CHAPTER - 2

REVIEW OF LITERATURE
Taxonomy of fungi has drawn the attention of mycologists for more than last two centuries and various systems of classifications have been proposed by authorities based on different criteria. In earlier systems of classification, the prime importance was given to the morphology of fungi and similar morphology were placed together. Therefore, the classification systems based on morphological variations are simple and convenient in application at various levels. As research progresses and more modern techniques are easily available in different laboratories, morphological criteria are replaced by molecular or ultra structure criteria for classification and phylogeny of fungi. The present chapter deals with the taxonomic and ecological review of zoosporic fungi with focus on saprolegniaceous aquatic organisms. Chart 2.1 gives outline of classification with respect to zoosporic fungi. Modern researchers have provided a natural scheme of classification representing phylogenetic relationship (Leclerc et al., 2000; Cooke et al., 2000). The Phycomycetes were treated as a unit and constitute the lowest of the three primary subdivisions. The name Phycomycetes means "algal fungi" often showing many similarities to algal forms in their life cycles and habitats.

Taxonomists like Hogg (1860), Haeckel (1878), Copeland (1956), Whittaker (1969) have made significant contribution by proposing various systems of classification. Copeland (1956) established a four-kingdom system in which he placed fungi with algae and protozoa in the kingdom Protista. Whittaker (1969) revised and simplified Copeland’s system by proposing a five-kingdom system. He classified the zoosporic fungi under the subdivision 'Mastigomycotina' of the kingdom fungi. Ainsworth (1966) treated fungi either as a separate kingdom or as a subkingdom of the plant kingdom with two divisions: the Myxomycota for plasmodial forms, and the Eumycota for non-plasmodial forms, which are frequently mycelial. Generally all coenocytic, aquatic,
semiaquatic or terrestrial fungi with zoospores produced in thin walled sporangia are included in Phycomycetes. However, Sparrow (1958) pointed out the importance of the structure of zoospores and recognized the four groups as follows:

1) Chytridiomycetes
2) Hypochytridiomycetes
3) Plasmodiophoromycetes
4) Phycomycetes

He further stated that Phycomycetes are not a homogenous, monophyletic group but rather an artificial category or group of fungi of highly discordant elements. He traced out interrelationship within zoosporic Phycomycetes. Though there is a controversy regarding the criteria for the classification of fungi and more specifically for that of the Phycomycetes, taxonomic arrangement is made according to the system established by Ainsworth (1973) in the present work. Ainsworth (1973) in ‘The Fungi’ divided the Mastigomycotina based on flagellation of zoospores:

Class I Chytridiomycetes
   Order 1) Harpochytriales
          2) Chytridiales
          3) Blastocladiales
          4) Monoblepharidales

Class II Hypochytridiomycetes
   Order 1) Hypochytriales

Class III Oomycetes
   Order 1) Saprolegniales
          2) Leptomitales
          3) Lagenidiales
          4) Perenosporales
In some cases, oomycetes are grouped with the ‘higher’ fungi (Whittaker, 1969) because of their filamentous growth, and because they feed on decaying matter like fungi. It was believed that they are merely degenerate algae, which, along with the assumption of the parasitic or saprophytic habit, have lost their ability to synthesis chlorophyll. Margulis and Schwartz (1982) later on classified all zoosporic fungi with algae and protozoa in the kingdom Protoctista, which is separated from zoymycetes and ‘higher’ fungi in the kingdom Fungi. In contrast, phylogenies inferred from similarities in cell wall composition and other biochemical markers suggest that fungi are at least diphyletic (Barfnicki-Garcia, 1970,1987). One line consists of oomycetes and hyphochytriomycetes; a second line contains the chytridiomycetes, which are thought to be the ancestors of zygomycetes, ascomycetes and basidiomycetes. In yet another scheme, based on their similarities with algae, oomycetes and hyphochytriomycetes have been separated from other fungi and together with chrysophytes, xanthophytes, phaeophytes, chloromonadophytes and diatoms put into one of the four plant kingdoms, the Chromophyta (later named Chromista; Cavalier-Smith. 1981,1986). Dick et al. (1984), divided Peronosporomycetes into subclasses Peronosporomycetidae (oomycetes with a translucent, solid ooplast) and Saprolegniomycetidae (oomycetes with a granular, fluid ooplast). Later on, a new taxonomic system for the Peronosporomycetes has been proposed by Dick (1995), in which he divided them into three sub classes, the Saprolegniomycetidae, Rhipidiomycetidae, and Peronosporomycetidae. His ordinal classification was based on morphological and ultrastructural characters, e.g., oosporogenesis, oospore wall, and protoplasmic structure of the oospore.

Though role of morphology in classification can not be denied, recent studies on various aspects showed that oomycetes did not belong to fungi (Dick, 2001). The recent grouping of oomycetes in kingdom Chromista (Stramenopila) arose largely from molecular studies which categorically confirmed that algae (previously referred to as Heterokonts or Chrysophytes) were related to a variety of non algal Protists - such as the heterotrophic bicosoecid flagellates and the fungal Oomycetes (Leipe et al., 1994).
Chart 2.1. Classification Outline of Some Fungi and Fungal-like Protists

This abbreviated classification is patterned after the broad scheme of Margulis and Schwartz (1987). The classification ranks below Phylum reflect interpretations of Hawksworth et al. (1995), Koch (1966) and Hanlin and Ulloa (1988).

Kingdom: Chromista (collection of eukaryotic heterokont, biflagellate microorganisms, which includes two fungal-like groups that are not currently considered to be part of the Kingdom Fungi)
- Phylum: Hyphochytridiomycota (hyphochytrids)
  - Class: Hyphochytridiomycetes
  - Order: Hyphochytridiales

- Phylum: Oomycota (egg-bearing aquatic phycomycetes)
  - Class: Oomycetes
  - Order: Saprolegniales (water molds)
  - Order: Leptomitales
  - Order: Lagenidiales
  - Order: Olpidiopsidales
  - Order: Peronosporales (damping-off fungi, white rusts, downy mildews)

Kingdom: Fungi [collection of eukaryotic microorganisms, which includes four or five Phylums that are mostly amastigote (lack flagella except for the Chytridiomycota) and usually form spores during their life cycle]
- Subkingdom: Mastigomycotera (flagellate sporangial fungi flagellate lower fungi, flagellate phycomycetes; aquatic phycomycetes)
- Phylum: Chytridiomycota (chytrids, the posteriorly unflagellate fungi)
  - Class: Chytridiomycetes
  - Order: Chytridiales
  - Order: Spizellomycetales
  - Order: Blastocladiales
  - Order: Monoblepharidales
  - Order: Neocallimastigales
Fungi belonging to Mastigomycotina (zoosporic fungi) form the prevalent group of fungi along with conidial fungi among aquatic fungi. The zoosporic fungi were found in diversified habitats. They are either saprophytes or parasites. All these fungi have been arbitrarily brought together in this sub-division on the basis of zoospore. The thallus of fungi varies from unicellular with or without rhizoid to form an extensive filamentous coenocytic mycelium.

Chytridiomycetous fungi occur as saprobes on plant and animal remains in water while some members being parasitic on algae and aquatic animals. No species monograph that includes all orders of the Chytrids has yet replaced that of Sparrow (1960). Longcore (1996) published a bibliography that included information on taxonomic changes since Sparrow’s Aquatic Phycomycetes. Dick (1976), Usha Kiran and Dayal (1980), and Manoharachary (1981) have compiled useful information on the taxonomy, morphology, ecology and physiology of these fungi. Members of Chytridiomycetes are characterized by extreme simplicity of form and structure, and are regarded as the lowest of all fungi. True mycelium is lacking. Rhizoids are present. Many of the Chytrids are parasitic on algae.

**Salient features are:**

**Chytridiomycota:**

Examples: *Allomyces, Olpidium, Rhizophlyctis.*

**Main distinguishing features:**

- **Somatic stage:** Typically unicellular or dichotomously branched chains of cells with tapering rhizoides for anchorage or absorption. Usually haploid. Wall composed of chitin and glucans.

- **Asexual reproduction:** By zoospores. In some cases the whole body (thallus) converts into a sporangium (e.g. *Rhizophlyctis*), in others the sporangia form on part of thallus.

- **Ecology and significance:** The Chytridiomycota are divided into the predominantly unicellular types (Chytridiales) and the (Blastocladiales) with usually branched chains of cells attached to a substrate by rhizoids (e.g. *Allomyces*). They accumulate on ‘baits’ such as pollen grains, insect...
exoskeletons etc. A few species are serious plant pathogens e.g. *Synchytrium endobioticum* which causes potato wart disease. *Catenaria anguillulae* parasitizes nematodes and fungal resting spores in soil. *Coelomomyces* spp. parasitize mosquito larvae and are of interest as biocontrol agents (Federici, 1995/Shoulkamy, 1997).

The thallus of Hypochytridiomycetes is holocarpic or eucarpic, monocentric or polycentric and their vegetative system is rhizoidal or hypha-like with intercalary swellings. This group of fungi is parasitic on algae and other fungi. Sparrow (1960) has discussed various aspects of Hypochytridiomycetes.

The Oomycetes contain 95 genera and 694 species (Hawksworth et al., 1995) which are mostly aquatic living either as parasites or saprophytes. These include the so-called watermoulds and downy mildews. The Oomycetes are the group of heterotrophic Stramenopiles (Dick, 2001). Members of Oomycetes are characterized by biflagellate zoospores. The spores in the lower form are usually motile (zoospores or swarm spores), but in the higher, they are usually non-motile (aplanospores). In some genera like *Achlya, Saprolegnia* an additional asexual spore form is present. These spores are termed as "gemmae or chlamydospores". It occurs chiefly on old thalli, and are merely vegetative cells with thickened walls. Most of the members of this class are typically aquatic, either free living or parasitic on algae, water mould, other forms of aquatic life. Members of *Leptomitales and Saprolegniiales* and other Oomycetes are important as they help in decomposition of organic matter, food chain cycle and productivity besides being pollution indicators and disease causing agents. They are found all over the world in fresh and salt-water habitats. Some of the terrestrial Oomycetes are among the most important plant pathogenic organisms that may be facultatively or obligately parasitic. The organisms range from those preferring well-aerated streams to some that occur in stagnant waters and that may gain most of their energy from anaerobic metabolism. Most members of the group are filamentous and lack septa except where reproductive cells are
produced, but holocarpic forms are found in the Saprolegniales and Lagenidiales.

The cell walls of the group consist mostly of β-1,3- and β-1,6-glucans with a small amount of cellulose. A few members also have chitin deposits. The group is characterized by oogamous reproduction that includes interaction of male antheridia with female oogonia. The main distinguishing features of Oomycota are;

**Oomycota :- the cellulose - walled fungi**

Examples : Achlya, Phytophthora, Pythium, Saprolegnia.

**Main distinguishing features :-**

- **Somatic stage :-** Mainly aseptate, diploid with a wall of cellulose and other glucans (polymers of glucose).

**Ecology and significance :-** The Oomycota contains at least four major subgroups. The Saprolegniales (watermoulds) includes species of Achlya and Saprolegnia that are common saprotrophs in fresh water habitats, but S. diclina and S. parasitica are important parasites of salmonid fish (Neish, 1977). The closely related Aphanomyces sp. are aggressive root pathogens, but A. astaci is a pathogen of crayfish. The Leptomitales is a small group of aquatic fungi, such as Leptomitus lacteus, which is common in sewage-polluted waters. The Lagenidiales is a small group of parasites of plant roots or parasites of algae, fungi or invertebrates (e.g. L. giganteum on nematodes, mosquito larvae etc.). The Perenosporales is the most important group, because it contains many serious plant pathogens (e.g. Pythium spp.). Also in this group are downy mildew pathogens that are obligate parasites.
ECOLOGY OF AQUATIC FUNGI:

Sparrow (1968) and Park (1972) have emphasized the importance of mycological studies of aquatic habitats. Considerable attention was given to study the ecology of aquatic fungi from various countries (Lund, 1934; Perrott, 1960; Willoughby, 1962; Hughes, 1962; Cooke, 1965; Sparrow, 1968, 1973; Alabi, 1971; Hunter 1975; Czeczuga, 1997; Steciow, 2002). Though considerable efforts have been made in this regard, these are mostly confined to the studies in lacustrine ecosystems (Fox and Wolf, 1977; el-Nagdy and Abdel-Hafez, 1990; Czeczuga and Muszynska, 1993). Therefore, relatively little is known about the effect of various physico-chemical factors on the distribution and occurrence of watermoulds in an open ecosystem (Maestres and Nolan, 1978; Barlocher, 1992; Steciow, 2001). Though much work has been done on the watermoulds of India (Dayal and Tandon, 1962, 1963; Khulbe and Bhargava, 1977; Manoharachary, 1981, 1991), these efforts were mostly confined to studies in closed ecosystems (Dayal and Thakur, 1966; Misra, 1982; Usha Kiran and Dayal, 1980, 1983; Khulbe, 1998). Hardly any attempt has been made to study the zoosporic fungi of Indian rivers (Gandhe and Desale, 1993; Khulbe, 1995; Gandhe, 2001). However, as compared to lentic habitats, Waterhouse (1942) found the lotic fungal flora less rich and more variable. Zebrowska (1976) also reported the difference in the mycoflora of lentic and lotic systems.

Seasonal periodicity and variation in occurrence was shown by certain species of Saprolegniaaceae. Coker first recorded this in 1923 and detected the importance of temperature as one of the important and major factors responsible for causing fluctuations and seasonal variations in the occurrence of the aquatic zoosporic fungi. In his conclusion, Coker stated that 'for the majority of species spring is the most favourable season for growth'. He did not stress various environmental factors that might be responsible for the seasonal periodicity. Lund (1934) published an extensive account of Danish fresh water Phycomycetes. In his detailed and comprehensive study of seasonal periodicity, he concluded that temperature might be one of the influencing factors.
determining seasonal occurrence of Saprolengniaceae. Waterhouse (1942) worked on watermoulds of the Hogmill river especially on Balstocladiaceae, Pythiaceae and Leptomitaceae and observed that most of the moulds reveal a seasonal rhythm. Igler (1958) observed the seasonal periodicity of Saprolengniaceae in Florida, U.S.A. Parrot (1960) in her ecological studies of Phycomycetes devoted a considerable attention to environmental factors that might be responsible for the seasonal periodicity of aquatic fungi. Two periods of germination and growth during the year, one in spring, and the other in autumn were found responsible for the seasonal occurrence of the aquatic fungi. During summer and winter fungi are dominant in occurrence. Temperature was the important factor for germination and growth of fungi and also for the degree of maturation of oospore. Roberts (1963) during his study on the distribution of Saprolengniaceae in 21 natural water habitats in the U.K. found that the number of species recorded during the warm season was low, while in autumn number began to rise again, reaching the maximum stage of growth in spring. Alabi (1971a, 1971b) reported seasonal periodicity of Saprolengniaceae in Nigeria. He grouped the species according to the season, dry seasons species (October to April), rainy season species (May to August) and all seasons species (isolated throughout the year). Hunter (1975) working on tributaries of river Ouse observed similar results as those reported by other workers in temperate regions. Seasonal fluctuations of freshwater fungi in the river Nile (Egypt) were studied by El-Hissy et al. (1982). The richest periods in aquatic fungal genera and species were September to March. These periods represent low or moderate temperature months. The poorest periods were April to August, which are almost summer months. El-Sharouny and Tiefenbrunner (1983) studied the occurrence and periodicity of fungal genera from the River Inn (Austria) and reported that species exhibited three different patterns during the different months.
In India, a number of research studies have also been carried out on various water bodies in relation to the seasonal occurrence of aquatic Phycomycetes with varying water quality. Butler initiated research on the ecology and taxonomy of aquatic fungi in India. He gave an account of some Chytridiaceae along with genus Pythium (1907). Chaudhuri with his team of coworkers Kochhar, Barerjee and Hamid made important contributions to the study of water moulds of India (1934, 1935, 1936, and 1941). Lacy’s work (1949, 1955) focused on the isolation of aquatic moulds from decaying substrates, algae from local water bodies in Patna. Das Gupta and John (1953) described several species of the aquatic Phycomycetes. Karling visited India as a participant in the U.S. Biology Programme of International Indian Ocean Expedition. During his brief stay in India he visited many States and reported the unexplored fungal flora from many aquatic habitats. He did pioneering work on Indian Chytrids, which is still treated as a monument work. He reported 71 species of aquatic Phycomycetes belonging to 30 genera of 6 orders (Karling, 1966a, 1966b). Indian researchers have made their valuable contribution through detailed study and seasonal periodicity of watermoulds with respect to the physico-chemical properties of water, which is mostly confined, to the studies in the lacustrine systems such as ponds, lakes etc. Dayal and Thakur Ji (1966, 1969) and Usha Kiran and Dayal in 1980 studied the effect of physico-chemical factors on the distribution of aquatic moulds in Ponds of Varanasi. A team of workers (Srivastava and Prabhuji) carried out applied work on watermoulds in relation to fish mycosis at Gorakhpur. It included work on the exploration of new fungal parasites of fish, limnological studies and susceptibility of fish to these fungi Srivastava and Srivastava (1977a, 1977b). Srivastava (1978) studied the host range of Saprolegniaceous fungi and Olpidiopsis species on fish. Sati (1991) explored the relationship between the fungal diseases and fish. Table 2.1 lists the contribution made by various Indian workers regarding studies on aquatic fungal ecology.
<table>
<thead>
<tr>
<th>Name</th>
<th>Year</th>
<th>Locality / Habitat</th>
<th>Topic of Investigation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rai and Misra</td>
<td>1977</td>
<td>Uttar Pradesh/alkaline ponds and soil</td>
<td>Periodicity of watermoulds and their relation with physico-chemical parameters</td>
</tr>
<tr>
<td>Ramarao and Manoharachary</td>
<td>1981</td>
<td>Hyderabad / ponds</td>
<td>Influence of water chemistry on occurrence of aquatic moulds</td>
</tr>
<tr>
<td>Mishra and Dwivedi</td>
<td>1986</td>
<td>Uttar Pradesh / alkaline lakes</td>
<td>Seasonal occurrence of aquatic fungi</td>
</tr>
<tr>
<td>Hasija and Khan</td>
<td>1987</td>
<td>Jabalpur / lakes</td>
<td>Occurrence and distribution of aquatic fungi</td>
</tr>
<tr>
<td>Gupta and Mehrotra</td>
<td>1989</td>
<td>Kurukshetra / water tanks</td>
<td>Correlation studies between aquatic fungal population and physico-chemical variables</td>
</tr>
<tr>
<td>Singh and Wadwani</td>
<td>1989</td>
<td>Uttar Pradesh / stagnant and flowing aquatic habitats</td>
<td>Diversity of aquatic fungi</td>
</tr>
<tr>
<td>Gandhe and Desale</td>
<td>1993</td>
<td>Pune / flowing water</td>
<td>Sewage mycoflora</td>
</tr>
<tr>
<td>Khulbe and Anjali</td>
<td>1994</td>
<td>Kumaun Himalaya / Naini tal lake</td>
<td>Ecological account of watermoulds</td>
</tr>
<tr>
<td>Khulbe</td>
<td>1995</td>
<td>Kumaun Himalaya / riverine waters</td>
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Considerable attention was given to study the seasonal variation of watermoulds in India. Dayal and Tandon (1962) found that the watermoulds showed seasonal periodicity, and growth was mainly restricted to the winter season and the important factor in the germination of the oospore was temperature and maturation of oospores. Srivastava (1967) working on the ponds of Gorkhpur also observed a marked seasonal periodicity of aquatic fungi. They were governed by temperature. Khulbe and Bhargava (1977) working on the tropical and subtropical ponds of Nainital observed very distinct seasonal variation in the occurrence of aquatic fungi and also reported periodicity is governed by temperature. Mer et al. (1980) studied the seasonal occurrence of the watermoulds in water and soil of the Sat Tal area. They observed that the maximum number of watermoulds was obtained in the rainy season in all the lakes. Manoharachary and Ramarao (1980), Madhusudanrao and Manoharachary (1983) studied the seasonal variation and the distribution of fungi from fresh water ponds in Andhra Pradesh. The fungi were divided into monsoon, winter, summer and constant types based on their occurrence and periodicity. Chowdhry and Agarwal (1980a, 1980b) observed that species of Saprolengniaceae were more frequently collected during the winter followed by the rainy and summer seasons. Misra (1983) studied the occurrence, distribution and seasonal fluctuations of aquatic fungi and he reported that the maximum fungal forms occur in the month of December, followed by a gradual decrease in the subsequent months as the temperature of the water bodies rises. Gupta and Dubey (1987) studied the seasonal occurrence of aquatic fungi at Hanuman Tal and found a correlation between the chemical composition of the water body and fungal distribution. Hasija and Khan (1987) studied the seasonal occurrence and distribution of aquatic fungi in relation to the quality of the water bodies and found that they were found in maximum numbers during rainy and winter seasons, while they could not be obtained during summer. It is seen that most of the work has been done on the seasonal occurrence, distribution and periodicity patterns of aquatic fungi, which was mostly confined to closed ecosystems. Very scanty information is available on occurrence, distribution and seasonality of aquatic fungi from open riverine systems.

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