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First qualitative survey of filamentous fungi in Dal Lake, Kashmir

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Filamentous fungi comprehend a heterogeneous group of heterotrophic microorganisms that act as saprobes or parasites or, less frequently as symbionts living in association with other organisms. Water samples obtained seasonally from April 2010 to March 2011 at sixteen different sites of Dal Lake, Kashmir were serially diluted five folds followed by spread plate technique for the isolation of filamentous fungi, spreading 0.1 ml inoculum from the serial dilution tubes on the Petri dishes containing Rose-Bengal Streptomycin Agar medium. Twenty three (23) species of fungi namely *Penicillium caseicolum, P. commune, P. chrysogenum, P. funiculosum, P. lilacinum, P. olivicolor, P. dimorphosporum, Penicillium* sp. I, *Penicillium* sp. II, *Penicillium* sp. III, *Penicillium* sp. IV, *Aspergillus flavus, A. fumigatus, A. japonicus, A. niger, A. terreus, A. versicolor, A. wentii, Aspergillus* sp. *Fusarium* sp. *Rhizopus* sp. *Acremonium* sp. and *Mucor* sp. belonging to five genera were recovered from the Lake water samples. *Penicillium* and *Aspergillus* were the most dominant genera with a total of 11 and 8 species respectively. The most prevalent species was *P. chrysogenum* with its occurrence at all sixteen (16) sampling stations and a highest total of seventeen species was recorded at site 16 (Pokhribal Nallah II).

Key words: Filamentous fungi, Dal Lake, serial dilution, qualitative.

INTRODUCTION

Fungi are a diverse group of organisms belonging to the kingdom Eumycota. This kingdom comprises five phyla namely Ascomycota, Basidiomycota, Zygomycota, Chytridiomycota, and Glomeromycota (Kirk et al., 2001; Schußler et al., 2001). As a practical approach to classification, fungi have been divided into groups, such as the filamentous fungi, also called moulds, the yeasts, and the mushrooms. Some fungi are primarily adapted to aquatic environments, and will therefore, naturally be found in water. These fungi are zoosporic and may belong in phyla Chytridiomycota. Fungi belonging to the other phyla in Eumycota are primarily adapted to terrestrial environments. They are present in soil, organic material, and air, and anything in contact with air (Kirk et al., 2001). These fungi can also enter water bodies from various locations, although this is considered an 'unnatural' habitat for them. The knowledge of the occurrence of fungi in water was limited, but has increased due to the various studies performed. The filamentous fungi are ubiquitous group of organisms which explore almost all ecological niches on earth. They are estimated to be responsible for the spoilage of up to 25% of all plant-derived foods produced annually (Geisen, 1998).

Filamentous fungi or moulds are vital for the maintenance of ecosystems. By breaking down dead organic material, they continue the cycle of nutrients through ecosystems. Some of them act as plant pathogens causing severe crop losses and post-harvest food spoilage. In the reagent industry and medicine areas, filamentous fungi are the source of commercial enzymes, organic acids, and numerous drugs, such as

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antibiotics (e.g. penicillin, cefalosporin). *Penicillium* species have been frequently recovered from water in the various studies performed. Several of the species in genus *Penicillium* and *Aspergillus* are known to produce mycotoxins in other substrates, such as food and beverages (Moreau 1979; Pitt and Hocking, 1999). Interestingly, detection of aflatoxins produced by *A. flavus* in water from a cold water storage tank was demonstrated by Paterson et al., (1997). *Aspergillus* species is one of the more commonly isolated genus in water. *A. niger* and *A. flavus* are common allergens and may cause opportunistic invasive infections (De Hoog et al., 2000; Denning, 1998).

Predominant fungal genera and species in treated and untreated water are: *Aspergillus, Cladosporium, Epicoccum, Penicillium, Trichoderma, Arthrinium phaeospermum, A. flavus, C. cladosporioides, Fusarium culmorum, Mucor hiemalis* and *Trichoderma harzianum* (Kinsey et al., 1999). Many other fungal genera isolated from Danube river water in Europe include: *Mortierella, Absidia, Rhizopus, Acremonium, Beauveria, Doratomyces, Monilia, Rhizopus arrhizus, Acremonium strictum, Fusarium oxysporum* and *Stemphyllium botryosum* (Tothova, 1999)

The ecology of aquatic fungi affects their distribution both locally and globally, and the factors influencing the fungi are complex and vary depending on the aquatic environment. What governs the distribution of freshwater fungi is difficult to determine, although some species appear to be more common either in temperate or tropical regions (Shearer et al., 2007; Raja et al., 2009).

MATERIAL AND METHODS

Location and site description: Dal Lake, located at 34° 07′ N, 74° 52′ E, 1584 m a.s.l in Srinagar, Jammu and Kashmir, India- a multi-basined lake with Hazratbal, Bod Dal, Gagribal and Nageen as its four basins, having two main inlets as Boathall Nallah and Tailbal Nallah and two main outlets as Dal Lock Gate and Pokhribal Nallah, was taken up for the current study. Sixteen (16) sites viz., Hazratbal Open, Hazratbal littoral, Nageen Open, Nageen near Houseboats, Gagribal Open, Gagribal near Houseboats, Nishat Open, Near Centeur, Boathall Nallah-I, Boathall Nallah-II, Tailbal Nallah-I, Tailbal Nallah-II, Dal Lock Gate-I, Dal Lock Gate-II, Pokhribal Nallah-I and Pokhribal Nallah-II were selected with eight (8) sites from the four basins, Four (4) sites from two inlets and Four (4) others from the two outlets.

Collection of water samples

The water samples were collected on seasonal basis for a period of 12 months between April 2010 to March 2011, from Dal Lake in white plastic containers, which were previously sterilized with 70% alcohol and rinsed with distilled water. At the lake, the containers were rinsed thrice with the lake water before being used to collect the samples.

Isolation of fungi

Water samples obtained from different sites were serially diluted five folds followed by spread plate technique for isolation of filamentous fungi, spreading 0.1 ml inoculum from the serial dilution tubes on the Petri dishes containing Rose-Bengal Streptomycin Agar medium. Growing colonies were transferred to Petri dishes containing different culture media like Potato Dextrose Agar (PDA) (MERCK, Germany), Malt Extract Agar (MEA) (Acumedia, USA), Czapek's dox Agar (CZ) (MERCK, Germany) and Czapek's Yeast Agar (CYA) (MERCK, Germany), 25% Glycerol nitrate Agar (G25A) for identification, and then transferred everything to PDA for stock cultures. Plates were incubated at 25 to 37°C for one week in dark.

Identification of fungi

Identification of fungi was performed mainly on the basis of the micro- and macromorphological features, reverse and surface coloration of colonies grown on CZ, MEA, CYA and PDA media. Fungi were identified to genus level using Barnett and Hunter (1999). Cultures were identified to species level using various mycological texts: *Penicillium* LINK, species were identified using colony diameters, macro- and micromorphology according to the standardized conditions of PITT's monograph (2000). These species were grown on various differential media all prepared according to the recipes of Pitt (2000). Each *Penicillium* culture was inoculated in triplicate on each medium and incubated at three different temperatures (5, 25 and 37 °C) for a period of 7 days in the dark. The monograph by Raper and Fennell (1965) was used for identification of *Aspergillus* species. In addition to these the morphological characteristics of these and various other species were studied by making slide cultures obtained by inoculating microfungi directly on a small square of agar medium.

RESULTS

Although there are many methods such as filtration, direct plating, baiting etc. for sampling fungi from water (Kinsey et al., 1999) we used direct plating method for its isolation in our study. Twenty three (23) species (Table 1) of filamentous fungi; *P. caseicolum, P. commune, P. chrysogenum, P. funiculosum, P. lilacinum, P. olivicolor, P. dimorphosporum, Penicillium* sp. I, *Penicillium* sp. II, *Penicillium* sp. III, *Penicillium* sp. IV, *A. flavus, A. fumigatus, A. japonicus, A. niger, A. terreus, A. versicolor, A. wentii, Aspergillus* sp. *Fusarium* sp. *Rhizopus* sp. *Acremonium* sp. and *Mucor* sp. belonging to five genera were recovered from sixteen sampling stations of the Lake. The prevailing genera were *Aspergillus*, *Penicillium*, *Fusarium, Rhizopus*, *Acremonium* and *Mucor*. The most frequent genera obtained were *Penicillium* (11 species) and *Aspergillus* (8 species). However, *Fusarium* (1 species), *Rhizopus* (1 species), *Acremonium* (1 species) and *Mucor* (1 species) were also reported during the study. The most prevalent species was *P. chrysogenum* with its occurrence at all sixteen (16) sampling stations followed by *P. funiculosum*, *A. niger and A. flavus* from 14 stations each, *A. fumigatus* and *Fusarium* sp. from thirteen (13) stations each, *P. caseicolum, P. lilacinum, P. olivicolor, A. terreus, A. wentii,* and *Mucor* sp. from eight (8) stations each, *P. commune, P. dimorphosporum, A. japonicus,* and *A. versicolor* from seven (7) stations each, *Penicillium* sp. II,

Table 1. Occurrence of filamentous fungi in different seasons at different sites.

S/N	Name of Fungi	Seasons			
		Spring 2010	Summer 2010	Autumn 2010	Winter 2010
1.	*Penicillium caseicolum* Bain.	5, 9, 10, 14	3, 11, 12	-	2
2.	*Penicillium commune* Thom.	1, 2, 11	14	6, 13, 16	-
3.	*Penicillium chrysogenum* Thom.	4, 6, 7, 9, 12, 13, 16	1, 2, 4, 6, 9, 11, 12, 14, 15, 16	3, 5, 8, 12, 14, 16	3, 4, 9, 10, 11, 13, 14, 16
4.	*Penicillium funiculosum* Thom.	1, 2, 5, 6, 7, 8, 9, 12, 15, 16	2, 3, 4, 6, 12, 14, 16	11, 15, 14	3, 4, 6
5.	*Penicillium lilacinum* Thom.	3, 14, 15	5, 6, 11, 12, 14	7, 6, 11, 12	-
6.	*Penicillium olivicolor* Pitt	1, 2, 3, 4, 9, 11, 12	3, 9, 10	-	-
7.	*Penicillium dimorphosporum* Swart	1, 2, 3, 4, 5	5, 6, 8	5	-
8.	*Penicillium* sp. I	-	-	-	9, 10
9.	*Penicillium* sp. II	16, 14, 15	2, 3, 4	-	-
10.	*Penicillium* sp. III	-	14, 15, 16	-	-
11.	*Penicillium* sp. IV	9	11, 12	-	-
12.	*Aspergillus flavus* Link: Fr	4, 5, 8, 16	1, 2, 3, 5, 6, 9, 10, 11, 15, 16	9, 13, 16	3, 4, 5, 14, 15
13.	*Aspergillus fumigatus* Fresenius	3, 4, 6, 9, 10, 11, 14, 16	1, 6, 8, 11, 14, 15, 16	3, 6, 14	8, 12, 13, 15
14.	*Aspergillus japonicus* Saito	-	3, 4, 15	-	5, 12, 13, 16
15.	*Aspergillus niger* Van Tieghem	6, 12, 13, 14, 16	2, 3, 4, 10, 11, 14, 16	1, 5, 9, 11, 15, 16	5
16.	*Aspergillus terreus* Thom.	-	4, 6, 9, 10, 12, 14	16	2
17.	*Aspergillus versicolor* gr.	2, 3, 13, 15, 16	1, 10, 16	-	-
18.	*Aspergillus wentii* gr.	4, 6, 13, 14, 16	5, 8, 13, 16	7	8
19.	*Aspergillus* sp.	-	13, 16	-	8, 9
20.	*Fusarium* sp.	1, 3, 5, 6, 10, 14, 16	2, 3, 5, 6, 7, 8, 16	1, 9, 15	1, 4, 9, 10 14, 15
21.	*Rhizopus* sp.	1, 9, 14, 16	1, 4, 9, 14	-	-
22.	*Acremonium* sp.	2, 6, 7	7, 16	-	-
23.	*Mucor* sp.	4, 6, 13, 14, 15	13, 14	5, 6, 7, 16	15, 16

1= Hazratbal open, 2= Hazratbal littoral, 3= Nageen open, 4= Nageen near houseboats, 5= Gagribal open, 6= Gagribal near houseboats, 7= Nishat open, 8= near Centeur, 9= Boathall Nallah-I, 10= Boathall Nallah-II, 11= Tailbal Nallah-I, 12= Tailbal Nallah-II, 13= Dal Lock Gate-I, 14= Dal Lock Gate-II, 15= Pokhribal Nallah-I, 16= Pokhribal Nallah-II.

from six (6) stations, *Rhizopus* sp. from five (5) stations, *Aspergillus* sp. and *Acremonium* sp. from four (4) stations each, *Penicillium* sp. III and *Penicillium* sp. IV from three (3) stations each and *Penicillium* sp. I from two (2) stations. The highest total of seventeen fungal species was recorded from site 16 (Pokhribal Nallah II), followed by site 14 (Dal Lock Gate II) with fifteen species, site 4 (Nageen Near Houseboat) with fourteen species, site 3 (Nageen Open) site 6 (Gagribal near Houseboats) site 9 (Boathall Nallah) with thirteen species each, site 2 (Hazratbal littoral) with twelve species, site 5 (Gagribal open) site 15 (Pokhribal Nallah II) with eleven species each, site 10 (Boathall Nallah) site 11 (Tailbal Nallah I) site 12 (Tailbal Nallah II) site 13 (Dal Lock Gate I) with ten species each, site 8 (Near Centeur) with eight species and site 7 (Nishat Open) with six species.

DISCUSSION

The overwhelming presence of these terrestrial moulds in water supports the paradigm that their

deposition is attributable to contamination of the water body due to the entry of sewage from the catchment areas, as they survive conventional treatment strategies and enter the distribution through the sewage coming out from the sewage treatment plants (Neimi et al., 1982). It can be attributed to the entry of sewage from the drains into the lake, as these genera have been reported frequently from the drain waters with maximum densities during higher pollution (Khulbe and Drugapal, 1994) and can therefore be inferred that these species are good indicators of pollution. The genera and species isolated in the present study were previously isolated, but with various numbers and frequencies, from different substrata in Saudi Arabia such as rainfall water and mud (El-Nagdy et al., 1992), soil (Abdel-Hafez, 1982a) and ferns (Abdel-Hafez, 1984). Almost all of the filamentous fungal genera recovered in this study had been found in various habitats in India (Saju 2011; Shafi et al., 2011, Bandh et al., 2011a) Egypt (Abdel-Hafez and Bagy, 1985; El-Hissy et al., 1990; Moharrum et al., 1990; El-Nagdy and AbdelHafez, 1990) Brazil (Gomes et al., 2008) and other countries (Barlocher and Kendrick, 1974; Bettucci et al., 1993; Bettucci and Roquebert, 1995). Terrestrial fungi in aquatic habitats are likely to originate from air (Sparrow, 1968), as well as from living or dead animal and plant, soil and litter being in contact with water (Park, 1972). These species have also been isolated from soils, water and other substrata in Saudi Arabia, Egypt and other countries (Abdel-Hafez et al., 1978; Abdel-Hafez, 1982b; Abdel-Kader et al., 1983; Abdel-Hafez and Bagy, 1985; Bandh et al., 2011b).

Aspergillus and *Penicillium* spores are the most widespread aeroallergens in the world. According to qualitative and quantitative reports, the former is the dominant species in tropical regions whilst the latter is dominant all over the world (Rosas et al., 1992).

A. fumigatus, found in our study, is one of the most ubiquitous airborne saprophytic fungi. Water fungi can play a vital role in the decomposition of some organic materials such as dead leaf and stem litter. The decomposition of fallen leaves and other detritus in streams is dominated by fungi (Garnett et al., 2000).

Penicillium was the most frequent and predominant genus detected in our study, followed by *Aspergillus*. According to the Kinsey et al. (1999), certain fungi such as *Aspergillus*, *Cladosporium*, *Epicoccum*, *Penicillium* and *Trichoderma* species appear more frequently than others in water. Our results concur with theirs except that we did not find *Epicoccum*, *Cladosporium* and *Trichoderma*. *A. fumigatus*, *A. niger*, *P. chrysogenum* and many other species belonging to the two genera observed in the current study with a high occurrence at different sampling stations have also been found to be widespread in Turkey and have been reported in many studies (Asan, 2000). *Aspergillus* spp and *Penicillium* spp are major contaminants of the environments and occur as ubiquitous saprophytes, with their spores able to survive and reproduce in water as well. The present results are confirmed by a Brazilian study on filamentous fungi of sand and water from “Bairro Novo” and “Casa Caiada” beaches in which *Aspergillus* and *Penicillium* were the most frequent genera in both sand and water, with a total of 11 and 19 species, respectively (Gomes et al., 2008)

Conclusion

The mycoflora of Dal Lake with reference to filamentous fungi investigated in the present work showed that the genus *Penicillium* was found to be widespread in the water samples indicating that the spores of this genus are most widespread in nature.

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