Mycopath (2007) **5**(2)**:** 65-70

Colonization pattern of aquatic hyphomycetes on persistent substrata in some irrigation channels of Lahore

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Abstract
The aquatic hyphomycetes showed a characteristic pattern of colonization on baited branches of *Callistemon citrinus* and culms of *Saccharum bengalense* in tropical irrigation water channels in Lahore. A total of twelve species of aquatic hyphomycetes colonized these substrata. Some of the species could be identified as early or late colonizers on these baited materials. *Sporidesmium ensiforme* and *Mycofalcella iqbalii* showed the most distinctive pattern. *Sporidesmium ensiforme* was the early colonizer and showed maximum colonization during the first two weeks and then it declined while *M. iqbalii* colonized it in the second week with increasing colonization till the end of baiting period. Other early colonizer species included *Lemonniera aquatica* and an unidentified species Sp C whereas *Articulospora proliferata* and *Flagellospora fusarioides* appeared to be the late colonizers.
Keywords: Aquatic hyphomycetes, colonization pattern, substrata, irrigation channels.

Introduction
Significant amounts of woody debris are found in stream channels. Shearer (1992) had reviewed the role of woody debris in the life cycles of aquatic hyphomycetes. One of the important features is its longer persistence in streams sometimes extending over several years. In a stream system, woody debris influences channel morphology, increases habitat diversity, affects transport of other materials by forming debris dams and serves as a carbon source, site of attachment and shelter for aquatic organisms (Harmon *et al.*, 1986). The collected decaying wood from a stream and estuary in Tai Ho Bay, Hong Kong was investigated for the biodiversity of saprobic fungi recording 55 taxa, including 33 ascomycetes and 22 anamorphic fungi (Tsui and Hyde, 2004). In low-order streams, woody substrata have a structuring effect on aquatic hyphomycete communities (Gönczöl and Révay, 2003). Despite its importance in rivers and streams little is known about how woody debris is processed by nature.

Aquatic hyphomycetes rapidly colonize the available substrata and within few days, releasing prodigious numbers of conidia for further dispersal (Bärlocher, 1992). Changes in chemical compositions during decomposition of submerged plant materials show selective inhibition or stimulation of fungal species and exhibit biological interactions, resulting in a succession (Shearer and Webster, 1991).

This study was carried out in order to observe the role of woody or enduring substrata with longer residence time in irrigation water channels which represent difficult ecological situation with intermittent dry periods. For this purpose, two water channels were selected flowing through the Quaid-e-Azam Campus, University of the Punjab, Lahore, at different places. The colonization pattern of aquatic hyphomycetes on baited submerged materials, namely branches of *Callistemon citrinus* (Curt.) Stapf. and culms of *Saccharum bengalense* Retz., was studied having a much longer residence time than leaves due to the slow rate of decomposition.

Materials and Methods

The Sampling Sites
The selected 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. For convenience these were named as Water Channel-1 and Water Channel-2. The first water channel WC – 1 passes along the Main Library of the Quaid-e-Azam Campus and irrigates the lawns and gardens around it. The main vegetation around this water channel includes *Broussonetia papyrifera* (L.) L'Hér. ex Vent., *Callistemon citrinus*, *Citrus* sp., *Dalbergia sissoo* Roxb., *Ehretia acuminata* Clarke and *Morus nigra* L. The second water channel WC-2 passes along

the row of Hostels used to irrigate fields cultivated on the opposite side. The main vegetation along this channel consists of *Broussonetia papyrifera, Bombax ceiba* L., *Ficus carica* L. and *Terminalia arjuna* Wight and Arn.

Leaf pack baiting experiments

The colonization by aquatic hyphomycetes on two substrata, the culms of *Saccharum bengalense,* and thin branches of *Callistemon citrinus* was studied by the leaf pack baiting technique of Shearer and Webster (1985). These were cut into 2 cm pieces and were packed in separate nylon mesh bags (6 × 6 cm; mesh size 1 mm) tied with nylon cords. Each set consists of fifteen bags of each plant material. These plant materials were then directly submerged under water against the direction of flow of water. The baited bags, one for each plant material, were collected thrice a week and transported to the laboratory for further processing.

The baited materials were washed with tap water to remove any surface mud or other debris. They were aerated through an aquarium air pump for 24 hours, in shallow distilled water in two separate conical flasks. The water in the flasks was then 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 ^{0}C for 5 minutes and then stained with 0.05% Trypan blue stain. Percentage frequency of occurrence of aquatic hyphomycetes was recorded.

Results

Study of Colonization Pattern of Aquatic Hyphomycetes in the Irrigation Water Channel – 1:

I. Baited branches of *Callistemon citrinus*

A total of nine species of aquatic hyphomycetes were observed on the baited branches during June 25 to September 17, 2007. Nine species of aquatic hyphomycetes were observed during 33 days of baiting on the branches of *C. citrinus* baited in the Irrigation Water Channel–1 during 25th June to 27th July, 2007. There was an intermittent desiccation period of four days from July 2 to July 5 during which lesser number of species were observed on the baited material. An unidentified species named as Sp. C was also observed on that plant material during this experimental period. Seven species were found on that substratum during 25 days of baiting from 24th August to 17th September, 2007. *Sporidesmium ensiforme* Desc. was dominant in occurrence in both the experiments (Table 1).

II. Baited culms of *Saccharum bengalense*

A total of eight species of aquatic hyphomycetes was observed on the baited culms of *S. bengalense* during June 18 to September 17, 2007. All these species were found on the baited culms during the 37 days of baiting from June 18 to July 24, 2007. There was an intermittent desiccation of four days from July 2 to July 5 during which the number of species became reduced. An unidentified species named as Sp. C was also observed on that plant material during this experimental period. *Sporidesmium ensiforme* was found to be dominant. Culms of *S. bengalense*, exposed for different periods, in the WC – 1, were studied for 25 days from 24th August to 17th September, 2007. Six species of aquatic hyphomycetes were found colonizing *S. bengalense*. During this period, both *Sporidesmium ensiforme* and *Mycofalcella iqbalii* Fird. & Braun were dominant (Table 1).

Study of Colonization Pattern of Aquatic Hyphomycetes in the Irrigation Water Channel – 2:

I. Baited branches of *Callistemon citrinus*

Eight species of aquatic Hyphomycetes were found colonizing baited branches of *C. citrinus* during 29 days of baiting from 28th June to 26th July, 2007 in the Irrigation Water Channel–2. *Sporidesmium ensiforme* was the dominant species with the maximum frequency of occurrence (Table 1).

II. Baited culms of *Saccharum bengalense*

In the Irrigation water channel – 2, ten species of aquatic hyphomycetes were found on *S. bengalense*. During the 29 days of baiting from June 28 to July 26, 2007, *S. ensiforme* was found to be dominant (Table 1). A species *Anguillospora* sp. B could be identified only on the generic level.

The colonization of aquatic hyphomycetes on these substrata showed a characteristic pattern. On introduction of branches and culms into the water, species richness was initially low, reached a specific peak and declined again (Figure 1). *Fusarium* sp., *Lemonniera aquatica, Sporidesmium ensiforme* and an unidentified species Species C were the early colonizers whereas *Articulospora proliferata* and *Mycofalcella iqbalii* were the late colonizers. *Flagellospora fusarioides* was found sporulating on *S. bengalense* only. The most distinct colonization pattern of colonization was observed for two species, *S. ensiforme* and *M. iqbalii* on both baited branches of *C. citrinus* and culms of *S. bengalense*. *Sporidesmium ensiforme* was the early colonizer of these baited materials and appeared within a week of baiting and its occurrence on these materials decreased to a minimum during the third week of baiting whereas *M. iqbalii* being the late colonizer, appeared after the second week of baiting and its frequency of occurrence increased with time along with a decrease in frequency of occurrence of *S. ensiforme*. The colonization pattern of these species on branches of *C. citrinus* and on culms of *S. bengalense* is shown in figures 2 and 3 respectively.

Discussion

Wood is an important component of the aquatic habitat sheltered by higher plants. The fungi occur commonly and abundantly on submerged wood (Tsui and Hyde, 2004). Wood is a complex of many types of chemicals whose concentrations vary among and within species, within parts of a single tree (e.g., sapwood, heartwood and bark) and with height (Findlay 1985) and the presence of aquatic hyphomycetes on decaying wood reveals that the chemical compounds present in wood are susceptible to these fungi or these species have enzymes to degrade them.

Abdel-Raheem and Ali (2004) observed that different aquatic hyphomycetes species vary in their susceptibility to soluble substances present in the plant materials. This depends upon their ability to produce enzymes to degrade the substances present in plant material. When isolated from woody substrates for their ability to produce extracellular lignocellulolytic enzymes on solid media, *Alatospora acuminata*, *Flagellospora penicillioides* and *Triscelophorus monosporus* were positive for all tested enzymes, endoglucanase, endoxylanase, β-glucosidase, laccase, peroxidase, polyphenoloxidase, tyrosinase and β-xylosidase. *Anguillospora longissima* was positive for all enzymes except lignin-peroxidase. The species show remarkable ability to produce cellulase while few species were positive for lignin-peroxidase. The ability of the species to produce other lignocellulotic enzymes ranged from 50% to 83%. Freshwater hyphomycetes have been shown to produce a rich array of enzymes able to degrade the polysaccharides of plant debris. In the present experiments, the presence of *Flagellospora pennicillioides* and *Triscelophorus monosporus* reveals the presence of chemical compound that could be degraded by these enzymes.

As the chemical composition of substrates changes during the decay (Maria *et al*., 2006) the species colonizing the decaying wood also changes with time named as succession. Sivichai and colleagues (2002) suggested the replacement of fungal species over time during decay of substratum. Such type of distinct species replacement is not usually observed on leaves, rather initial colonizers persist as new species are added and then some members of the community disappear as the substratum is degraded (Chamier and Dixon, 1982; Bärlocher and Schweizer, 1983). The longer persistence of wood as compared to leaves and the gradual release of new substrata through the sloughing off old colonized substrata may permit successive colonization of wood. Whether the species replacement observed by Shearer and Webster (1991) are truly successional or whether they are cyclic and related to species seasonality is not known. However, in the present study, early and late colonizers were clearly observed. *Fusarium* sp., *Lemonniera aquatica*, *Sporidesmium ensiforme* and a new species named as Species C were the early colonizers whereas *Articulospora proliferata* and *Mycofalcella iqbalii* were the late colonizers in both *Callistemon citrinus* and *Saccharum bengalense*. *Flagellospora fusarioides* was also a late colonizer of *S. bengalense*. Other species occurred randomly on both substrata. These results show that the chemical composition of the decomposing wood may change over time thus changing the types of colonized species. However, the complete replacement of one community with another was not observed in any of the experiments, though the replacement of one specific species by the other was observed. As the colonization by *Sporidesmium ensiforme* gradually declined, *Mycofalcella iqbalii* invaded the baited materials. *Sporidesmium ensiforme* may disappear after one month of baiting. This decrease and disappearance of *Sporidesmium ensiforme* may be due to the decrease or removal of the compounds necessary for the survival of *Sporidesmium ensiforme* on the substratum. Similarly the gradual appearance or increase in number of conidia of *Mycofalcella iqbalii* indicates that the compounds that can be degraded by this species became available when *Sporidesmium ensiforme* exhausted its required substances.

Previous studies indicated that when a specific substratum is introduced into the stream, species richness is initially low, rises to a peak within a few weeks, then remains stable or declines slightly (Chamier *et al*., 1984). The two plant materials baited in these experiments also show this basic pattern, however, their behaviour slightly differ from each other. On *Callistemon citrinus*, the species richness first declined a little and then rose to a maximum during the second week of introduction in water and declined again. This may be due to the fact that as the wood decomposed and released new substrata the susceptibility of substrata to number of species increased or decreased according to the type of species present in the aquatic environment in which the plant material is baited. The baited plant material has two peaks which means that the species richness first increase to a specific number and then decrease to a minimum and again increased to get another peak of species richness and finally decreased to a minimum. This shows that the gradual sloughing off plant material released the substratum susceptible to most of the species present in these tropical water channels and these substances were utilized by the aquatic hyphomycetes species. Again when these newly available substances were utilized by other species having different enzymes to degrade them, another peak was obtained after two weeks of the first peak.

The importance of study of aquatic hyphomycetes in these irrigation water channels is the role of woody debris during the desiccation period which is the most important characteristic feature of these water channels. 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 desiccation in persistent substrata. However, their conidial numbers become reduced with time on baited culms of *Saccharum bengalense* but in the case of baited branches of *Callistemon citrinus* there was no effect on the number of species colonized. According to Umm-ul-Banin (2006), aquatic hyphomycetes show better survival on hard and persistent substrata. This shows that harder and persistent debris plays a role in the survival of aquatic hyphomycetes during harsh dry periods.

Table 1: Aquatic Hyphomycetes species detected by baited branches of *Callistemon citrinus* and culms of *Saccharum bengalense* in the Irrigation Water Channels

Hyphomycetes species	**Occurrence on**			
	Callistemon citrinus		*Saccharum bengalense*	
	WC-1	WC-2	WC-1	WC-2
Anguillospora sp. B	-	-	-	+
Articulospora proliferata Rold. & Merwe.	+	+	+	+
Bacillispora inflata Iqbal & Bhatty	+	+	-	-
Flagellospora fusarioides Iqbal	-	+	-	+
Flagellospora penicillioides Ingold	++++	++	+++	++
Fusarium sp.	++	+	++	+
Lemonniera aquatica de Wild.	+	-	+	+
Lunulospora curvula Ingold	++	+	+	+
Mycofalcella iqbalii Fird. & Braun	+++	++	+++	++
Sporidesmium ensiforme Desc.	+++++	++	+++++	++
Triscelophorus monosporus Ingold	-	-	-	+
Sp. C	+	-	+	-

* Each + indicates the frequency of occurrence of conidial species between range 1-4.

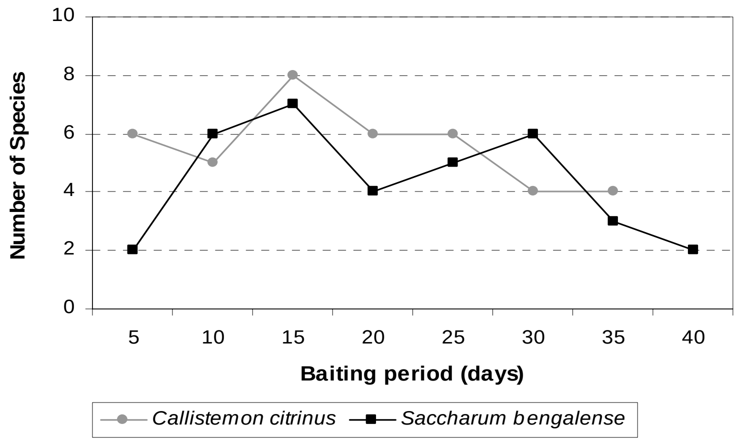

Fig. 1: Species richness on baited branches of *Callistemon citrinus* and culms of *Saccharum bengalense* during the baiting period

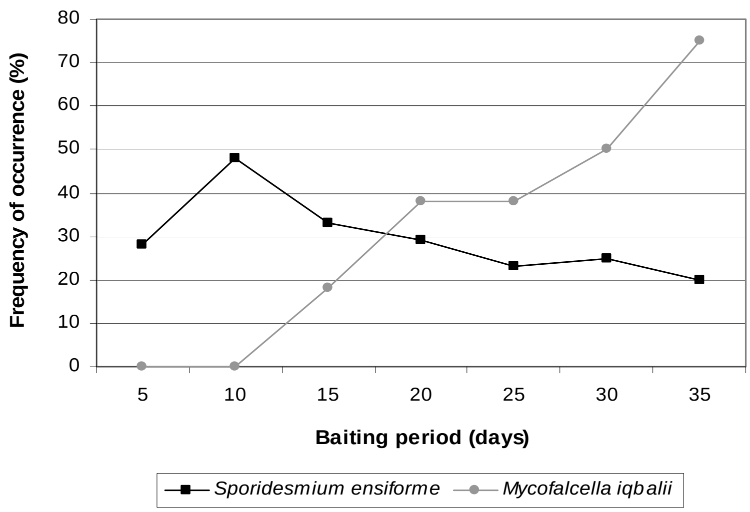

Fig. 2: Colonization pattern of *Sporidesmium ensiforme* and *Mycofalcella iqbalii* on the baited branches of *Callistemon citrinus*

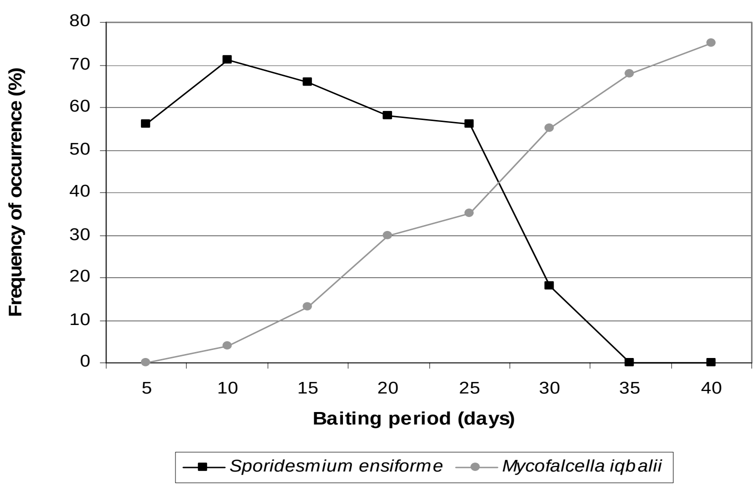

Fig. 3: Colonization pattern of *Sporidesmium ensiforme* and *Mycofalcella iqbalii* on baited culms of *Saccharum bengalense*

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