C H A P T E R - 2

MATERIAL AND METHODS

This should go in chapter-3.

CHAPTER - 2

MATERIALS AND METHODS

Materials, methods, classification are adopted according to floristic and taxonomic investigation of Hymenomycetous (Basidiomycetous) Fungi as per Ainsworth (1973).

2.1 COLLECTIONS AND PRELIMINARY OBSERVATIONS:

The Hymenomycetous (Basidiomycetous) fungi studied in the present investigation were collected from various localities of Western Ghats of Sahyadri ranges, as well as from plain of Deccan plateau as follows :

Satara District - Satara, Satara timber market, Yawateshwar, Mahuli, Kas, Karad, Koyana-nagar, Shivasagar, Mahabaleshwar, Panchgani.

Poona District - Lonawala, Khandala

Raigad District - Panvel College campus, Panvel, Panvel timber market, Khopoli timber market. Khopoli, Karnala, Aranyeshwar.

The Hymenomycetous fungi were collected from these various localities are lignicolous, foli-colous, terricolous, humicolous, and obligate or facultative saprophytes.

The ecological features greatly influence the myco-

logical fungal flora from region to region and from locality to locality. The above mentioned localities were visited periodically from June 1987 to October 1988 throughout the year. Excursion were arranged mainly during the monsoon, from June to September as well as throughout the year whenever necessary. Fungal flora belonging to Hymenomycetes have been found to be mostly as lignicolous saprophytes on decaying twigs, branches, bark, wood, leaves (dead) and rarely terricolous or humicolous saprophytes. Saprophytic fungal flora of Hymenomycetous have been found to be more rich from the end of June to the middle of September and found to be abundantly and luxuriantly growing on various decaying substrates and humous rich soil during rainy season under the shade of forest trees as well as in open land areas. Most of the jelly and fleshy forms are ephemerals; therefore, regular and careful explorations were made from time to time. Jelly and fleshy saprobic forms occurring on decaying twigs, branches, wood, bark, leaves, were collected into polythene bags, plastic or glass containers, cardboard boxes etc. as per their nature along with their substrates; especially the members of the order Tremellales, Auriculariales, Dacrymycetales and Aphyllophorales. Identification of host and substrates have been confirmed by studying the morphology by the living plant parts in the vicinity which the organism were collected. Collection were carefully brought into the laboratory and a special care has also been taken to observe

their colour, shape, size, texture, consistency as well as other possible features and noted in the fields. The area under investigation, of course limited, but very rich as far as its mycoflora is concerned.

2.2 LABORATORY PREPARATIONS :

Specimens and their substrates were cleaned in laboratory and immature Jelly forms and some members of Aphyllophorales were placed in damp culture chambers (Petri-dishes, or larger glass containers lined with moist filter paper or under the bell Jars). The collections were examined by usual laboratory methods. Specific modified laboratory techniques were used for the order Tremellales, Auriculariales and Dacrymycetales. In these cases, most of the forms were studied by making observations of fresh specimens and important characters like texture, consistency, colour, size, growth pattern, place of growth, smell etc. were recorded and then further microscopic observations were carried in laboratory. A simple technique was used to study the micro-scopic details of the Jelly and fleshy forms by taking a minute piece or scraping of the superficial part of fructi-fication by a sharp blade with the help of needle and slides were prepared simply by teasing and squash method; while micropreparations of Aphyllophorales were made by free hand sections and stained with cotton blue. Better results were observed with mixture of cotton blue and slightly acidified 1% aniline blue in 50% glycerine, which stains nuclei, as well as basidia and other parts (Tu and Kimbrough, 1973),

then mounted in lactophenol and sealed with wax by steel rod. Preparations were carefully examined for detailed study of shape, size, ornamentation, hymeium, generative hyphae, skeletal-hyphae clamps, various types of basidia, epibasidia, sterigmata, basidiospores, ballistospores, conidia, conidiospores, paraphyses, dikaryophyses, cystidia, oleocystidia, gloeocystidia, hairs, incrustations, capillitium, setae etc. In some cases it was found better to make permanent preparations by usual laboratory method, rather than temporary preparations.

In addition to usual laboratory techniques, some specific chemical tests were also made for the specific fuhgal groups in fresh condition viz. 10% $FeSO_4$ solution for clavariod forms and corticoid Aphyllophorales, which makes greening of fructifications; 10% KOH solution for corticoid Aphyllophorales which dissolves the incrustation or warts of spores and makes swellings of hairs in specific genera; Melzer's Reagent was also used to confirm amyloid or inamyloid nature of basidiospores.

2.3 PRESERVATION OF MATERIALS :

Method of preservation depends upon the nature of organism. Jelly and fleshy forms were preserved by usual laboratory methods in 4% formalin, 70% spirt or in F.A.A. fixative. As well as some forms were dried for reconfirmation;

while other arid forms were preserved in dried forms. Specimens and their micropreparations were labelled properly and preserved in proper containers.

2.4 MEASUREMENTS, CAMERA-LUCIDA DRAWINGS AND PHOTOGRAPHY:

Metric units were used for macro and micro measurements. Microscopic measurements were made by calibrated 'Ernst Leitz Wetzlar occular' by using 10x, 20x, 40x 45x, 100x objectives and 5x, 10x and 15x eye pieces. Choice of objectives and eye pieces were according to the size of the fungal structure. All the camera lucida drawings have been drawn with the help of prism type of 'Erma Camera – lucida' (Mirror type) at a stage level. Most of the camera lucida drawings of jelly fungi were made by using 45x objective and 15x eye piece. Photography of the habit of the fresh specimens were made immediately after collections. Photomicrographs of the micropreparations were taken by using 'Olympus pm-6 unit' of photomicrography with the help of proper combinations of objectives and eye pieces according to fungal structure.

2.5 DESCRIPTION :

Hymenium hyphae, basidiospores, ballistospores, conidia, conidiospores, cystidia, gloeocystidia, oleocystidia, paraphyses, capitillium, peridiole, peridium, setae etc.

have been described by usual pattern. Basidial morphology of the order Tremellales, Auriculariales and Dacrymycetales show varied ranges of morphology viz. probasidia, metabasidia, hypobasidia epibasidia, sterigmata etc. Therefore, basidial terminology has been adopted according to Martin (1945). Donk (1958), Talbot (1965) as well as the recent concepts of later workers.

2.6 IDENTIFICATION :

Identifications were confirmed with the help of keys given by Ainsworth et al., (1973). Monographs on Tremellales and Auriculariales by Wojewoda (1977, 1981), Monograph on Clavaria and allied genera of Corner (1950) Domanski (1984) and uptodate scientific communication as for the author is aware of. The terminology was confirmed with 'Dictionary of fungi (Ainsworth, 1961). Latin diagnosis of proposed new species were made with the help of "A Mycological English-Latin Glossary" by Cash, E.K. (1965). Some genera and most of the species belonging to the order Tremellales, Auriculariales, Dacrymycetales are new to India; as well as poorly reported from different countries.

Most of the forms studied in the present investigations have been described in details and some were only recorded and listed alphabetically. All the collected materials and micropreparations (slides were properly labelled and sketches,

(camera lucida drawings) have been deposited in the Mycological Herbarium, Department of Botany, Yashwantrao Chavan Institute of Science, Satara under the code WIF Nos. (Fungi of Western India).