cavity block.

Seed germination and cotyledonary leaf study:

1. The seeds of different 14 species of *Ipomoea, Merremia* and *Jacquemontia* were selected for the cotyledonary leaf studied under laboratory condition. Other species were not germinated.
2. The seed coat is very hard in *Ipomoea and Merremia*. Seeds were treated with Conc. H_2SO_4 for 10 minutes then washed with distilled water for removing the traces of acid. There was no any treatment given to the seeds of *Jacquemontia*.

After this 10 seeds of each species were kept in petriplates containing moist blotting paper/ in plastic pots containing cocopit for germination. After 4-6 days seeds were germinated, the cotyledons were photographed under Carl Zeiss Microscope using Nikon coolpix 4500 camera for further study. Also cotyledonary leaf of each species was illustrated on graph paper for studying their shape, size variation and venation pattern.

Protein estimation:

Mature seeds were washed with water, dry and crushed to make fine powder. Seed powder defatted with hexane, air dried and stored at 4^0C in refrigerator for further use. Seed proteins from mature defatted seed powder were kept for extraction in 1:6 proportion of distilled water with 1% (PVP) Polyvinyl polypyrrolidone and allowed to stand overnight. The suspension was centrifuged at 12,000 rpm at 4^0C for 20 minutes to remove the particulate matter and clear supernatant was used for further protein estimation by the biuret method (Layne, 1957).