



Ecological studies of aquatic hyphomycetes in a canal and its connecting irrigation channels

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Abstract

Ecological studies of the aquatic hyphomycetes were carried out at the Lahore Branch of the BRB (Bombarwali Ravi Badian) Canal and its associated irrigation water channels at Quaid-e-Azam Campus, University of the Punjab, Lahore, Pakistan. A comparison was made between the canal and its connecting irrigation channels for the physico-chemical properties and the spora of the aquatic hyphomycetes. Two water channels, WC-1 and WC-2 were selected for this purpose. The canal and the irrigation water channels differed from each other in their riparian vegetation and the water chemistry.

The comparison revealed a greater similarity in aquatic hyphomycete communities of the irrigation water channels as compared to that of canal. A total of nineteen aquatic hyphomycete species were detected from the canal, nineteen from WC-1 and fifteen from WC-2 by using three techniques, leaf pack baiting technique, random sampling of submerged plant materials and membrane filtration of water. The absence of some hyphomycete species and the presence of some unidentified species in irrigation channels showed the absence of a favorable stimulus for survival and presence of some inhibiting factor in these channels. This could be due to the presence of a new set of environmental conditions and also the different riparian vegetation along the channels.

Key words: Aquatic hyphomycetes, BRB canal, irrigation channels, environmental factors

1. Introduction

Aquatic hyphomycetes being the most active group of organisms in the process of decomposition of leaf litter play an important role in the trophic chain (Scholein-Crusius and Grandi, 2003). Many studies have been done on the aquatic hyphomycetes owing to their importance in the aquatic environment. The previous studies on aquatic hyphomycetes have been done in freshwater temperate streams in countries like Canada, England, France, Germany, Hungary, Italy, Pakistan, Switzerland and USA (Bärlocher, 1992). Only a few studies have been done in semi-tropical and tropical areas of world like Egypt (Abdel-Raheem, 1997; 2004), Texas, America (Akeridge and Koehn, 1987), Western Ghats, India (Rajashekhar and Kaveriappa, 1996), Morocco (Chergui, 1990) and South Africa (Van der Merwe and Jooste, 1988). The Lahore Branch of the BRB Canal is a good example of a semitropical water course. The most important character of canal water is its higher temperature regime. This canal represents a massive body of turbid water with steady and uniform flow rate, having its origin from the famous rivers of the Punjab which in turn are fed by freshwater streams in the mountainous areas of Pakistan. Thus this canal has its ultimate origin from freshwater streams but with a different set of physico-chemical characteristics (Firdaus-e-Bareen and Iqbal, 1994).

In the present study, irrigation water channels originating from the canal to feed the cultivated fields directly were studied to determine their hyphomycete communities having a different water chemistry as well as riparian vegetation. The study was aimed at comparing the fungal community of canal with that of irrigation water channels and to observe the fate of aquatic hyphomycetes after their entrance into a new habitat. All the possible techniques were used to study the aquatic hyphomycetes in order to estimate the maximum number of conidial species present in the water. Physical and chemical properties of the water were also measured to determine the differences in water chemistry of three selected sites for aquatic hyphomycetes studies.

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2. Materials and methods

The study was carried out in Lahore (located between longitudes 74 ° and 75 °, and latitudes 31 ° and 32 °). It is located in the plains of the province Punjab in the sub-tropical zone. The details of the sampling site of canal are given by Firdaus-e-Bareen and Iqbal (1994). The irrigation water channels are located at the Quaid-e-Azam Campus, University of the Punjab and are the tributaries of the Lahore Branch of the BRB Canal passing through the campus and reaching the irrigation channels. For convenience, these were named as Water Channel-1 (WC-1) and Water Channel-2 (WC-2). The WC-1 passes along the Main Library of the Quaid-e-Azam Campus and irrigates the lawns and gardens around it. It is about 0.8 m in width and 0.7 m in depth. The rate of flow of water is 7.17 m/min. The main vegetation around this water channel includes *Broussonetia papyrifera* (L.) L'Hér. ex Vent., *Callistemon citrinus* (Curt.) Stapf., *Citrus* sp., *Dalbergia sissoo* Roxb., *Ehretia acuminata* Clarke and *Morus nigra* L. The ecological studies were done in this water channel from June 18, 2007 to September 21, 2007. The second water channel, WC-2 passes along the hostels and is used to irrigate fields cultivated on the opposite side and was studied during June 28 to July 26, 2007. The water channel is 1.3 m in width and 0.5 m in depth. The rate of flow of water in this irrigation channel is 14.13 m/min. The main vegetation along this channel consists of *B. papyrifera*, *Bombax ceiba* L., *Ficus carica* L. and *Terminalia arjuna* Wight & Arn.

2.2. Study of physico-chemical properties of irrigation channels

To compare the water chemistry of canal and irrigation channels, the physical and chemical properties of water were monitored. The water analyses were done weekly. The physico-chemical properties of water were monitored by observing temperature, pH, Electrical conductivity (EC), Total Dissolved Solids (TDS) and Total Solids (TS). The NaCl percentage, chlorides, bicarbonates, Biochemical Oxygen Demand (BOD₅), Chemical Oxygen Demand (COD), sodium, potassium and calcium contents were estimated to determine the chemical properties of the water of these three water courses.

2.3. Baiting experiments

The aquatic hyphomycetes in the canal were studied by leaf pack baits (Iqbal, 1994; Abdel-Raheem, 2004) during a period from March 15, 2007 to September 17, 2007. For the first experiment, dead, brown and disease free leaves of *Populus euramericana* CV-1-214 (Dade) Guinier, culms of *Saccharum bengalense* Retz. and blades of *Imperata cylindrica* (L.) Beauv. were selected as the baiting material. Leaves with visible signs of fungal attack were discarded. The leaves and grass blades were dried at room temperature for three weeks before introducing into the canal water. The outer covering of *S. bengalense* was removed and cut into pieces of 2 cm approx. Leaves, grass blades and culms of *S. bengalense* (5 - 7 pieces in each) were packed in separate nylon mesh bags (6 × 6 cm; mesh size 1mm) which were then tied with three different nylon cords in order to make three individual sets of plant materials. The plant materials were then directly exposed to canal water by fixing with an iron rod submerged perpendicular to the direction of water flow. In the other experiments, culms of *S. bengalense* and blades of four types of grasses, *Cenchrus pennisetiformis* Hochst. & Steud. ex Steud., *Cynodon dactylon* (L.) Pers., *Cyprus rotundus* L. and *I. cylindrica* were used for baiting in the same way as described above. The bags were collected twice a week and transported to the laboratory for further processing.

2.4. Study of aquatic hyphomycetes

The leaves were washed thoroughly in running tap water after collection. Some leaves were randomly selected and square discs of one square cm were cut from each leaf having the margin of leaf lamina as one side of the disc. These discs were incubated in shallow distilled water, one disc in each Petri dish, at room temperature for 24 hours. After sporulation, the leaves were observed under light microscope in Petri plates as well as on glass slides mounted in 0.05% Trypan Blue stain under a cover slip. The developing and released conidia were observed. The percentage frequency of occurrence was recorded. Similarly the discs of *S. bengalense* were washed with tap water to remove any surface mud or other debris. Then the discs were forcefully aerated through an aquarium air pump for 24 hours, in a conical flask of 250 ml filled one third with distilled water. The water in the flask was filtered through Millipore membrane filters of 8 micron pore size. After filtration, the membrane filters were processed on glass slides by heating in an oven at 30°C for 30 minutes and then stained with 0.05% Trypan Blue stain. Percentage frequency of occurrence of aquatic hyphomycetes was recorded by observing these filters microscopically.

Three techniques were used to study aquatic hyphomycetes in the irrigation water channels in order to observe the maximum numbers of species present in these newly studied water courses. To access the conidial number present in water, the water was filtered at the spot through Millipore membrane filters of 8 micron pore size, thrice a week. The water was taken up in a 50 ml syringe and then filtered through a filter assembly by exerting pressure on the piston of the syringe until it became difficult to exert more pressure. After filtration, the membrane filters were taken on glass slides and stained with 0.05% Trypan Blue. They were heated to make the filter membrane colorless. The total numbers

of conidia of each species were recorded. The percentage frequency of occurrence of each species was calculated from the total number of conidia observed. The total numbers of conidia obtained on all filters were pooled and the amount of conidia per litre of water was calculated.

The submerged fallen leaves, branches, submerged roots and runners were randomly sampled from the water channels and transported to the laboratory. The leaves were studied microscopically after incubating in distilled water in Petri plates and the hard materials such as branches, roots and runners were aerated through aquarium air pump in separate conical flasks. The water in the flask was then filtered through Millipore membrane filters of 8 micron pore size. Percentage frequency of occurrence of aquatic hyphomycetes was recorded from these filters.

2.5. Application of ecological software

For the observations on ecological trends, the software Community Analysis Package (CAP) version 4 (Pisces Conservation Ltd., 2008) was used. The Dendrogram of agglomerative clustering showing full linkage between physico-chemical data of the irrigation channels through the Euclidean linkage distance was prepared. A multivariate Principal Component Analysis (PCA) was carried out on square root transformed data of physico-chemical characteristics and aquatic hyphomycetes of canal and its irrigation channels. The PCA plots of covariance were prepared.

3. Results and Discussion

The experiments were conducted to investigate the effects of new environments on aquatic hyphomycetes community and to observe how these fungi tolerate the challenges of a new environment. With the passage of time, canal water has become polluted with point and non-point sources. Addition of sewerage water in the canal is also a source of pollution in it and people also used to dispose off their garbage in the flowing water of canal. In a previous study, in the Lahore Branch of the BRB Canal, a total of sixty seven species were observed during a period from October 1991 to September 1993 by using three possible techniques, water filtration, random collection of submerged fallen leaves and immersion of leaves of some known tree species as baits. A total of thirty five species were observed by baited leaves only. Three genera namely, *Entomophthora*, *Fusarium* and *Helicomyces* belonged to other groups of conidial fungi while all others belonged to freshwater hyphomycetes (Firdaus-e-Bareen and Iqbal, 2003). This was an indication of the rich inoculum of aquatic hyphomycetes present in the canal at that time, which could colonize and decompose the allochthonous plant materials. The physico-chemical properties of canal and irrigation water channels are tabulated in Table 1. The canal water was studied during the period from March 15, 2007 to September 17, 2007.

Table 1. Physico-chemical properties of the Lahore Branch of the BRB canal and its connecting irrigation water channels during the study period.

Parameters studied	Canal		Water Channel-1		Water Channel-2	
	Range	Mean	Range	Mean	Range	Mean
Temperature ($^{\circ}$ C)	19.5 – 27.4	23.45	22 – 27	24.5	22 – 27	24.5
pH	5.59 – 6.0	5.79	5.09-7.25	6.17	5.80-6.02	5.91
Electrical Conductivity (EC) (μ S/cm)	242.3 – 258.5	250.4	251.1-257.7	254.4	206.7-261.1	233.9
Total Solids (TS) (mg/L)	300 – 800	550	400-800	600	450-750	600
Total Dissolved Solids (TDS) (mg/L)	116.4 – 134.9	125.6	127.4-132.0	129.7	111-151	131.0
Sodium chloride (NaCl) (%)	0.3 – 1.5	0.9	0.4	0.4	0.4	0.4
Carbonates (CO_3) (mg/L)	----	----	----	----	----	----
Bicarbonates (HCO_3) (mg/L)	91.5 – 108.6	100.1	85.1-106.1	95.6	71.2-123.6	97.4
Chlorides (Cl) (mg/L)	21.3 – 35.5	28.4	22.3-28.7	25.5	19.1-33.5	26.3
Biological Oxygen Demand (BOD_5) (mg/L)	7.4 – 9.5	8.45	8-12	10	5-7	6
Chemical Oxygen Demand (COD) (ppm)	110 – 124	117	109-133	121	106-112	109
Sodium (Na) (mg/L)	3.45 – 9.5	6.47	4.4-4.8	4.6	4.5-4.9	4.7
Calcium (Ca) (mg/L)	12.5 – 24.5	18.5	10.6-16.2	13.4	10.5-15.3	12.9
Potassium (K) (mg/L)	7.45 – 9.5	8.47	4.9-8.1	6.5	4.39-8.41	6.4

If only a few of these important physical parameters, observed in canal, were compared with that of the previous data by Firdaus-e-Bareen and Iqbal (2003), a remarkable difference between the water of two different periods are observed. The range of temperature from March to September 1993 was 15-27 °C whereas its range was 19.5 °C to 27.4 °C during 2007. The comparison of the individual months from March to September also showed that during the passage of time, the temperature of the canal water had increased and due to the increase in temperature hyphomycetes community of the canal was disturbed in such a way that high temperature sensitive species were eradicated from the canal. According to Belwal et al., (2008) some hyphomycetes species are specific for high temperatures whereas others are sensitive to high temperatures and can tolerate low temperatures only. Similar differences were observed in other factors like pH which has become more acidic as the average recorded pH of 1993 ranged between 6.12-8.19 whereas it was 5.59-6.00 in 2007. Complete linkage by the Euclidean distance measure of agglomerative clustering according to the data on physico-chemical characteristics of the canal and its irrigation channels is shown in Figure 1.

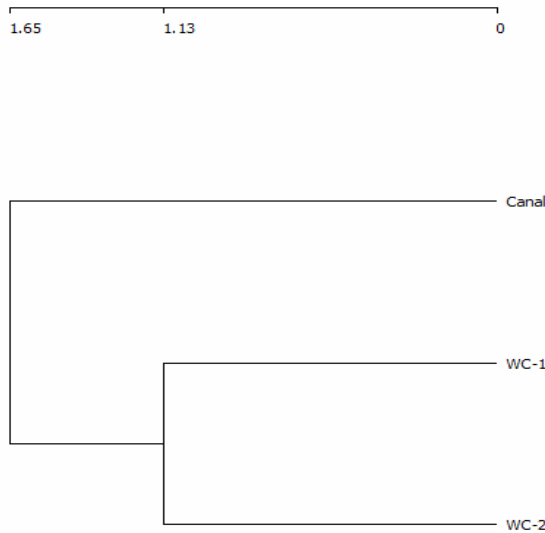


Figure 1. Agglomerative clustering of canal and its associated irrigation channels showing full linkage separated by Euclidean distance.

This figure reveals that the irrigation water channels showed a distinct set of physico-chemical characteristics in comparison to canal. Principal Component Analysis (PCA) of covariance between the physico-chemical characteristics versus the three irrigation channels, sorted out the two irrigation channels from the canal itself (Figure 2), due to deviation in pH, TDS and especially TS.

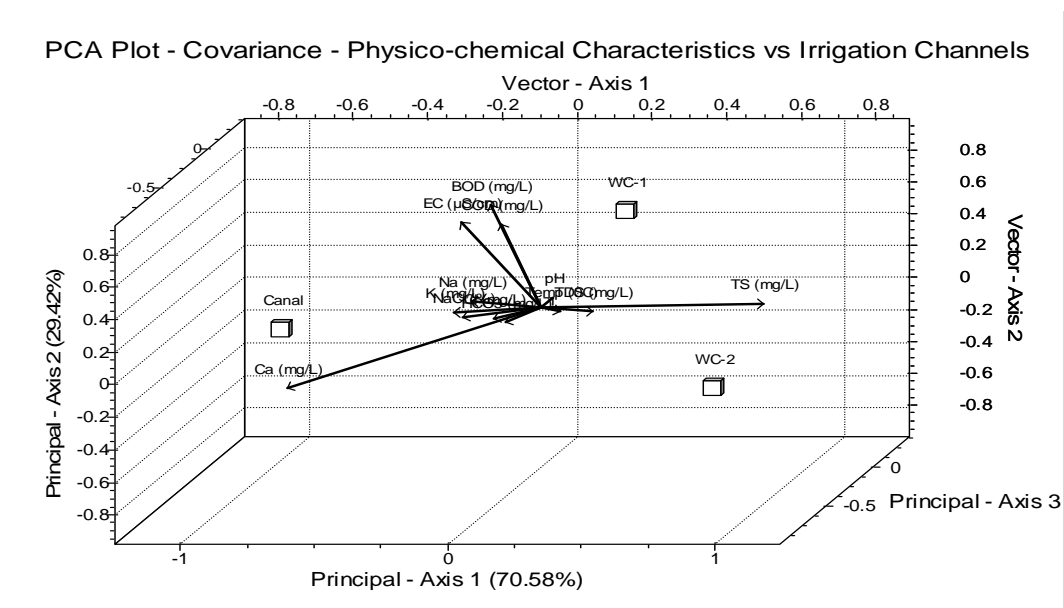


Figure 2. Principal Component Analysis (PCA) showing covariance between the aquatic hyphomyete spora of the canal and its associated water channels.

A total of nineteen species were observed from March 15, 2007 to September 17, 2007 from the canal by leaf pack baiting technique of selected plant materials. *Anguillospora* sp. A, *Anguillospora* sp. B and *Scorpiosporium* sp. could not be identified to the species level. Four species namely Species D, E, F and G could not be identified even up to generic level. These were observed on baited culms of *Saccharum bengalense*. All these unidentified species were multiradiate (Table 2).

Table 2. Aquatic hyphomycetes species observed in the Lahore Branch of the BRB Canal and its connecting irrigation water channels (+ = 20% frequency of occurrence).

Aquatic Hyphomycetes Species	Canal	Water Channel-1	Water Channel-2
<i>Anguillospora</i> sp. A	+	+	+
<i>Anguillospora</i> sp. B	+	+	+
<i>Articulospora proliferata</i> Jooste <i>et al.</i>	+++	++	+
<i>Bacillispora inflata</i> Iqbal & Bhatti	-	+	+
<i>Clavariopsis aquatica</i> de Wild.	-	+	-
<i>Cylindrocarpon aquaticum</i> (Nilss.) Marv. & Desc.	-	+	+
<i>Dimorphospora foliicola</i> Tubaki	+	-	-
<i>Flagellospora curvula</i> Ingold	+	-	-
<i>Flagellospora fusarioides</i> Iqbal	+	++	+
<i>Flagellospora penicillioides</i> Ingold	+++	+++	+
<i>Fusarium</i> sp.	++	++	+
<i>Heliscus lugdunensis</i> Sacc. & Therry	-	+	+
<i>Lemonniera aquatica</i> de Wild.	+	+	+
<i>Lunulospora curvula</i> Ingold	+++	+++	+
<i>Mycofalcella iqbalii</i> Fird. & Braun	+++++	+++	+
<i>Sporidesmium ensiforme</i> Desc.	+++	++	+
<i>Scorpiosporium</i> sp.	+	-	-
<i>Tetracladium marchalianum</i> de Wild.	+	+	-
<i>Triscelophorus monosporus</i> Ingold	+	+	+
Species A	-	+	+
Species B	-	+	-
Species C	-	+	-
Species D	+	-	-
Species E	+	-	-
Species F	+	-	-
Species G	+	-	-
Total number of species	19	19	15

This indicates that the change in environmental factors has reduced the numbers of species in the canal water. The freshwater hyphomycetes are apparently temperature dependent (Bärlocher *et al.*, 2007). Similarly pH of water also affects the presence of aquatic hyphomycetes in water and ultimately rate of decomposition. Dangles and Chauvet (2003) concluded that water with high pH enhanced the rate of decomposition as compared to that of low pH. Low pH elevates the concentration of the heavy metals in the water that retards the fungal metabolism. As the canal is located beside one of the busiest roads of Lahore, there is a possibility that the concentration of lead may be high in its water and with increasing vehicles this concentration might be higher than in the past.

The data of physico-chemical properties of WC-1 showed the range of temperature between 22 to 27°C whereas the mean temperature was 24.5°C. The pH of WC-1 was higher than that of the canal water. All other characteristics were somewhat similar to canal. Carbonates were absent from both the canal as well as the WC-1. Biochemical Oxygen Demand (BOD) was higher whereas K was low as compared to canal water. Similar temperature variation was observed in WC-2. The pH of WC-2 was lower than that of WC-1 resembled more to canal. Electric conductivity was much lower than both the canal and the WC-1. In WC-2 TDS, bicarbonates and Na were higher than WC-1 whereas chlorides, COD, Ca and K were lower. Carbonates were also absent from that water channel. BOD₅ of WC-2 was much lower than the canal and the WC-1.

The most important characteristic feature of irrigation water channels is the intermittent desiccation due to channel closure as these are used for irrigation in fields and gardens and are opened according to the need of the irrigation water in respective fields and gardens. The rate of flow of water in these water channels is also quite low which reduces the aeration necessary for survival of aquatic hyphomycetes in these water courses. The riparian vegetation as well as water chemistry of these channels differs from the canal to some extent. As these water channels represent a habitat of occasional drought, the presence of these aquatic hyphomycetes in these water channels shows their abilities to tolerate

intermittent desiccation but it is certain that their number becomes reduced with time. As in the first baiting experiment in WC-1, there was an intermittent drought of four days in July. The number of species in all cases became reduced as detected by baiting, random collection of submerged plant materials or membrane filtration except in the case of roots and baited culms of *Saccharum bengalense*.

From water channel-1 and water channel-2, nineteen and fifteen species were observed respectively (Table 2). *Fusarium* sp. belonged to other conidial group of fungi, thus, eighteen species were of aquatic hyphomycetes, among them, *Anguillospora* sp. A and *Anguillospora* sp. B could not be identified up to the species level. Three unidentified species, Species A, Species B and Species C were observed. Species A was observed on membrane filters in both the water channels, Species B was observed on baited leaves of *Salix babylonica* whereas Species C was observed on membrane filters and on hard substrata randomly collected as well as baited in water channel-1 only. All of these unidentified species were sigmoid. The conidiophores of Species B resembled the genus *Flagellospora*, however, the species could not be identified. With reference to PCA covariance between the aquatic hyphomycete species versus the irrigation channels. The water channel-1 showed greater similarity of hyphomycete spore to the canal, while water channel-2 distinctly differentiated from both (Fig. 3). The species showing greater affiliation with the canal included *Mycofalcella iqbalii*, *Sporidesmium ensiforme* and *Articulospora proliferata*, while the species showing more association with the water channels included. *Bacillispora inflata*, *Heliscus lugdunensis* and *Anguillospora* sp. B. Water channel-1 showed a greater similarity to the canal as compared to channel-2.

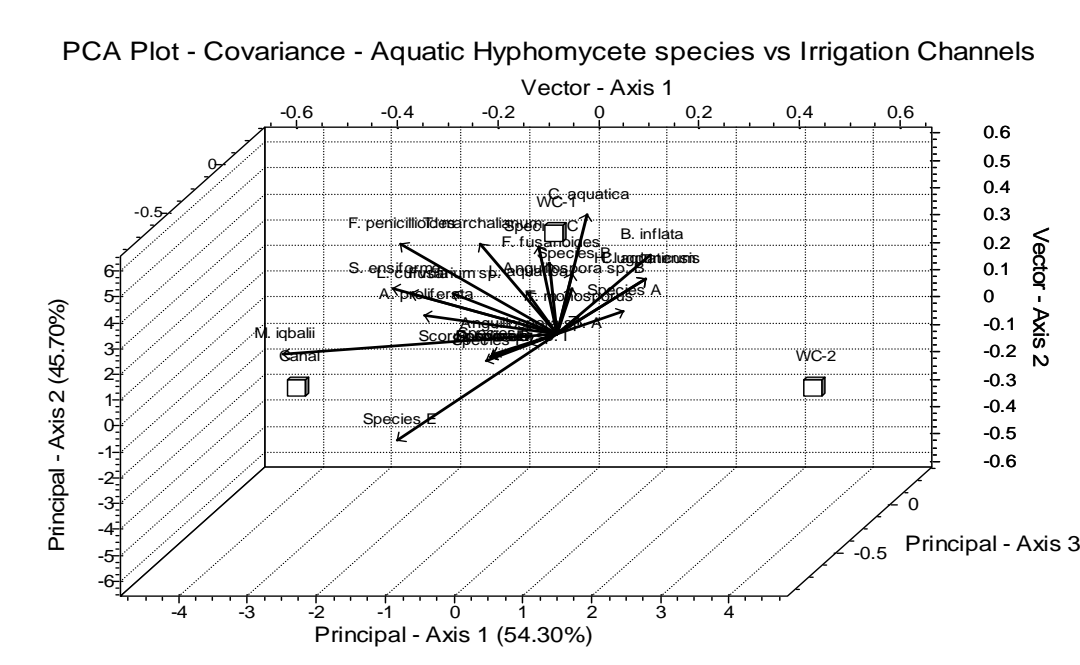


Figure 3. Principal Component Analysis (PCA) showing covariance between the aquatic hyphomycete spora of the canal and its associated water channels.

The comparison of similarity indices between the canal and water channels indicated that the species present in these irrigation water channels were quite similar to those of canal except for three unidentified species only found in water channels and were not reported from the canal. The other species that were absent from the canal but were present in the irrigation water channels, were observed in the canal in previous studies by Firdaus-e-Bareen and Iqbal (2003). These species were also observed in the canal during 2005-2006 on membrane filters as well as on randomly collected submerged plant debris (unpublished). However, a total of seven species of aquatic hyphomycetes found in the canal were absent from both the irrigation water channels.

The fungal community in these two irrigation water channels was very similar to each other showing high similarity indices. Most of the species were similar in these channels but *Clavariopsis aquatica*, *Tetracladium marchalianum* and two unidentified species, Species B and C were not observed in WC-2, neither on any plant material nor by the membrane filtration technique. The difference in fungal species may be due to the environmental conditions present in these irrigation water channels and the riparian vegetation along these water channels. The effect of riparian vegetation on fungal community can be seen by replacing one community type by another. This shows that there is a significant correlation between fungal community and riparian vegetation (Ferreira et al., 2006).

Wood-Eggenschwiler and Bärlocher (1983) found that streams in the same region with similar water chemistry had a higher percentage of species in common than streams of contrasting water chemistry. The differences in the fungal communities of the canal as well as the irrigation water channels are less because the differences in the water chemistry which are less pronounced between canal and water channels.

The studies in water channels were used to define the aquatic hyphomycetes community of these newly studied water bodies. The ecological studies of freshwater hyphomycetes depend upon the techniques used to study them in a habitat. The fungal communities can be efficiently characterized by using data generated by all the techniques simultaneously (Iqbal, 1994). In the present study of water channels, an attempt has been made to use the results obtained by using three techniques to describe the communities of aquatic hyphomycetes. The dominant species of aquatic hyphomycetes were *Mycofalcella iqbalii* and *Sporidesmium ensiforme* by almost all techniques (Table 3).

Table 3. Top ranking species of Aquatic Hyphomycetes detected by different techniques in water channel-1 and water channel-2.

Techniques used to study Aquatic Hyphomycetes		Habitats studied	
		Water Channel-1	Water Channel-2
Membrane filtration technique		<i>Flagellospora pennicillioides</i>	<i>Flagellospora pennicillioides</i> & <i>Mycofalcella iqbalii</i>
Random collection of submerged plant materials	Branches	<i>Sporidesmium ensiforme</i>	<i>Mycofalcella iqbalii</i> & <i>Sporidesmium ensiforme</i>
	Leaves	<i>Lunulospora curvula</i>	<i>Lunulospora curvula</i>
	Roots	<i>Sporidesmium ensiforme</i>	<i>Sporidesmium ensiforme</i>
	Runners	<i>Sporidesmium ensiforme</i>	<i>Sporidesmium ensiforme</i>
Baited plant materials	Branches of <i>Callistemon citrinus</i>	<i>Sporidesmium ensiforme</i>	<i>Sporidesmium ensiforme</i>

In water channel-1, seventeen species were observed on membrane filters, fourteen on randomly collected plant materials and eleven on baited plant materials. In water channel-2, fifteen species were found in running water as detected by membrane filters, eleven on each randomly collected and baited plant materials. Among the randomly collected plant materials in WC-1, a total of eleven species were observed on submerged branches, ten on submerged leaves, nine on roots and nine on runners also. On baited plant materials, four and six species were observed on leaves of *Populus euramericana* and *Salix babylonica* respectively, nine on branches of *Callistemon citrinus* and eight on *Saccharum bengalense* were observed. In WC-2, a total of ten species were observed on randomly collected branches, five on leaves, seven on roots and six on runners. On baited leaves of *Populus euramericana* and *Salix babylonica* four and five species were observed respectively, eight on branches of *Callistemon citrinus* and ten on *Saccharum bengalense*. There were little differences in the number of species present on individual plant material. However, the top ranking species on membrane filtration and on plant materials were the same in both the water channels. Thus it can be concluded that the differences in the water bodies, connected to each other, may effect the number and presence of species but do not alter the complete fungal community or replacement of one community with another.

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