CHAPTER V

MATERIAL AND METHODS

A real interest in systemic mycology and to a appropriate its problems, it needs to acquire a general knowledge of various groups of fungi to provide a background for future study. The most easy and satisfactory way to do this is to acquaint the common species occurred in the neighbourhood throughout the year. For this, a keen and careful search repeatedly and periodically throughout the year is essential for a particular area or locality under investigation. Systematic collecting the material provides the basis of all taxonomic research.

The way how to collect the material ideally is also an art. The quality of collections obtained and the number of species collected or found tend to be in inverse proportion to the distance travelled during collection of the material. In the beginning, it was attempted to collect at random anything which catches the attention but later on with the experience it was only the best and different collections from previous collection collected. This, no doubt avoids the repetation but also gives nature of the occurrence of different groups of fungi in the same locality or the area under study.

The procedures for collecting and examining the fungi of

different groups are different. Presence of mycoparasitic fungi in some cases was recognisable with a hand lens and in others the fungi in general without any specification for any group or kind of fungi or in substrate were collected and the collections were thoroughly combed in the laboratory to find their presence. For mycoparasitic fungi, it was made sure that they were overgrowing other fungi and limited to them.

Material collected from the field when brought in to the laboratory for further macroscopic and microscopic observations, the whole collections can not be examined the same day or next. Therefore, the materials have been dried well under blotting paper with press or in boxes for drying at room temperature for following successive changes.

Examination of the individual material starts with a study of the microscopic features visible to unaided eye, hand lens. Accordingly, all the collections were examined and were sorted into their main groups.

Microscopic examination followed after the gross microscopic examination groupwise. For the routine examination a minute portion of sporing tissue is removed with the aid of a mounted needle, needle knife, razor blade or scalpel point and placed in a small drop of mounting fluid, Lactophenol on a

microscope slide. Stained with stain cotton blue.

Ingredients of Lactophenol :

Phenol	100 ml.
Lactic acid	100 ml.
Glycerine	100 ml
D.W.	100 ml.

Preparation - Take above ingredients and mix well

Ingredients of cotton blue :

Phenol	100 ml.
Lactic acid	100 ml.
Glycerine	100 ml.
D.W.	100 ml.
Aniline Blue	2 g.

Preparation - Take above ingredients and mix well.

If the material is bulky then gently teased apart and coverslip applied. Some light pressure (e.g. by tapping with the butt of a pencil or needle holder) serves to disperse the structures in a thin optical plane. Warming the slide over a spirit lamp for any air bubbles that present disappeared. But care should be taken that the mounting medium should not allowed to boil.

Semipermanent well stained prepared slides required

sealing by a proper and suitable sealents. The slides in lactophenol were warm for 3-4 minutes to ensure that all air bubbles were completely eliminated. Excess of mounting fluid was thoroughly clean off and then were sealed by melted paraffin or nail warnish. Slide prepared in this way are reasonably permanent and were labelled and numbered and kept in the slide boxes for further study.

Microscopic examinations by normal transmitted light is quite adequate for most purposes. Accurate measurement of microscopic structures which are necessary to identify the material are essential were taken by already calibrated Meta Olympus microscope. A set of calibration for each eyepiece and objective (10X, 45X) has been already prepared is used for direct combinations (eyepiece and objective). Duriing measurements, the largest and smallest matured structures [conidia, spores, conidiophores etc.] were searched for and measured to ascertain the range of variation that occurred.

All the materials well dried and of good quality in duplicate were kept in standard size packets, first inserted into transperent paper envelops and were labelled printed in block and glued to each packet carrying all pertinent collection data (Herberium No. or Collection No., Name, Host locality, Collection date, Reference etc.). And all these collections and slide boxes

were kept carefully. The identification of the angiospermic hosts were made with the help of Cooke's Flora of Bombay Presidency.

All the materials were identified upto the species level with the help of upto date taxonomic literature, keys, monographs or from the same standard text books, journals, proceedings, reports etc. Efforts have been made to take into consideration the taxonomic criteria like -

1) Mycelium - Colour, smooth or warty, septate or non septate, diameter, mycelial setae etc. Mycelial setae may be smooth or warty, simple or branched, straight or flexous, uncinate or irregularly curved, length, diameter etc.

2) Characteristics of fruiting bodies, sporangia, pycnidia, colour, size, shape etc. Presence and morphology of appendages, outgrowths etc.

3) Characteristics of conidiophores, conidia, their attachments etc.

4) Characteristics of spore, conidia - number, size, shape, septation, constrictions, straight or curved, nature of wall, smooth, spiny, warty etc. terminal, discrete, phialdic, scars etc.

5) Characteristics of Colonies.

6) Host.

Illustrations (drawings, graphs, plates etc.) are drawn from the essential part of taxonomic study. As they are able to convey considerable amount of data which can not be as readily apparent from the written text.

Some camera lucida pencil sketches of the materials have been prepared in line drawings with Black India ink and also some colour photographs of collected specimens were taken by using Pentax Camera of our department and microphotographs were taken by using MFAK's system of Jenavel Carlzeiss microscope of our department. Photographs and microphotographs have been presented by setting them into plates. These specimen materials are deposited at National Mycology Herbarium "Herbaria Crypto Indiae Orientalis" I.A.R.I. New Delhi and the numbers received from them are given in the following text.

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