Materials and Methods

"Western Ghats" is a continuous source of water and also is rich in vegetation. Western part of Satara is a part of Western Ghats. Satara district is with number of water bodies viz. various streams, rivers, lakes, ponds and ditches which are full of water throughout year. In present investigation, aquatic fungi around Satara were studied. Visits were made fortnightly, to different water bodies in and around Satara during different seasons, viz. Kas, Yavteshwar, Krishna river and pond like Moti talav, Mangalwar talav, Phutaka talav and Godoli lake. Also frequent visits were made to the different streams of Mahabaleshwar (Lingmala fall), Venna lake, urmodi river at Pateghar etc. The various groups of Aquatic fungi, were isolated and studied and preserved.

1. Collection of foam samples:

An excellent source for the isolation of aquatic fungi is, collection of foam samples in streams and rivers. Usually, fungi develop around barriers behind fallen logs and contain hundreds of spores. Air bubbles are present in fast or slow flowing streams which are collected at the edges of the river and streams. Clean wide mouth glass bottles were used for collecting the foam samples. The fresh, natural foam is white in colour and after agitating the foam, a small amount of cream coloured solution remains. The few ml of foam samples were placed in the petriplates containing agar medium and remaining foam samples were fixed in 5% FAA fixative which arrest the germination of conidia 5 %. FAA is also used as preservative, [5 ml, Formaldehyde; 5 ml Acetic acid; 90 ml of 90 % Ethyl alcohol].

2. Collection of litter samples:

The submerged litter samples, viz. petiole, barks, leaf, dried flowers, fruits, damaged roots, wood pieces etc. were collected separately from the water bodies in clean polythene bags. The samples were rinsed thoroughly in water before placed in the bags. The necessary informations viz. colour, habit, date of collection, locality and diam., of colony etc. was also recorded. These samples were brought into the laboratory.

3. Collection of water samples:

The water samples were also collected in clean bottles from various localities. They were collected from different depths of water from surface downwards. These samples were found brought into the laboratory.

4. Laboratory Observations:

1. The foam samples were thoroughly and carefully observed under microscope in the laboratory. Different types of conidia, conidiophores, mycelium etc. were observed under the microscope. Then the detailed microscopic observations were made by preparing semipermanent micropreparations either in water or in lactophenol, as a mounting medium. They were stained with 1 % cotton blue stain. Depending on season, quality of water, kind of litter and physico – chemical features of the surrounding environment, the number of conidia present in unit volume of water, found change (Bhat, 1998).

2. The litter samples were placed in clean petriplates containing 10 ml water samples, collected from the same locality of the litter sample and was incubated at 20^{0}c or at room temperature for 4 - many days. Then, the detailed microscopic observations were made by preparing semipermanent micropreparations, stained in 1 % cotton blue and mounted in lactophenol, as mounting medium.

3. Baiting technique: The water samples from different localities were brought in laboratory. The baiting technique was used. 10 ml of the collected water sample was added to each petridish. Various baits were used. They were first sterilized, with distilled water and also autoclaved. These baits were put in each petridish. They were incubated at 20^{0}c for 4 - 14 day. Autoclaved insects viz. cockroaches, ants, boiled been seeds, autoclaved wood pieces, collected in submerged water were used as baits. Apart from these, the other baits were also used viz. human hair, Drosophila fly, boiled hemp seeds and grass leaves etc. (Sparrow, 1960). Fungi which grown, on these baits were collected and semipermanent slides were prepared.

5. Culture technique:

The aquatic fungi or spores were observed from the litter and foam samples, baits and water samples. These aquatic fungi were transferred to the culture media for study of growth (pure culture). The foam samples which were poured, on petriplates and growth was observed under microscope. Other samples, were again transferred to a fresh media for proper culture medium for fungal growth. Various types of culture media were used, viz. PDA (Potato Dextrose Agar medium), Sabouraud's Agar medium, CDA (Czapeck Dox Agar medium), 0.05 % Agar medium and MEA (Malt Extract Agar medium) etc. Among, these MEA was found be ideal for the growth of aquatic fungi. After few days, the aquatic fungi sporulate. Fungal spores were very peculiar with their morphology and distribution.

The components of various media are as follows:

1. **Potato dextrose Agar medium (PDA):**

   Potato - 20 g.
   Dextrose Sugar – 20 g
   Agar Agar - 14 g
   Distilled water - 1000 ml

2. **Sabouraud's Agar Medium:**

   Peptone – 10 g
   Dextrose - 40g
   Distilled water - 1000 ml

3. **0.05% Agar medium:**

   Agar Agar - 15 g
   Distilled water - 1000 ml

4. **Czapeck Dox Agar medium (CDA):**

   $NaNo_3$ - 0.75 gm
   K_2HPO_4 - 0.25 gm
   Mg $SO_4.7H_20$- 0.125 gm
   KCl - 0.125 gm
   $FeSO_4.7H_2O$ - 0.0025 gm
   Sucrose - 7.5 gm
   Agar Agar - 3.75 gm
   Distilled water – 250 ml.

5. Malt Extract Agar medium (MEA):

Malt Extract - 10 g

Agar Agar - 20 g

Distilled water- 1000ml.

6. Mycological stains:

The mycological stains are used to stain different fungal structures such as mycelium, conidiophores, conidia, their appendages, setae, conidiogenous cells etc. Aquatic fungi are filamentous (particularly hyphomycetes) and mostly moniliaceous conidia forming fungi. Almost all conidia, are colourless or hyaline. In the present study, stain has been used as per nature of fungus structure. Composition of stain used to study the fungi, is as follows:

1. Cotton blue:

It is an acidic stain used to study fungal structures. It is a cytoplasmic one. The cytoplasm turns blue in colour, leaving the hyphal or spore wall and the septa hyaline. Therefore, the size, shape, septation, structure of conidia and mycelium can be well marked and studied.

Composition of cotton blue in lactophenol is:

Lactic acid - 20.0 ml

Phenol crystals - 20.0 g

Glycerol - 40.00 ml

Distilled water - 20.0 ml

Cotton blue 1 % aqueous – 2.00 ml.

2. Lactophenol :

It is used as a mounting medium. Semi permanent slides were made in lactophenol and also used for emphasizing hyphal, spore characteristics.

Composition of lactophenol is as follows:

Lactic acid - 20.0 ml

Phenol crystals - 20.0 g

Glycerol - 40.0 ml

Distilled water - 20.0 ml

7. Measurements:

The microscopic measurements were made with the ocular micrometer. "Ernot Leitz Wetzlar" ocular was used. The ocular scale was calibrated with the stage micrometer and was reduced to 1 division. Thus, each eye piece of 5X, 10X and 15X magnifications, was calibrated with the objectives of 5X, 10X, 45X and oil immersion. Both of them were used in combination, as per the nature of the fungus.

8. Description:

All the characters, which are useful in the classification are distinctive, viz. shape, size, colour, position and other morphological characters of colony, mycelium, hyphae, setae, conidia, septation, position of conidia, conidiogenous cells and conidiophores etc. have been described as per mycological terminology stated by Hawksworth, 1991.

9. Identification:

The 'aquatic fungi' is the only ecological group in the fungal kingdom where, one can comfortably identify the fungus upto species level, simply by examining the conidia [Ingold,1975; Bhat,1998]. Latest and recent literature is used for the nomenclature and identification of the fungi and their hosts. The scheme of classification proposed by Ingold (1942), Ellis (1971), Barnett and Hunter (1973) and von Arx and Muller (1975), has been followed with the addition of modern taxonomic concepts of Alexopoulos and Mims (1979).

10. Illustrations:

a) Camera lucida drawings:

All the drawings of micropreparations were drawn with the help of prism type 'Erma' camera Lucida at stage level using 10 X, 45 X, 100 X or oil immersion objectives and 10 X, 15 X, eyepieces.

b) Drawings 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 pen of various thickness of 0.1mm, 0.2mm and 0.3mm diam., etc as per nature of the part, to be sketched.

Pigmentation, if present, dark or faint, was represented 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.

c) 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 comparison between the 'types species' and the present collection. Where ever, the new variety has been proposed, as well as to summarize the floristic pattern.

d) Photomicrography :

The photomicrography of the material shows, the natural habits on the substratum. The photomicrography was done with the help of 'Nikon" automatic unit of photomicrography, at the department of Botany, Shivaji University, Kolhapur. The photomicrography was done with the help of 10X, 15X, magnifications of eye piece in combination with 10X, 45X, 100X, or oil immersion. Conidia, conidiophores, mycelium etc. were mostly photomicrographed, under oil immersion to see the details.

e) Citations and References:

In citing the reference in journals, generally the list of the references or bibliography is given at the end of the dissertation in which, it includes title of article, year, volume then first and last pages are given. Journal is cited in Italic type, volume in bold type. In the present investigation, the references which are cited in the work, have been listed. The system has been followed here as, 'Authors 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, paginations, title, have been checked and confirmed. All the references, have been properly arranged, year wise and alphabetically.

f) Preservation and deposition of the materials:

The foam samples were preserved in 5 % FAA. It prevents the germination of conidia. While, the other samples were preserved in the 4 % formaline. Each bottle was marked well and placed in boxes. The bottles were properly marked with all the

information regarding locality, date of collection, order, family, genus, species etc. 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 form 1 to 20.

11. Micropreparation: The semipermanent micropreparations were cleaned, properly, labelled with black India ink and arranged genus wise in wooden slide boxes and were deposited in Mycological Herbarium, Botany Deapartment, Yashvantrao Chavan Institute of Science, Satara (M.H.B.D.Y.C.I.S.Satara). They are number form 1 to 20.