CHAPTER-2

MATERIALS AND METHODS

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2.1. FLORISTIC SURVEY:

The floristic survey for the present work was of special type because it was the survey of plants of a special habit group which lives in special but diverse habitat structure. At the same time, starting from the selection of vegetation to the mounting, at every stage some special care had to be taken for their proper an effective documentation.

2.1.1. SELECTION OF VEGETATION FOR SURVEY

The dissertation on the angiospermic climbers of Darjeeling and Sikkim Himalayas included extensive survey work in different types of vegetation spreading all over these two places during 1994 to 1996. Different locations/vegetation's have been selected accordingly except in the permanent snow covered region of North Sikkim. In fact, quite a large number of places have been visited during over 80 field trips. These places include Bengdubi (110m), Bagdogra (126m), Sukna (132m), Kurseong (1500m), Dow Hill (2000m), Sonada (2000m), Jalapahar (2250m), Birch Hill-Lebong (2000-1900m), Senchal- Tiger Hill (2350-2450m), Manebhanjan (2100m), Meghma-Tonglu (above 3000m), Sevoke (450m), Kalimpong (1500m), Ryang (500m), Neora Valley National Park (2500- 3100m), Lava (2050m), Latpunchor (1200m and above), etc of Darjeeling Himalaya; Jorethang (300m and above), Tadong (1200-1300m), Mangan (1600m), Chungthang (above 2000m), Soreng (1500-1600m), Gyalshing-Pelling (1600-1900m), Pangthang (2000m), etc of Sikkim Himalaya. For the selection of major localities following points were considered:

(i) Places should represent all subdivisions (for Darjeeling or districts (for Sikkim);

(ii) should spreaded over a very wide altitudinal gradient (upto 3200m);

(iii) should represent different types of vegetation, e.g,. (a) herbaceous, (b) shrubby, (c) forested, (d) degraded forests, (c) cultivated land, etc and

(iv) should represent different types of exposure in respect of light, precipitation and wind.

2.1.2. COLLECTION AND PRESERVATION OF MATERIALS

During field trips angiospermic climbers were traced on visual observation and

representative specimen were collected. A representative specimen include leaves, climbing organ, flowers or fruits and/or fruits, etc. In case flowers or fruits were not available, specimen with healthy and mature leaves were collected. In the field, specimen were recorded in the **Field Note Book** and kept in polythene bag. Specimen were cleaned trimed to the size and transferred to wooden plant press for drying. In some cases specimen were dipped in formalin for a few minutes or some amount of formalin were added directly on the blotters to check fragmentation of different parts, mainly leaves, flowers and fruits. During rainy season, for quick drying a Hot Air Oven was also used.

In most cases specimen were completely dried within ten days. However, after proper drying, all specimen were poisoned by soaking with 4% solution of Mercuric Chloride in rectified spirit, dried again under pressure using blotting papers.

Dried and poisoned specimen were then pasted on standard herbarium mounts, labelled properly and temporarily stored in a cabinet in the Plant Taxonomy Laboratory, N.B.U.

2.1.3. THE FIELD NOTE BOOK

All the collected specimen were recorded in a **Field Note Book** which include specific location, date of collection, availability, habit and habitat structure, flower colour, aroma, pecularities, etc. Field *notes* were transferred to herbarium labels for ready reference. After the conclusion of the dissertation, the Note Book will be deposited at the NBU-Herbarium.

2.1.4. IDENTIFICATION

Specimen were primarily identified in the Plant Taxonomy Laboratory of North Bengal University, using literature and matching with the available predetermined specimen in NBU-Herbarium. Identification was confirmed by matching all specimen at Central National Herbarium, Howrah (CAL) and at the herbarium of Sikkim Himalayan Circle of Botanical Survey of India (BSHC).

2.1.5. DEPOSITION OF SPECIMEN

The first set of specimen will be stored at NBU-herbarium and the duplicates will be deposited mainly in CAL and BSHC

2.1.6. ENUMERATION AND DESCRIPTION

For the enumeration of different recorded species of angiospermic climbers no any accepted system of classification has been followed. Instead, all families, genera under each family and all species under a genus have been arranged alphabetically

for easy handling. Climbers are not representing a very high number of families, so their placement within the empty structure of a giant classificatory system would not have been much meaningful.

At the beginning of a family an artificial key for the recorded genera have been provided vvhenever more than one genera have been reported. A similar key have been provided for each genus recorded with more than one species. Keys for 'varieties 'and 'forma' were included within the species key.

Species keys are followed by the enumeration of different recorded species (also alphabetically). For each species a correct name may be followed by basionym and other available synonyms. And, each name is followed with proper author citation, references to protologue and record in floristic and taxonomic work as a correct name. Key to the acronyms and abbreviation used to denote various journals and books have been provided at the beginning of the enumeration.

.The scientific naming part is followed by a brief description for the species. In case of new species or new records or a species with much diversity in the population a detailed description have been provided. All measurement provided in the metric system only.

A description is followed by local name(s)- if available any. The language in which the name is given also have been indicated in majority of cases.

For flowering and fruit ripening periods a month to month range have been provided. Plants growing over an wide altitudinal range shows variation in flowering time at various levels of altitude. In such cases a total range have been provided. Plant, which were collected once only bearing either flower or fruit- the recorded month of collection have been provided in the parenthesis.

To cite a specimen (voucher or type), place of collection is followed by altitude of the place, date of collection, collector's names and field number. Also, the specimen from Darjeeling and Sikkim have been provided separately.

Local distribution provided in the enumeration are not only based on collected specimen but also on observations in the field. The cited local distribution is not literature based but on observation during the present work only. On the other hand, the cited general distribution of a species has been determined either from literature or from the study of deposited specimen in different herbaria.

At the end of enumeration for some species a 'NOTE' has been added which covered mainly (i) local uses of plants and / or their economic importance; and the (ii) cytological information.

Photographs on habitat, different interesting and/ or rare species of plants and herbarium species have been provided along with the line drawing of some other interesting species.

2.2 PHYTOSOCIOLOGICAL STUDIES:

To know the present status of availability, including their distribution, of different species of angiospermic climbers in the hills of Darjeeling and Sikkim Himalayas and to know their natural associates, phytosociological studies were conducted in different localities.

2.2.1. DISTRIBUTION OF STUDY AREAS

For better understanding of the situation different localities were selected against four altitudinal zones: (I) upto 800m; (II) 800-1600m; (III) 1600-2400m and (IV) 2400-3200m study areas selected for phytosociological studies in Darjeeling include Sukna, Tindharia, Kurseong, Birch Hill, Jalapahar, Jorebungalow, Senchal-Tiger Hill, Sukiapokhari-Manebhanja, Meghma -Tonglu, Kalimpong, Lava, Jorepukri and Rechilachak in Neora Valley National Park, etc. And, the areas in Sikkim include Jorethang, Namchi, Legship, Soreng, Gyalshing- Pelling, Yaksum, Pedong, Rhenek, Singtam, Gangtok, Mangan, Chunthang, etc.

2.2.2. METHODS OF SAMPLING

Different types of herbaceous, shrubby and degraded vegetations are more rich in climber flora than a continuous dense undisturbed forest. So, sampling were made mainly in these types of vegetation. While only 1x1m size of quadrats were used for survey in herbaceous vegetation, for shrubby vegetation quadrats of two sizes were employed. Quadrats measuring 5x5m were used for recording shrubs and shrubby climbers, and quadrats of 1x1m size for recording ground cover vegetation's. In each tire 25 quadrats were studied. Whenever a 5 x 5m quadrats was taken, an 1x1m quadrat was also taken inside it keeping the quadrat number same. The site selected for laying quadrats were mainly at random except that there should have one or more species of angiospermic climber.

The quadrats were studied mainly for List and Count information.

2.2.3. DATA PROCESSING

Data collected List-count quadrats were used for determining Relative Density (RD), Relative Frequency (RF) and Important Value (IV) of different recorded species using following formulae:

$$1.\ RD = \frac{\text{Number of individuals of the species}}{\text{Number of individuals of all the plant species}} \times 100$$

$$2.\ RF = \frac{\text{Number of points of occurrence of the species}}{\text{Number of points of occurrence of all the plant species}} \times 100$$

3. IV = RD+ RF.

In the present work "Importance Value "(IV) was calculated by summing up the values 1& 2 above for each of the recorded species. [Actually, IV should have been calculated by summing up the values of RD, RF and RDm (Relative Dominance), but in the present work it was simplified as sufficient data for the calculation of RDm was not available. Das (1986) and Das and Lahiri (1997) also calculated IV in the similar way].

2.3. ANATOMICAL STUDIES:

The main purpose of taking up the anatomical work is to determine the basic morphology of climbing organs. In the present work the studies were restricted to tendril-climbers only. In different species tendrils might be the modification of stem or leaf or stipule or petiole or petiolule, etc. but how far these ideas are true was the question raised in mind. For understanding this, a very simple internal structure was studied, i.e. the structure of stele.

For this purpose, specimen were preserved immediately after collection. Appropriate portion of shoot having stem, leaf tendril, etc. were fixed in 5:5:90 Formal-Aceto-Alcohol (70% ethanol) or FAA for overnight and then stored in 70% ethanol.

Transverse sections from appropriate regions in stem, petiole, petiolule, different portions of tendril were differentially stained with saffranin and light green, dehydrated and mounted in DPX (Johansen 1940). The slides are now stored in the Taxonomy and Environmental Biology Laboratory of Department of Botany, N.B.U.

For the method of preparation of diagram and terminology Metcalfe and Chalk (1950) was followed.

2.4. POLLEN MORPHOLOGICAL STUDIES:

External morphology of pollen grains of 77 species of angiospermic climbers have been studied. Anthers from flowers and /or mature buds (just before blooming) were collected, dried under sun and preserved in a dessicator with silica gel.

Pollen for all species (including monocotyledonous) were acetolysed, chlorinated (half of the stock), mounted in glycerine jelly with inserted wax sealing. The acetolysis method of Erdtman (1960) with modifications of Chanda (1966) and Nair (1970) were followed.

2.4.1. PREPARATION OF ACETOLYSIS MIXTURE

For pollen preparations, freshly prepared acetolysis mixture is essential and it was made by adding slowly one part of Sulphuric acid to nine parts of Acetic anhydride.

2.4.2 DETAILS OF ACETOLYSIS METHOD

I. Dry or fresh polliniferous materials were crushed over a finely meshed stainless steel sieve (0.11sq mm) resting on a funnel; setting on a hard glass centrifuge tube;

II. Before each treatment the stainless steel sieve was flamed for avoiding species to species contamination;

III. After crushing the material, 10ml of acetolysis mixture was slowly poured over it and then stirred with a clean and dry glass rod;

IV. Centrifuge tube containing pollen-mixture were heated in a water -bath or kept in an oven at 60^0 c until the mixture turns brown;

V. The tubes were then allowed to cool down, centrifuge at 4,000 r.p.m. for 5 minutes before decanting;

VI. Little amount of distilled water was added to the sediment, shaken thoroughly and centrifuge at 4,000 r.p.m. for 5 minutes;

VII. This process was repeated for 2-3 times for complete wash out of the chemicals;

VIII. Distilled water added to the sediment once again along with a few drops of acetone, shaken thoroughly and sieved through the finely meshed stainless-steel net;

IX. 10ml of distilled water was added to the sediment and divided into two equal parts after shaking. One part was taken for chlorination and the other part 2ml of 50% glycerine was added after centrifuging and decanting;

X. Chlorination was effected by adding 5ml of distilled water, two drops of concentrated hydrochloric acid, a few drops of sodium hypochloride solution (4%) and stirring with a clean glass-rod;

XI. After washing the pollen grains by repeated centrifugation and decantation this stock was mixed with the other stock (kept with glycerine);

XII. Washing again for 2 minutes;

XIII. A drop of 50% glycerine was added to the sediment;

XIV. Placed the tubes in inverted position over a piece of blotting paper for few hours or over night in order to dry the materials.

2.4.3. PREPARATION OF POLLEN SLIDES

I. To prepare permanent pollen slides a very small piece of glycerine jelly (After Kisser's 1935) was taken on a platinum needle, touched the surface of the pollen bearing sediment in centrifuge tube, transferred to the centre of a clean glass slides and then covered with a 18mm round or square cover-glass;

II. Gently heated the slide just below the jelly on a pointed flame to spread the jelly along with the specimen;

III. A few chips of wax (melting point 60°- 62°c) was placed on one margin of cover-glass, heated gently to enter the malten wax inside for sealing;

IV. Kept the slide on a flat and horizontal surface and allowed to cool down;

V. Scrap off excess wax from the surface and then cleaned by rubbing with a piece of soft cloth. All the slides were labelled properly with reference to the specimen, date of preparation, identity the plant, etc.

2.5. PHENOLOGICAL STUDIES:

Phenology of some of the common climbers were studied to understand their life-style which include: germination, leaf-flash, flowering, fruiting, senescence, breaking of dormancy, etc. Most of the observations were made with the plants in their habitat and in some cases under garden environment.

2.6. CYTOLOGICAL INFORMATION :

Haploid and /or diploid chromosome number for 96 species of the recorded angiospermic climbers have been recorded from the literature and were cited in the enumeration, at the end of morphological description. For most of the plants chromosome number were collected from the *Chromosome Atlas of Flowering Plants of the Indian Subcontinent* (Kumar and Subramaniam 1986) and for these species wise references have not been provided. But, in cases where chromosome numbers were collected from other publications were duly indicated and detailed in the bibliography.

2.7. ETHNOBOTANICAL INFORMATION :

While working in the field ethnobotanical information were collected mainly in two ways: (I) by observing how local people using angiospermic climbers, and (ii) by interviewing local people. These information include their use as food, fodder, medicine, ornamental, poison and for miscellaneous purpose. Some ethnobotanical information were also collected from the literation.