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Abundance and diversity of major cultivable fungal flora of River Jhelum in Kashmir Himalaya

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The present work was carried out in the in river Jhelum of Kashmir Himalaya to assess the density and diversity of fungal flora, to isolate and identify the fungi from the water along with some physical parameters like pH and temperature which was carried out between June-November, 2011 at four sites differing from each other markedly in terms of biotic and abiotic factors. During the study, a variety of fungal strains were isolated and identified from the water of river at the four sites. The highest viable count of fungi was observed at site III with a cfu/ml of 3.6 x 10^2 in the month of July and the lowest viable count at site IV with a cfu/ml of 2.7×10^2 in the month of November. Among most dominant of the isolate identified 20% were *Aspergillus* spp. followed by 4% *Pencillium* spp. and 4% *Candida* spp. Comparative analysis of different types of colonies found at the four sites during the study indicates that the fungal density was dominant in the month of July.

Key words: River Jhelum, fungi, *Aspergillus* spp., *Pencillium* spp., and *Candida* spp.

INTRODUCTION

The valley of Kashmir is a lacustrine basin with an average altitude of 1585 m a.s.l. Both the valley and its surrounding mountains are home to a large number of aquatic habitats like lakes, ponds, streams, rivers and wetlands. It is estimated that 6% of the land area of Jammu and Kashmir is under aquatic habitats (Zutshi and Gopal, 2000). Water is essential to sustain life, and without it life becomes impossible, it is an indispensable commodity, which should be easily accessible, adequate in quantity, free of contamination, safe, affordable and available throughout the year in order to sustain life (Al Khatib and Salah, 2003). Fungi are among the most diverse groups of living organisms on earth, though inadequately studied worldwide (Grover et al., 2007). Aquatic fungi play a crucial role in the freshwater ecosystem in nutrient cycling by breaking down leaves and woody substrates and also as symbionts (Barlocher and Kendrick, 1981). Physical chemical factors of ecosystem play important role on the growth, multiplication, distribution and seasonal periodicity of aquatic fungi (Park, 1972). Fungi belong to the kingdom Eumycota. This kingdom comprises five phyla namely Ascomycota, Basidiomycota, Zygomycota, Chytridiomycota, and Glomeromycota (Kirk et al., 2001;

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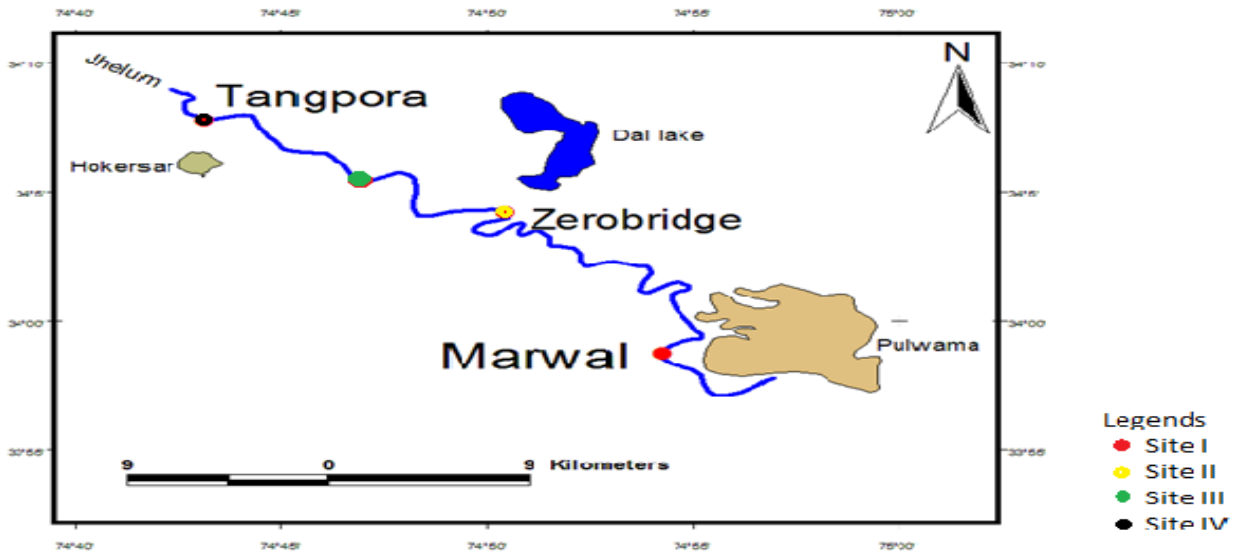

Figure 1. Geographical map of study areashowing the location of study area and sampling site.

Schußler et al., 2001). *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) and detection of aflatoxins produced by *A. flavus* in water from a cold water storage tank was demonstrated by Paterson et al. (1997). 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). 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).

Since no substantial work has been carried out regarding the current understanding and distribution of fungal flora in the Jhelum River. Therefore the objective of this study was to focus on the isolation and identification of the fungal flora from this important river

MATERIALS AND METHODS

Study area and study sites

Jhelum, the major waterway of Kashmir, originates from the spring Verinag located in the foot of a spur of the Pir Panjal Mountains in the district Anantnag from where a number of tributaries join the Jhelum and make it navigable from Khannabal to Wular Lake. The river runs a course of 203 km through the valley and the hydrology of River Jhelum is largely controlled by snowmelt in spring season and heavy rains from June to September. A total of four study sites (Figure 1) markedly different in respect of geographical and demographical features were selected for the sampling. These sites were characterized by having the moderate human population on both the banks along with the agricultural fields:

Site I: It was near Marval, Pampore lying between geographical coordinates 33˚ 58’ 45.4” N and 74˚ 54’ 16.5” E with an elevation of 1601 m. a.s.l. This site was located about 32 km from the main city centre (Lal chowk). On both sides of the bank the land was used for agricultural purposes and was bordered with residential hamlets around. The average depth of river at this site was about 1.6 m.

Site II: It was located (about 1.2 km from the city centre) at Zero bridge in Srinagar city lying between the geographical coordinates of 34˚ 4’ 9.2” N and 74˚ 50’ 20.88” E and having an elevation of 1582 m. a.s.l. At this stretch of the River, Jhelum congested human population and commercial activities takes place along the both sides of the banks. All along its course from Marval to Srinagar the river receives significant quantities of domestic wastes from human settlements and army cantonment areas. The average depth at this site was about 2 m.

Site III: This site was located about 10 km from main city centre at Qamarwari in Srinagar city lying between the geographical coordinates of 34˚ 05’ 35.9” N and 74˚ 46’ 45.4” E and having an elevation of 1579 m. a.s.l. At this site both commercial and residential activities take place along both sides of river, which directly release the sewage and other solid wastes directly into the river. The average depth at this site was about 1.2 m.

Site IV: This site was located at Tengpora about 26 km from the main city centre lying between the geographical coordinates of 74˚ 43’ 11” E and 34˚ 7’47.1” N and having an elevation of 1577 m. a.s.l. This stretch of River Jhelum was characterized by moderate human population on both the banks along with the vegetable cultivation. Human interference like emission of domestic sewage,

washing of clothes and other activities usually takes place at this particular site.

Laboratory analysis

Surface water samples were collected aseptically in pre-sterilized bottle on the monthly basis from June to November, 2011. During the present study Rose Bengal Agar media was used for isolation of Water fungi. At the end of the incubation period, the percentage frequency and percentage contribution of the fungal flora was calculated (Hogg and Hudson, 1966).

Dilution plate method

The water samples were mixed with sterile distilled water and a series of dilutions were made. From the dilutions, 0.1 ml inoculums was poured onto Rose Bengal agar and incubated at 28±2°C for 1 week to assess the growth of colonies (Waksman, 1922; Warcup, 1950; Bandh et al., 2011; Dar et al., 2013). The number of colonies counted was expressed as (colony forming units) cfu/ml and were calculated by using the formula:

Cfu/ml=n×d

Where, n= number of colonies; d = dilution factor = 1/dilution.

Identification of the fungal isolates was done upto genera level using standard fungal identification key of Barnett and Hunter (1999), Khulbe (2001).

RESULTS AND DISCUSSION

The study was carried out between in four months June-November, 2011 and a total of 40 isolates were obtained during the study. The results of isolation and enumeration are indicated in Table 1. The isolates were identified on the basis of difference in some morphological features like colony appearance, elevation, margin, condia colour and reverse colour of the colonies. The colony appearance ranged from circular, irregular and filamentous. The margin varied from entire filamentous, undulate and lobate. There was also considerable difference in the colour of colonies (dark green, sea green, yellowish white, cream white etc.). The individual colony count of different fungal isolates reveals that isolate type F23 was having the highest number of colonies (n=48) at site I followed by F6, F33, F30 and others. The colony count of isolate F23 was highest (n=24) in the month of July at site 2, isolate F33 had the highest number of colonies (n=38) followed by F30, F23 and others. The colony count of isolate F33 was highest (n=21) in the month of July at site 3, isolate F23 had the highest number of colonies (n=40) followed by F33, F4, F3 and others, and the colony count of isolate F23 and F33 was highest (n=22) in the month of July. At site 4, isolate F23 was had the highest number of colonies (n=40) followed by F33, F8, F5 and others. The colony count of isolate F23 was highest (n=22) in the month of July. The total colony count given indicates that isolate F23 had the highest total colony count (n=156) for all months followed by isolate F33 (n=119). The total monthly fungal population (Cfu/ml) was also recorded for all four months as indicated in Table 2 and the data recorded reveals that the maximum population during July was 3.6×10^2 for site 3 and least for site 1 (1.1×10^2) in November as shown in Table 3. The diameter of the identified species was recorded and ranged from 1.5 to 1.63 cm; the elevation of these species included flat, raised, convex and filamentous. The margin included filamentous entire convex undulate and entire. The colour of the colonies on both upper and reverse sides varied from cream, white, yellow, pink etc. Aspergillus sp. contributed 20% followed by Pencillium sp. (4%) and *Candida* (5%) as shown in Figure 2.

The results obtained regarding the Aspergillus and Penicillium species during the study are in agreement with the study of Kellerman and McBeth (1912) who mentioned that the species of genus *Aspergillus* and *Pencillium* are found in polluted lake waters and act as cellulose decomposer. These genera have also been reported frequently from the drain waters with maximum densities during high pollution (Khulbe and Durgapal, 1994). *Candida* spp. obtained during the study was found to be pathogenic to humans which are a concern but these species can thus act as good indicators of water pollution (Cooke, 1954). The data recorded for water indicates higher temperature during July and lower during November (Table 3). The fungal load also shows decreasing trend from June to November. The higher temperature in July may be the reason for better growth of fungal population. Similar results were suggested by Bock (1956) and Dar et al. (2013), who reported optimum temperature for growth fungi bacteria has to be between 15˚C and 31˚C thus confirming the results obtained during the study. The maximum fungal population in July may possibly be due to more feasible temperature and increase in organic matter (Khulbe and Durgapal, 1992). Increase of fungi indicates increasing organic loading in water (APHA, 1998). During our study in the month of November, fungal population was minimum which may be attributed to the low temperature. These results are in agreement with the study of Khulbe and Durgapal (1992) who in his studies on Naintal Lake, has reported that fungal population was maximum in August during high temperature while it was lowest in the month of January when temperature was relatively low.

Conclusions

From the study, it may be concluded that *Aspergillus* sp. and *Pencillium* sp. were present at all four sites during the study while as *Candida sp.* was found at site I in October and November. However, the Site III, on the River Jhelum showed the highest density of cultivable fungal population.

Table 1. Comparative analysis of different types of colonies found at the four sites in 2011.

Isolate	Site 1				Site 2				Site 3				Site 4			
	Jun.	Jul.	Oct.	Nov.	Jun.	Jul.	Oct.	Nov.	Jun.	Jul.	Oct.	Nov.	Jun.	Jul.	Oct.	Nov.
F_1	+	+	-	-	+	+	-	-	-	-	-	-	+	+	-	-
F_2	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
F_3	-	-	+	-	-	-	+	-	+	+	+	-	-	-	+	-
F_4	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-
F_5	+	+	-	+	+	+	-	+	-	-	+	-	-	-	+	-
F_6	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
F_7	+	+	+	-	+	+	+	-	-	-	+	-	-	-	-	-
F_8	-	-	+	-	-	-	+	-	-	+	-	-	+	+	-	-
F_9	-	-	+	-	-	-	+	-	-	-	-	-	-	-	+	-
F_{10}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
F_{11}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
F_{12}	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-
F_{13}	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-
F_{14}	-	-	-	-	-	-	-	-	+	-	-	-	-	+	+	-
F_{15}	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-
F_{16}	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
F_{17}	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
F_{18}	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
F_{19}	-	-	-	-	-	-	-	-	+	+	-	-	-	+	-	+
F_{20}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
F_{21}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
F_{22}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F_{23}	+	+	+	-	+	+	+	-	+	+	+	-	+	+	-	-
F_{24}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
F_{25}	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+
F_{26}	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+
F_{27}	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
F_{28}	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-
F_{29}	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-
F_{30}	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-
F_{31}	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
F_{32}	-	+	-	+	-	+	-	+	-	-	-	-	-	-	-	-
F_{33}	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
F_{34}	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
F_{35}	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
F_{36}	-	-	+	+	-	-	+	+	-	-	-	-	-	-	+	-
F_{37}	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	+
F_{38}	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-
F_{39}	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-
F_{40}	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-

Table 2. Colony count and cfu/ml at the four sites during the study.

Site	June		July		October		November	
	Colony count	Cfu/ml	Colony count	Cfu/ml	Colony count	Cfu/ml	Colony count	Cfu/ml
I	24	2.4×10^2	30	3.0×10^2	15	1.5×10^2	11	1.1×10^2
II	21	2.1×10^2	29	2.9×10^2	21	2.1×10^2	19	1.9×10^2
III	23	2.3×10^2	36	3.6×10^2	30	3.0×10^2	23	2.3×10^2
IV	22	2.2×10^2	28	2.8×10^2	27	2.7×10^2	18	1.8×10^2

Table 3. Water temperature (°C) and pH recorded at four sites during June and November 2010.

Site	June		July		October		November	
	Temp.	pH	Temp.	pH	Temp.	pH	Temp.	pH
I	17	7.6	20.1	7.1	11	7.6	6	7.0
II	19.1	7.8	19	7.1	11.9	7.7	6.6	7.1
III	21.7	7.7	19	7.0	12.3	7.7	6.2	7.1
IV	21	7.7	22	7.2	11.2	7.8	5.8	7.1

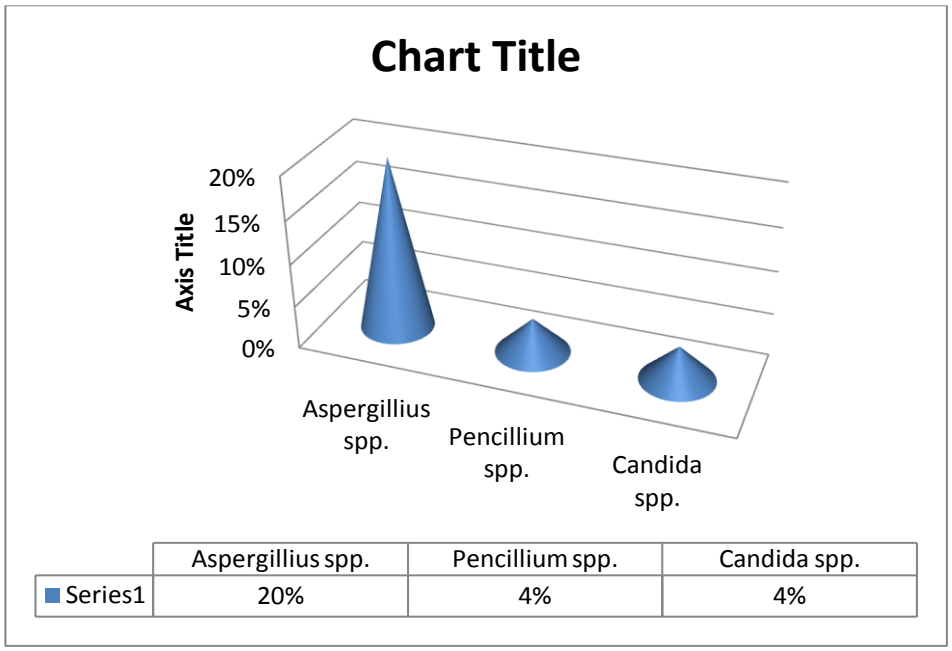

Figure 2. Percentages of identified species of fungi.

Conflict of Interests

The author(s) declare there is no conflict of interests.

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