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RESEARCH ARTICLE

ISOLATION, IDENTIFICATION AND SEASONAL DISTRIBUTION OF *PENICILLIUM* AND *ASPERGILLUS* SPECIES IN DAL LAKE, KASHMIR

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ABSTRACT

Water samples obtained seasonally from April 2010 to March 2011 at eight different sites of Dal Lake, Kashmir were serially diluted five folds followed by spread plate technique for the isolation of *Penicillium* and *Aspergillus* species, spreading 0.1ml inoculum from the serial dilution tubes on the Petri dishes containing Rose-Bengal Streptomycin Agar medium. Out of a total 213 fungal colonies isolated six (6) species of *Penicillium* viz, *P. caseicolum*, *P. commune*, *P. chrysogenum*, *P. funiculosum*, *P. lilacinum*, *Penicillium* spp. and six (6) species of *Aspergillus* viz, *A. flavus*, *A. fumigatus*, *A. japonicus*, *A. terreus*, *A. niger* and *Aspergillus* spp. were obtained. Out of these species *P. chrysogenum* was the most abundant (30.99%) followed by *P. funiculosum* (16.43%), *A. fumigatus* (14.09%), *A. niger* (13.15%), *A. flavus* (9.39%), *A. terreus* (3.76%), *P. lilacinum* (3.27%), *P. caseicolum* (2.82%), *P. commune* (2.35%), *A. japonicus* (1.88%), *Penicillium* spp. and *Aspergillus* spp. (0.94%) each. Highest number of colonies 60 (28.17%) was obtained from site Pokhribal Nallah (PKB) followed by Tailbal Nallah (TBN) - 45(21.13%), Dal Lock Gate (DLG) and Gagribal (GB) - 28(13.14%) each, Nageen Lake (NL) - 1(8.45%), Boathall Nallah (BHN) - 15 (7.04%), Hazratbal (HB) - 11(5.17%) and Bod Dal (BD) - 8(3.76%).

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INTRODUCTION

Fresh water bodies receive various types of waste materials, with many of them being organic in nature. These organic wastes are easily degraded by microbes like fungi and bacteria, naturally present in the water bodies. Fungi are an integral part of any ecosystem biota and play an important role in the energy transfer from allochthonous and autochthonous particles to other components of the food chain. The aquatic fungi play a key role in the decomposition of leaf litter in aquatic environments (Gessner *et al*., 1999). Fungal activity on the organic wastes is affected by several environmental factors, such as dissolved nutrients in water (Suberkropp and Chauvet, 1995; Gulis and Suberkropp, 2003), temperature (Chauvet and Suberkropp, 1998), turbulence (Webster, 1975) and pH (Dangles *et al*., 2004). Generally, low to moderate nutrient concentrations stimulate fungal activity (Gulis *et al*., 2006). Majority of the world's fungi are microscopic, and do not usually produce structures which are visible to the naked eye, unless the hyphae form a thick growth (Often referred to as 'moulds'). Zoosporic fungi are universally present in all types of freshwater ecosystems and occur as saprotrophs on a wide variety of substrata, playing a key role in those ecosystems as decomposers of organic materials (Müeller *et al*., 2004). Aquatic hyphomycetes generally dominate leaf decomposition in streams, and play an important role as intermediaries between decaying leaves and leaf eating invertebrates (Suberkropp, 1992). *Penicillium* is a well known cosmopolitan genus of moulds with more than 250 accepted species. It is one of the dominant fungal genera in soil (Thom, 1930; Christensen *et al*., 2000) where it is mainly responsible for decomposing organic matter and assists in the maintenance of soil nitrogen fertility in concert with other organisms (Seneviratne and Jayasinghearachchi, 2005). They function as decomposers of dead materials and are especially important as post-harvest organisms, where they spoil various food commodities (Janisiewicz, 1987; Pitt and Hocking, 1997; Holmes and Eckert, 1999; Morales *et al*., 2007). Aspergilli, ubiquitous in nature are geographically widely distributed and have been observed in a broad range of habitats principally in soils and decaying vegetation. Some *Aspergillus* species such as *A. niger* and *A. temari* were isolated from soil of forest and cave ecosystems of Taiwan by Hsu and Agoramoorthy (2001). Some species of genus Aspergillus are medically and commercially important while as some are important plants and animal pathogens (Gregory *et al.,* 1997). The present

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study was carried out to study the distribution and seasonality of *Penicillium* and *Aspergillus* species in an aquatic habitat Dal Lake, of Kashmir.

MATERIAL AND METHODS

Location and site description

The 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 Nagin as its four basins, having two main inlets - Boathall Nallah and Tailbal Nallah and two main outlets as Dal Lock Gate and Pokhribal Nallah, was taken up for the current study. Eight (8) sites with four (4) sites in the four basins, two (2) sites in the two inlets and two (2) more sites in the two outlets were selected for the present study. The selected sites were HB (Hazratbal), NL (Nagin Lake), GB (Gagribal), BD (Bod Dal), TBN (Tailbal Nallah), BHN (Boathall Nallah), DLG (Dal Lock Gate) and PKB (Pokhribal Nallah).

Collection of Water Samples

The water samples were collected on seasonal basis for a period of 12 months between April 2010-March 2011, at eight (8) different sites of the lake in white plastic containers, which were previously rinsed with distilled water and sterilized with 70% alcohol. 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 and then spread plate technique was followed for isolation of *Penicillium* and *Aspergillus* species in the study, spreading 0.1ml 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 25–37 °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 and PDA media. Fungi were identified to genus level using Barnett and Hunter's work (1999). Cultures were identified to species level using the following mycological texts: *Penicillium* LINK, species were identified using colony diameters, macro- and micromorphology according to the standardized conditions of PITT's monograph (1979). These species were grown on three different media all prepared according to the recipes of Pitt (1979). So, Czapek Yeast Agar (CYA), Malt Extract Agar (MEA), and 25% Glycerol nitrate Agar (G25A) were used for cultivation of *Penicillium* species and prepared according to Pitt (1979). Each *Penicillium* culture was inoculated in triplicate on each medium and incubated at three different temperatures (5 °C, 25 °C 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 were

Table 1: Individual colony count of *Penicillium* spp., and *Aspergillus* spp., in different seasons at different sites of Dal Lake, Kashmir

S. No	Name of Fungi	HB				NL				GB				BD			
		Spr	Sum	Aut	Win	Spr	Sum	Aut	Win	Spr	Sum	Aut	Win	Spr	Sum	Aut	Win
1.	*Penicillium caseicolum*	0	0	0	1	0	1	0	0	1	0	0	0	0	0	0	0
2.	*Penicillium commune*	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
3.	*Penicillium chrysogenum*	0	2	0	0	2	1	1	1	1	3	1	0	0	0	2	0
4.	*Penicillium funiculosum*	1	1	0	0	0	1	0	1	1	4	0	1	1	0	0	0
5.	*Penicillium lilacinum*	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0
6.	*Penicillium* sp.	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
7.	*Aspergillus flavus*	0	1	0	0	1	2	0	1	2	1	0	1	1	0	0	0
8.	*Aspergillus fumigatus*	0	1	0	0	1	0	1	0	2	1	1	0	0	1	0	1
9.	*Aspergillus japonicus*	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0
10.	*Aspergillus niger*	0	1	1	0	0	1	0	0	1	0	1	1	1	1	0	0
11.	*Aspergillus terreus*	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0
12.	*Aspergillus* sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S. No	Name of Fungi	TBN				BHN				DLG				PKB			
		Spr	Sum	Aut	Win	Spr	Sum	Aut	Win	Spr	Sum	Aut	Win	Spr	Sum	Aut	Win
1.	*Penicillium caseicolum*	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0
2.	*Penicillium commune*	1	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
3.	*Penicillium chrysogenum*	3	9	3	1	2	2	0	1	5	2	1	1	11	6	3	2
4.	*Penicillium funiculosum*	3	6	1	0	1	0	0	0	1	2	0	0	2	8	1	0
5.	*Penicillium lilacinum*	0	1	1	0	0	0	0	0	0	0	0	0	1	1	0	0
6.	*Penicillium* sp.	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
7.	*Aspergillus flavus*	0	1	0	0	0	1	1	0	1	2	1	0	0	2	0	1
8.	*Aspergillus fumigatus*	2	1	0	1	1	0	0	0	2	1	0	1	8	2	1	1
9.	*Aspergillus japonicus*	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0
10.	*Aspergillus niger*	3	4	1	0	0	1	1	0	1	2	1	0	2	3	1	0
11.	*Aspergillus terreus*	0	1	0	0	0	1	0	0	0	0	1	0	0	1	0	0
12.	*Aspergillus* sp.	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0

HB = Hazratbal; NL = Nageen Lake; GB = Gagribal; BD = Bod Dal; TBN = Tailbal Nallah; BHN = Boathall Nallah; DLG = Dal Lock Gate; PKB = Pokhribal Nallah; Spr = spring season; Sum =summer season; Aut = autumn season; Win = winter season

Table 2: Colony count and percentage occurrence of *Penicillium* spp., and *Aspergillus* spp., at different sites of Dal Lake, Kashmir

S. No	Name of Fungi	TC								GT	% Occurrence	Genus %age
		HB	NL	GB	BD	TBN	BHN	DLG	PKB			
1.	*Penicillium caseicolum*	1(16.67%)	1(16.67%)	1(16.67%)	0(0%)	1(16.67%)	1(16.67%)	0(0%)	1(16.67%)	6	2.82	
2.	*Penicillium commune*	1(20%)	0(0%)	1(20%)	0(0%)	1(20%)	0(0%)	1(20%)	1(20%)	5	2.35	
3.	*Penicillium chrysogenum*	2 (3.03)	5 (7.58)	5 (7.58)	2 (3.03)	16 (24.24)	5 (7.58)	9 (13.64)	22 (33.33)	66	30.99	
4.	*Penicillium funiculosum*	1(2.86%)	2(5.71%)	6(17.14%)	1(2.86%)	10(28.57%)	1(2.86%)	3(8.57%)	11(31.43%)	35	16.43	
5.	*Penicillium lilacinum*	0(0%)	1(14.29%)	2(28.57%)	0(0%)	2(28.57%)	0(0%)	0(0%)	2(28.57%)	7	3.27	121 (56.80%)
6.	*Penicillium* sp.	1(50%)	0(0%)	0(0%)	0(0%)	0(0%)	1(50%)	0(0%)	0(0%)	2	0.94	
7.	*Aspergillus flavus*	1(5%)	4(20%)	4(20%)	1(5%)	1(5%)	2(10%)	4(20%)	3(15%)	20	9.39	
8.	*Aspergillus fumigatus*	1(3.33%)	2(6.67%)	4(13.33%)	2(6.67%)	4(13.33%)	1(3.33%)	4(13.33%)	12(40%)	30	14.09	
9.	*Aspergillus japonicus*	0(0%)	1(25%)	1(25%)	0(0%)	1(25%)	0(0%)	1(25%)	0(0%)	4	1.88	
10.	*Aspergillus niger*	2(7.14%)	1(3.57%)	3(10.71%)	2(7.14%)	8(28.57%)	2(7.14%)	4(14.29%)	6(21.43%)	28	13.15	
11.	*Aspergillus terreus*	1(12.5%)	1(12.5%)	1(12.5%)	0(0%)	1(12.5%)	1(12.5%)	1(12.5%)	2(25%)	8	3.76	92 (43.20%)
12.	*Aspergillus* sp.	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	1(50%)	1(50%)	0(0%)	2	0.94	
	GT	11(5.16%)	18(8.45%)	28(13.15%)	8(8.45%)	45(21.13%)	15(7.04%)	28(13.15%)	60(28.17%)	213	100	100

TC = Total Count; GT = Grand Total

Table 3: Total Colony count and percentage occurrence of fungal colonies in different seasons at different sites of Dal Lake, Kashmir

Seasons / Sites	Total no. of Colonies in Different Seasons				
	Spr	Sum	Aut	Win	Total
HB	2	6	1	3	12
NL	5	8	2	3	18
GB	8	11	5	4	28
BD	3	2	2	1	8
TBN	12	24	6	3	45
BHN	5	5	2	3	15
DLG	10	10	5	3	28
PKB	25	24	6	4	59
Total	70(32.87%)	90(42.26%)	29(13.61%)	24(11.27%)	213

studied by making slide cultures obtained by inoculating microfungi directly on a small square of agar medium.

RESULTS

During the study a total of 213 fungal colonies were isolated and quantified to determine the individual and total percentage occurrence (Table 1 and 2). The study revealed that the most dominant species of fungi in Dal lake water was *P. chrysogenum* with a maximum percentage occurrence of 30.99% followed by *P. funiculosum* with 16.43%, *A. fumigatus with* 14.09%, *A. niger* with 13.15%, *A. flavus* with 9.39%, *A. terreus* with 3.76%, *P. lilacinum* with 3.27%, *P. caseicolum* with 2.82%, *P. commune* with 2.35%, *A. japonicus* with 1.88%, *Penicillium* sp. and *Aspergillus* sp. with 0.94% each. The maximum percentage of fungal colonies (28.17%) was observed at site PKB followed by TBN- (21.13%), DLG and GB- (13.14%) each, NL- (8.45%), BHN- (7.04%), HB- (5.17%) and BD- (3.76%). The genus *Penicillium* (6 out of 12 species, 121 out of 213 total fungal colonies) had the higher abundance of the isolated species while as the genus *Aspergillus* (6 species, 92 colonies) had a little lower. The occurrence of fungal species showed considerable variation in different seasons (table 3) in all the eight stations of

Dal Lake, water. Highest number of colonies 90 (42.26%) was found in summer season followed by 70 (32.87%) colonies in spring, 29 (13.61%) colonies in autumn and 24 (11.27%) colonies in winter.

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). The highest percentage occurrence of the fungi at site PKB followed by DLG, GB, NL and BHN is because of the sewage entering the lake system from the drains, as these genera have been reported frequently from the drain waters with maximum densities during higher pollution (Khulbe and Durgapal, 1994). It could therefore be inferred that these species are good indicators of pollution due to the sewage from the adjoining residential and commercial catchment area. The species of genus *Penicillium* and *Aspergillus* are usually found in polluted lake waters and act as cellulose decomposers as opined by Kellermann and McBeth, 1912.

Seasonal variation observed in the fungal population during the study is possibly due to the temperature variations, as the maximum percentage of fungal colonies was found during high temperature (summer) and minimum during low temperature (winter). Similar results were obtained by Khulbe and Durgapal, 1992 in their study on the population dynamics of Geofungi in a polluted freshwater body at Nainital. A preliminary mycological study on the same lake by Bhat and Kamili, 2004 had also reported the presence of these fungal genera with a similar fluctuation in their numbers during different seasons. The seasonal variation found in the study is also in consonance with the results of Sharma and Parveen, 2011. The total percentage occurrence of the genus *Penicillium* (56.80%) and genus *Aspergillus* (43.20%) (Table 2) obtained in the present study was confirmed by a recent Nigerian study of Eze and Ogbaran, 2010 who in their study on the fish pond water found that species of genus *Penicillium* showed a maximum of 55.8% followed by species of genus *Aspergillus* with 44.1%. This can be attributed to the fact that *Penicillium* and *Aspergillus* are biologically most successful of all fungi and are expected to occur in all sorts of habitats. Their 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 with a maximum percentage occurrence among the species of genus *Aspergillus* in our study, is one the most ubiquitous of the airborne saprophytic fungi (Asan *et al*., 2003). *Penicillium*, the most dominant genus as compared to *Aspergillus* observed in the study concurs with the results of Kinsey *et al*. (1999), who reported that certain fungi such as *Penicillium*, *Aspergillus*, *Cladosporium*, *Epicoccum*, and *Trichoderma* species appear more frequently than others in water. The results corroborate with theirs except that this study was confined to the isolation and distribution of the *Penicillium* and *Aspergillus* genus only. *A. fumigatus*, *A. niger*, *P. chrysogenum* and many other species belonging to the two genera, isolated and identified in the current study with a high percentage occurrence and colony number have also been found to be widespread in Turkey and have been reported in many studies (Asan, 2000).

CONCLUSION

The mycoflora of Dal Lake with reference to *Penicillium* spp., and *Aspergillus* spp., 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. The results of percentage occurrence of the two fungal genera further implied that the spores of genus *Aspergillus* are equally widespread and distributed in nature.

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