MATERIALS AND METHODS

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The Ascomycetes fungi studied in the present investigation were collected from various localities by frequent visits to the different places during different seasons. Most of the fungi collected are either parasites or saprophytes growing on varied substractes like living or dead leaves, stems, fruits, bark, woods or in/on the humus rich soils under shade of the forest trees. Method of collection of these forms depend on the nature of the organism. Forms occuring on the dead or living leaves were collected in the paper envelop, those occuring on the twigs, stems or wood were collected into small polythene bags along with living part of the host, with their reproductive structures like flowers, fruits or seeds to confirm the identity of the host, Delicate fleshy forms were collected in plastic or glass bottles along with their substrate. The hosts, especially dead fallen twigs, branches or woods were identified tentatively as far as possible at the place of collection and confirmed in laboratory and noted on the envelop containing the material.

The collection collected were brought into the laboratory for further examination, first cleaned and sorted. Both host and fungus were examined together and

their identity were confirmed. The macroscopic characters were recorded for fleshy and delicate forms like colour, texture, size, shape etc. in the field.

For the microscopic study, microprepdrations were made by scrapping of superficial material by needle, blade or squash mount of asci and ascospores or by taking free hand sections. Following stains and mounting media were used.

Stains and Staining techniques :

Micro-organisms in their living state are so nearly transperent that little can be seen of the details of their structures unless the phase microscope is employed. For this reasons a variety of stains and staining methods have been devised. The simple stains which employ a single dye such as cotton blue, methylene blue, carbol fuchsia etc. and differential stains which through the use of a mixture or a sequence of different dyes combined with special treatment devised to bring out in clear detail certain structures.

The stains belong to the group of aniline (coal tar) dyes and include crystal violet, basic fuchsin, safranin, methylene blue, cotton blue, eosine, etc. The idea of staining microbes was first introduced into microbiology by Carl von Weigert in 1871, when he first stained bacteria with carmine and later by aniline dyes. Dozen of dyes have come into

common use since these early experiments of Weigert. A dye is a colour organic compound which has the ability to combine with certain substances and to impart colour to them. It is possible to enhance staining ability by adding intensifers, wetting agents, depressants, carbolic acid (phenol). Staining is a chemical or physical union between the dye and component of the cell. If it is a chemical reaction, a new compound is formed, and simple washing in water does not liberate the bound dye. Many staining reactions are undoubtly a combination of physical and chemical unions.

Stains are classified variously on the basis of their characteristics as : simple and differential stains, Positive and Negative stains, Acidic, basic or neutral stains, specific stains, counter-stains etc. The preparations of stains are very simple or a complex process and also the same of their uses. Today hundreds of stains are available and the stains and stain techniques became, now a day most complex and modern technological procedures to help in our understanding in most modern disciplines like bacteriology, mycology, histochemistry, microbiology etc.

i) Lactophenol :

Lactic acid	-	100 ml
Phenol	-	100 g
Glycerine	-	100 ml
Distilled water	-	100 ml

It is used as mounting medium.

ii) 2% Aquaous KoH : KoH - 2 g
Distilled water - 100 ml

It is used for swelling dried material and Ionomidotic reaction (some fungi release purple dye like substance).

iii) Cotton blue : Cotton blue - 1 g
Lactophenol - 100 ml

It is used to study ascospore markings.

iv) Congo-Red : Congo-Red - 1 g
10% NH_4OH (aquaous) 100 ml
or
Congo Red - 1 g
2% KoH (aquaous) - 100 ml

It is used for differential staining for ascus wall layers, general wall stain and ascospore ornamentation.

v) Melzer's reagent: Iodine - 0.5 g
Potassium iodide - 1.5 g
Chloral hydrate - 20 g
Distilled water - 20 ml

It is general stain to test iodine reaction (J +ve or J -ve); amyloid nature of asci; iodine imparts yellowish brown colour to non-gelatinous hyphal walls, which helps to decide presence of thin septa in ascospores.

vi) Phloxine B dye : Phloxine B dye - 1.0 g
Distilled water - 100 ml

It is excellent cytoplasmic stain to distinguish septa in the ascospores.

Asci, paraphysis, ascospores, ascocarp and mycelium etc. were examined microscopically by mounting them in water to know the truest colour of spore wall and sap. The semi-permanent slides were prepared and sealed with sealent.

Method of preservation depends upon the nature of the organism. Fleshy and delicate forms were preserved by the usual laboratory preservative i.e. 5% formalin or 70% spirit or formalin-acetic-alcohol. Dried foliicolous and lignicolous forms were preserved in the paper envelop or paper boxes. Name of fungus, host family to which it belongs, locality, date of collection etc. were recorded and micropreparations were also labelled.

Metric units were used for macro and micro-measurements. Microscopic measurements were made by calibrated " Ernst Leitz Wetzlar " ocular by using 6X, 10X, 15X eye piecies and 10X, 45X and 100X objectives. Choice of the objective and eye-pieces were according to the size of fungal structure.

All camera lucida drawings have been drawn with the help of "Erma camera lucida" mirror type at stage level, using proper combination of eye-piece and objectives. The inking

of the plate was made by rottring pen using rottring camel ink and scale was marked.

Photomicrography of the semi-permanent slides were taken by using "Olympus PM-6 unit" with the help of proper combinations of objectives and eye-pieces. The specimen were also photographed as far as in their fresh and good form.

Identification of these fungi and their reports from Maharashtra State and India were confirmed with the help of following literature :-

Ainsworth, Sparrow and Sussman (1973); Bhide, Pande, Sathe, Rao and Patwardhan (1987); Bilgrami, Jamaluddin and Rizwi (1979); Butler and Bishy (1960); Clements and Shear (1981); Chen et al., (1987); Dennis (1968, 81); Desai and Patwardhan (1974); Ellis (1971, 76); Eriksson and Hawksworth (1987); Hirata (1966); Hosagoudar (1987); Kamat (1976); Kamat, Patwardhan, Rao and Sathe (1971); Kamat, Patel and Bhide (1949); Kamat, Uppal and Patel (1935); Kamat, Sheshadri and Pande (1978); Katumato (1975); Patil and Pawar (1986); Parbery and Langdon (1963); Parbery (1967); Rogersoh (1970); Rogerson and Samuels (1985); Subramanian (1986); Sawada and Yamamoto (1956, 57, 59); Tommerup (1970); Tai (1946); Thite and Patil (1986); Vasudeva (1962); von Arx and E.Muller (1975); von Arx (1970); Webster (1951); Yamamoto (1975).

All collected materials and micropreparations were properly labelled and have been deposited in the Mycological Herbarium, Department of Botany, Shivaji University, Kolhapur under the Code Number WIF (Fungi of Western India) and HCIO, New Delhi.

Some of the doubtful taxa were confirmed by sending the materials to an experts e.g. Dr. Ove E. Eriksson, Department of Ecological Botany, University of Umea, Sweden; Dr.Braun, U. Padagogische Hochschule "Wlofgang Ratke" Sektion Biologue/Chemie. WB. Botanik II, Germany and Rogerson, J.D., Department of Plant Pathology, Washington State University, Washington America.