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ORIGINAL ARTICLE

ISOLATION & IDENTIFICATION OF AQUATIC FUNGI

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Abstract:

Water moulds were isolated from different ponds, ditches and lakes around Pune city. They were cultured and purified for identification.
Among all Saprolegnia was dominant. Mechanism of zoospore release was observed.

KEYWORDS:

Aquatic fungi, spores, Zoosporic fungi.

INTRODUCTION

'Zoosporic fungi' is a group of those fungi, which complete almost all life cycle events in water systems and thus are commonly called 'watermoulds'. Several research workers, especially to the members of Saprolegniaceae, apply the term 'watermoulds'. Many mycologists, however, referred 'zoosporic fungi' especially 'aquatic Phycomycetes', mostly, the members of Chytridiomycetes and Oomycetes as 'watermoulds'.

Watermoulds showed their diverse habitats. They play very important role in decomposition and as saprophytes they decompose various types of organic matter including cellulose, chitin, keratin, etc. into simpler substances (Powell, 1993), thereby, forming a nutrient pool and make it available to the producers so as to make water ecosystem a self regulating system (Bhairavnath and Manoharachary, 1985).

Sparrow (1968) studied worldwide geographic distribution of watermoulds and collected the exhaustive data of their occurrence.

Fresh water free flowing or open water systems (Lotic system) such as rivers, streams, brooks and small moving water bodies exhibit a diversified group of watermoulds with several genera and species, whereas, closed freshwater systems (Lentic systems) such as ponds, lakes, ditches, puddles do not show much diversity as in lotic system, however, they show more frequency and richness. (Waterhouse, 1942; Willoughby and Collins, 1966 and Dick, 1971).

The occurrence and frequency distribution of watermoulds varies from place to place. Their presence depends on the kind of water reservoir, organic matter added, rainfall in that area, environmental conditions and parameters, run off soil and water etc. Thus their distribution varies at global, regional and local level.

Ecology plays an important role in the occurrence of watermoulds. In such conditions, environmental parameters fluctuate, influence and develop correlation with their occurrence. Some environmental factors may act differently on the various species of watermoulds, and even on the vegetative and reproductive phases of same individuals (Johnson et al., 2002).

A very significant contribution on ecology of watermoulds from closed and open waterbodies has been done throughout the world (Coker, 1923; Hohnk, 1933; Lund, 1934; Waterhouse, 1942; Johnson, 1956; Ziegler, 1958; Perrot, 1960; Suzuki, 1960; Dick and Newby, 1961; Willoughby, 1962; Hughes, 1962;

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Roberts, 1963; Seymour, 1970; Alabi, 1971; Park, 1972; Hunter, 1975; Sparrow, 1960, 1968, 1973; Dick, 1962; 1966; 1973; El – Hissy and El – Nagdy, 1984; Klich and Tiffany, 1985; Letcher et al., 2004).

In India, many research workers made significant contribution on the ecology of watermoulds from lotic, lentic environment and terrestrial habitat (Das Gupta and John, 1953; Dayal and Tandon, 1962; Thakurji, 1967; Thakurji, 1970; Khulbe and Bhargava, 1977;Prabhuji and Srivatava, 1977; Manoharachary, 1977; 1979a,b; Rai, and Misra, 1977; Chowdhary and Agarwal, 1980; Kapadnis, 1980; Sati and Khulbe, 1980;Manoharachary and Ramarao, 1981; Misra, 1982; Mer and Khulbe, 1984; Prabhuji, 1984; Manoharachary, Bhairavnath and Madhusudan Rao,1983; Bhairabnath and Manoharachary,1985; Misra and Dwivedi,1987; Das Gupta and John,1988; Gupta and Mehrotra, 1989,a,b; Gupta and Mehrotra,1992; Khare,1992; Khulbe,1980;1985;1991;2001; Gandhe and Gandhe, 2002, 2003; Patwardhan, 2004).

Diversity of species in zoosporic fungi is studied by isolating them from water and damp soil near water reservoir by baiting technique.

Watermoulds grow luxuriantly on natural substrates or baits.

REVIEW

International status of the work

Watermoulds occur in temperate as well as in tropical and subtropical countries. Saprophytic and parasitic nature of watermoulds has economic significance, which gives them an international status.

The parasitic nature of watermoulds on aquatic organisms bound research workers to study the watermoulds in detail. The first record of watermoulds was Saprolegniaceous fungus on the tail of a fish (Arderon, 1748). Many research workers like, Ledermueller (1760), Spallanzani (1776), Schrank (1798), Meyen (1835), Braun (1856), Pringsheim (1860), Griffith and Henfrey (1875) noted members of Oomycetes and Chytridiomycetes parasitic on aquatic organisms.

Nees von Esenbeck started taxonomic studies in 1823 on Oomycetes (Saprolegniaceae) with the establishment of Saprolegnia and Achlya. Many research workers like Coker (1923), Johnson (1956), Scott (1961), Waterhouse (1962), Seymour (1970), Dick (2001), Khulbe (2001) published an illustrative monograph on Oomycetes. Significant contributions to Oomycetes were also made all over the world by de Bary (1852,1881), Cornu (1872), Humphrey (1893), Schroter (1893), Zopf (1893), Minden (1915, 1916), Fitzpatric (1930), Couch (1931), Matthews (1931), Hohnk (1933), Lund (1934), Coker (1935), Forbes (1935a), Wolf (1939), Wolf and Wolf (1941), Hamid (1942), Middleton (1943), Johnson (1950, 1974), Dasgupa and John (1953), Copeland (1956), Saksena and Rajgopalan (1958), Ziegler (1958), Beneke and Schmitt (1961), Barksdale (1962), Dick (1962,1963,1966,2001), Bhargava and Singh (1965), Unestam (1965), Dayal and Thakur Ji (1968,a,b), Milanez(1970), Padgett and Seymour (1974), Ho (1975), Hunter (1975), Prabhuji and Srivastava(1977 a,b), Rai and Misra (1977), Ismail et al. (1979), Manoharachary (1977, 1979,a, b), Chowdhary and Agarwal (1980a,b), Sati and Khulbe (1980), Misra (1982), El-Hissy and El – Nagdy (1983), Manoharachary et al.(1983), Usha Kiran and Dayal (1983), Mer and Khulbe (1984), Prabhuji (1984), Misra and Dwivedi (1987), Dayal and Usha Kiran (1988), Gupta and Mehrotra (1992), Steciow (1997), Johnson et al. (2002), Spencer et al. (2002) and Padgett and Johnson (2004).

MATERIALS & METHODS

General survey of closed waterbodies in and around Pune.

Lakaki Pond :
Lakaki Pond is also referred as Model Colony Pond. It is situated somewhat in the centre of the city.

Pashan Lake :
Pashan Lake is situated at the west side of the Pune City.

University Garden Pond :
University Garden Pond is situated in the garden near old canteen, which is at north side of the Pune City.

Modern College Pond :
It is situated in the campus of modern college.

COLLECTION OF WATER SAMPLES :

To study the watermoulds, water samples were collected at the interval of 15 days from the sampling sites in between 9.00 a.m. – 11.30 a.m. During the study period, a total number of 4 samples were collected from each established sampling site. Temperature and pH of the water were recorded at the sites. The samples were collected in sterile glass-stopper bottle (120 ml capacity) by holding the bottles horizontally without allowing any air bubble to pass in it for recording the concentration of dissolved oxygen. In addition water samples were collected separately in sterilized polythene jars (two-litre capacity) for quantitative analysis of other physicochemical parameters. The water samples were also collected in autoclaved polythene vials (25 ml capacity) and boiled Opium seeds were added as baits in them.

The water samples were brought to the laboratory for further analysis. Ten millilitres water sample along with few Opium baits from the small vials were transferred into sterilized petri plates containing fresh Opium baits (Butler, 1907). The water sample was mixed with the tap water in 1:1 proportion for dilution. Petriplates were then placed for incubation at room temperature. The collected submerged flowers; fruits and fragments of plant parts were washed with water thoroughly and transferred into sterile plates having sterile distilled water. Different baits such as: Opium seeds, Acacia nilotica L. pods (Photo Plate VIII, No.–7), guava, boiled grass leaves, insect body parts, boiled snake cast, cellulose paper, boiled onion skin were used, but, watermoulds show luxuriant growth on Opium seeds.

Fungal colonies mostly developed within a week or two on baits, which then washed with sterile distilled water to remove the contaminants if any and then transferred again to the sterile petriplate with sterile distilled water and fresh baits to obtain pure cultures of watermoulds. Preliminary growth of the colonies was observed under low power objective of the microscope (10X) every day. The colony growth was measured in centimeters at the interval of two days. Colonies, after sufficient growth (the growth of zoosporangia), were separated with the help of forceps or needles for purification and were placed in sterile petriplates with sterile distilled water and sterile Opium baits.

MICROSCOPIC MOUNT PREPARATIONS :

For morphological observations and to measure dimensions of fungal organisms, the fully-grown watermould colonies were selected from the pure culture. For the semi permanent preservation, a part of the mycelium containing reproductive structures was cut and stained with 1 % cotton blue prepared in lacto phenol and mounted it in lacto phenol. For permanent storage, colonies were preserved in injection bottles (10 ml capacity) containing sufficient amount of clear lacto phenol sealed with cellophane and then labeled.

IDENTIFICATION :

Monographs (Coker, 1923; Johnson, 1956; Sparrow, 1960; Seymour, 1970; Dick, 10973; Dayal and Usha Kiran, 1988; Khulbe, 2001; Johnson et al., 2002). Information on inter net was also referred to obtain recent views regarding zoosporic fungi.

MECHANISM OF ZOOSPORE RELEASE :

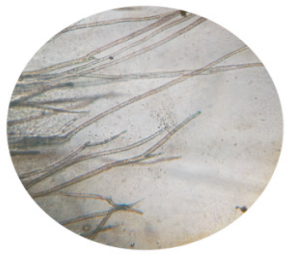
The morphogenesis of sporangium, spore and spore discharge mechanism with the help of microscopic observations and traditional microphotographs were studied. Johnson et al. (2002) documented and discussed these observations in zoosporic fungi.

The zoospore release mehanism in different genera would be another probable criterion for new system of classification.

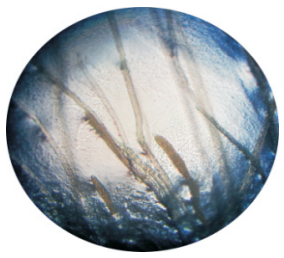
CONCLUSION

Morphological observation shows that in Saprolegnia after discharge, zoospores swim away in the surrounding water without forming a cluster at the tip of zoosporangium, which is the common characteristic feature.

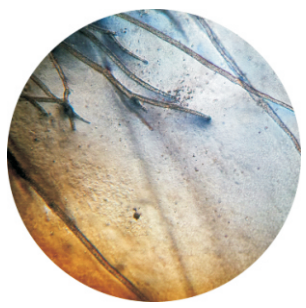
Structure like zoosporangia are developed with a great frequency at earlier stage of culture.



Zoospore release - Saprolegnia



Sporangia



Zoospore release mechanism

Baits showing radial growth

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