C H A P T E R III

MATERIAL AND METHODS

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All the dung samples used for isolation of fungi in the present work were collected by the auther himself. Collections were made from various localities viz. Radhanagari, Kas, Sangli, Satara, Amboli, Kolhapur and around the Kolhapur paying frequent visits to different places during different seasons. The dung samples of Cow, Buffelo, Rabbit, Goat, Donkey, Horse, Dog, Fox, Ox, Samber, Deer, Valture, Sparrow, Rodents, Pea-cock, Turtle, Zebra were collected. Podospora observed on dung of Cow, Rabbit, Goat, Donkey and Horse. Dung samples were collected separately either dried or semi-dried or fresh as well as from dung hill in polythene bags and brought into laboratory and were incubated seperately in duplicate in moist filter-paper in petriplates or under the bell-jar at room temperature as per the size of droppings.

Each sample was observed for the growth and development of the fungi daily. Natural droppings incubated under moist chamber generally produced fungi from surface of the droppings or slightly bellow the surface layer but from the interior if spores were present never get a chance to germinate due to different reasons. Therefore dry-droppings were crushed into powder, powder kept in petriplates, then moistened and then kept for incubation. But this technique gives very poor yield of fungi, even though kept for 2-3 months.

As per growth of the organismps on dung or individual pellet observing under binocular or hand lense removed from the petriplate and carefully the fungus was removed by forcep and needle or glass slide in water for clearing the fungus or fruiting. During the temparary micropreperations cotton blue is used as stain, attension also had been given to note the position of the ascocarps in or on the substrate, gross morphology, shape, size, presence or absence of hairs, setae etc. These primary observations were made in laboratory by hand lens or under binocular or dissecting microscope.

Before the use ofcotten blue the first stain used is Iodine. This is best supplied in the form of Melzer's reagent which is made as follow :-

Potassium Iodide ... 1.5 g.

Iddine ... 0.5 g.

Distilled water ...20.0 ml.

Some times chloral hydrate hydrate is added to this reagents which act as clearing agent. It gives blue cclour to the mycelium and ascus tip of some members of the Ascomycetes. Especially in the identification of the pezizales. It also imparts a yellowish-brown-colour to the nongelatinous hyphal walls in general and may help to decide the presence of thin septa in ascospores.

Coprophilous fungi (especially Ascomycetes fungi) were cultured on various culture media viz. Leonian's yeast extract agar, Oatmeal agar (with wheat germ powder agar), potato-carrot agar (PCA) and potato-dextrosa agar (PDA). The species of Podospora were tried to culture on the above media but didnot yield good results.

For the study of appendages Black-India ink is suggested but in our present investigation did not give + ve results.

The slides were made semi-permanent by staining them by cotton blue. All the drawing have been drawn with the help of prism type of"Erma" camera-lucida at the stage level using 10 X, 40 X, objectives and 5 X, 10 X , 15 X eye pieces. Choice of objective and eye piece were according to the size of the structure (Perithecia, asci and ascospores). Measurements were made by Ernst-Leitzwetzlar occular.

Identification of the species of the genus Podospora were carried out by the most upto date recent literature available.

Microphotography were made by using Olympus PM-6 unit of photomicrography by 5 X, 10 X, 15 X eye pieces and 10 X,40 X, 100 X objectives, which were based on the size of the specimens.

All the specimen studied in this work were labelled under the code number WIF.(Fungi of Western India), Materials micropreperations are deposited in the Mycological Herbarium,

Botany Department, Shivaji University, Kolhapur (Maharashtra, India) under WIF.Nos.

FAMILY SORDARIACEAE

Family sordariaceae is characterised by perithecia either produced on dung or saprophyte on wood, bark or plant debris.most of the genera are coprophilous in nature amongs which Podospora is one of them.

Ascospores are with or without appendages, one or two celled, germpore is apical or basal. It includes 22 genera (Dennis 1981).

GENUS - PODOSPORA CESATI

Hedwigia 1 : 103, 1856.

The genus Podospora was established by Cesati in 1856. It belongs to the family sordariaceae of the order sphaeriales. The genus Podospora characterized by ascospores distinctly clavate, at first hyaline, cells longer than broad, mostly cylindrical, matured spores dark-brown or black opaque, germ pore is apical and circular, a hyaline basal cell without visible content and hyaline with gelatinous dense and light refractive appendages, occasionly the upper end also provided with appendages. In some species the gelationous appendages are reduced, absent or appears as partial gelationous Sheath (Lundquist, 1970).

The ascus apex is funnel shaped, the base of the funnel is connected with the ascus lumen by a pore which can be surounded by a cushion-like structure included in the thickened wall; Perithecia scattered, sunkan or superficial in the substrate, ostiolate, wall of the perithecium dark, opaque, britle, smooth, bulbous, hairy. All species are saprophytic either growing on or in the dungs of various animals i.e. coprophilous or on dead herbaceous organic matter.

The genus Podospore Cesati is known by its 107 valid species out of these, 101 are coprophilous and remaining non-coprophious. Six species have been known from India and single from Maharashtra State.

Sixty four species of Podospora Cesati have been key out by Mirza and Cain (1969) on the basis of characters viz. Peridium, type of hairs, number of spore per ascus & their arrangement, ~~nature~~ size and shape of ascospore, nature of appendages (primary and secondary).

Type species :- P. fimicola Cesati