MATERIALS AND METHODS

Area under coverage: In the present investigation, collections were made mainly from the different localities of Satara district. Western part of Satara district is botanically rich viz. Mahabaleshwar, Jarendeshwar hills, Janai-malai hills, Pateghar, Yevteshwar, Kas, Koyana-back water areas etc. The part of Western Ghats in Satara district is with variety of biodiversity of plant species including fungi.

Collection: Systematic collection provides a basis for taxonomic research. There are different methods used for collecting different fungi. The fungi may have different types of habits as foliicolous, lignicolous etc. Some may be parasites or saprophytes. In present investigation, the more attention is given towards the foliicolous, ectoparasitic fungi and like wise, the method of collection has been adopted. The infected leaves, were collected and brought in the laboratory for further study. The fungi, which have been studied here were collected throughout the year. Extensive and repeated excursions were made by periodical visits to the different localities in different seasons. The materials were collected and properly placed in the paper or polythene bags. 10x field hand lens was used. The host plants were also collected separately along with their flowers and fruits. The necessary informations such as parasite, colour, habit, shape, date of collection, type of forest, locality etc. was also recorded at the time of collection in the field note-book. The materials were brought into the laboratory and were pressed in the blotting papers with field press, for further study. The fresh leaf materials were photomicrographed with their fungal parasites.

Laboratory observations: Each leaf was thoroughly and carefully observed under the binacular dissecting microscope, so as to know the morphology, colour, distribution of colony and also the position of the ascocarps or the colony i.e epiphyllous, hypophyllous or amphigenous nature (habits), were noted. The detailed microscopic observations were made by preparing semipermanent micropreparations either in water or in lactophenol as mounting medium. The morphological features such as size, shape, colour of the ascocarp, mycelium, hyphae, setae, ostiole, pseudo-ostiole, asci, ascospores and pseudoparaphyses, conidia, conidiophores, acervuli etc. were recorded.

The bitunicate nature of the ascus has got more importance in order-Dothideales. Luttrell (1973), Barr (1979), von Arx and Muller (1975) and Eriksson (1984) have used this character in their classification to differentiate from rest of the Ascomycetes fungi. Therefore, this feature has been carefully studied by mounting the asci in water or staining them with the different stains.

Funk and Shoemaker (1967), characterized the two layers of the ascus as:

a) Outer layer (Ectotunica or Exoascus)

b) Inner layer (Endotunica or Endoascus)

a) Outer layer: It is also called as ectotunica, or exoascus. It is thick, rigid but easily broken. This layer has different property of light transmission than the inner one and remains unstained with either basic or acidic or neutral dye. It is protective in nature and has a little role in ascospore discharge.

b) Inner layer: It is also called as endotunica or endoascus. It is thin. It has a property of staining with different stains as 1% Congo red reagent etc.

For the study of these two layers of the ascus the asci were mounted in water and were also stained with 1% Congo red and cotton blue.

The different stains and the staining techniques used for the study of the internal structure. The colouring agents used in the microtechniques are of various kinds but majority of them are dyes so called as stain. They stain tissue, cells, cell components or cell contents.

Mycological stains: The mycological stains are used to stain different fungal structures such as mycelium, asci, ascospores, conidia, conidiophores, pseudoparaphyses, periphyses, cells of ascocarp etc. In the present investigation different stains have been used as per the nature of fungus structure. The bitunicate asci, ascus tips, ascospores, pseudoparaphyses, mycelium, hyphae, septations of ascospores, colour of conidia etc. are important and stained with cotton blue and Congo red.

The different stains and mounting medium used with their composition are as follows:

1) Lactophenol: It is used as a mounting medium. Semipermanent slides were made in Lactophenol and also used for emphasizing hyphal, spore characteristics.

Components used in the preparation of mounting medium:

Lactic acid- 20.0 ml.

Phenol crystals- 20.0 g.
Glycerol- 40.0ml.
Distilled water- 20.0ml.

2) Cotton blue: It is an acidic stain used to study fungal structures. It is a cytoplasmic stain. The cytoplasm turns blue in colour leaving the hyphal or spore wall and the septa hyaline. Therefore, the size, shape, septation, structure of the ascospores, conidia and mycelium can be studied. The bitunicate nature of the ascus can be seen with this stain.

Components used in the preparation of stain cotton blue lactophenol are:

Lactic acid- 20.0 ml.
Phenol crystals- 20.0 g.
Glycerol- 40.0 ml.
Distilled water- 20.0ml.
Cotton blue (1% aqueous)- 2.0 ml.

3) Congo red: It is an important stain used to differentiate the two layers of the bitunicate ascus by colouring it. It is an amylloid stain and when treated, the inner layer of the ascus wall colour pink or red.

1% Congo red was prepared by dissolving 1g of Congo red in 100ml of distilled water. One drop of the stain was applied in the micropreparations. After few minutes, it gives good results.

To study the nature of association of the parasite on the leaf, it is necessary to take transverse section of both host and parasite. The internal structure of the ascocarp i.e. the arrangement of asci, pseudoparaphyses, hymenium, internal mycelium, attachment of conidia to conidiophores can be seen only in the vertical sections. These structures were studied by the hand cut thin sections of the ascocarps passing through the host leaf and morphological structure viz. pseudoparaphyses, asci and their arrangement of conidiophores etc. were studied.

Measurements: The microscopic measurement were made with the occular micrometer. Ernst Leitz Wetzlar occular was used. The occular scale was calibrated with the stage micrometer and was reduced to 1division. Thus, each eye piece of 5x, 10x, 45x and 100x magnifications were used in combination with objectives and as per the nature of the fungus.

Description: All the characters which are useful in the classification and were distinctive such as mycelium, hyphae, hyphopodia, septation, cells of pellicle and their ornamentation, colour, size, shape and cells of ascocarp, shield, ostiole, pseudo-ostiole, setae, asci, ascospores, pseudoparaphyses, conidia, conidiophores and acervulus etc. have been described as per the mycological terminology adopted by Butler and Bishy (1960) and Snell and Dick (1957).

Identification: Latest nomenclature and identification of fungi and their hosts have been adopted. The scheme of classification proposed for the order-Dothideales by von Arx and Muller (1975), has been followed with the addition of modern taxonomic concepts of Barr (1979) and Eriksson (1984). There is a great controversy regarding the status of the different genera. Luttrell (1973) classified the different orders, on the basis of character as bitunicate asci, ascostroma and ascospores.

For the study of Deutromycetous fungi various schemes proposed by Saccardo's (1886), Ellis, M.B. (1971 and 1976), Barnet, H.L (1973), Ainsworth, G.C. (1973), von Arx and Muller (1974) and Patil, M.S. et al. (2006) etc. were used.

Illustrations:

i) Camera Lucida drawing: All the drawing of micropreparations were drawn with the help of prism type Erma (Japan) Camera Lucida at stage levels using 5x,10x,15x eyes pieces.

ii) Drawing and text plates: The drawing of the sketches made on the Ivory sheets of A_4 size of paper with the help of pencil and later on it was inked with rotring pens of various thicknesses of 0.1 mm to 0.3 mm. Pigmentation if present, dark or faint was presented by splitting. Thus, all the figures, sketches were made so as to reflect the natural presentation of the taxa. The scales are directly drawn near each figure.

iii) Tables: Tables are valuable in the taxonomic studies for summarizing the statistical data of the taxa. In the present investigation, tables were made showing the comparision between the original species and the present collection, wherever the new species or a new variety has been proposed, as well as to summarize the fungal systematics pattern.

iv) Photomicrography: The photomicrography of the material shows the natural habits on the substratum. The close-up photographs are taken to clear the structure of sketches to give the real idea of habits. The photomicrography was done with the help

of Nikon camera model E8-400 at Department of Botany, Shivaji University, Kolhapur. The photomicrography was done with the help of 5x, 10x and 15x magnifications of eye piece in combination with 5x, 45x and 100x or oil immersion. Asci and ascospores were mostly photomicrographed under oil immersion to see the details.

v) Citations and References: In citing the reference in journals,generally the list of the references or bibliography is given at the ends in which it includes title of article, date, volume or first and last pages are given. Journal is given in the italic type, volume in bold type and the part numbers following the volume in the normal type. In the present investigation, the references which are cited in the work have been listed. The system has been followed here as Author's name was followed by fore name (initials), the year and then the title of the article following the journal abbreviations, the volume number and the pagination. The journal abbreviations are underlined and volume number has been double underlined. All the volumes, pagination, title have been checked and confirmed.

Deposition of the Material: Herbarium of the collected material was done. The materials were placed in the packets. The hosts were identified and confirmed with the help of recent taxonomic literature as far as the author is aware. The packets were properly labeled with all the information regarding the taxon such as class, order, sub-order, family, genus, species, host, locality, name of collector and date of collection. They were arranged as genera, family order and were deposited in Mycological Herbarium, Botany Department, Yashavantrao Chavan Institute of Science, Satara under the code number of (M.H.B.D.Y.C.I.S. Satara). They were numbered from1 to 31.

Micropreparations: The semipermanent micropreparations were cleaned, properly labeled with black Indian Ink and arranged genus wise in wooden cabinets and were deposited in Mycological Herbarium, Botany Department, Yashavantrao Chavan Institute of Science, Satara.