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Freshwater hyphomycete spora of the River Ravi

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Abstract

In a study of the river spora of Ravi, Lahore, twenty-nine conidial species mostly belonging to freshwater hyphomycetes were recorded by four techniques from January to June, 1999. These species were recorded by filtration of water, sporulation on submerged plant material and trapping in natural or artificial foam. The natural foam provided the richest data on the river spora. A few species were observed colonizing the submerged plant material. The data obtained by direct filtration and artificial foam trapping was quite similar. *Articulospora proliferata* and *Tetracladium marchalianum* were the only species detected by all the four techniques.

Key Words: Freshwater hyphomycetes, foam spora, the River Ravi.

Introduction

The freshwater hyphomycetes are distributed geographically extending from arctic to equator (Bärlocher, 1992). There are a number of reports on the occurrence of freshwater hyphomycetes in the tropical and temperate regions of the world.

The plains of Punjab in Pakistan have five large rivers connecting with the largest canal irrigation system of the world. This network of canals has its origin from the large rivers in the Punjab, one of which is Ravi near Lahore. These rivers are formed by the pooling of water from the freshwater streams. So, the rivers here have their ultimate origin from the freshwater streams which are quite rich in hyphomycete spora.

The dynamics of freshwater hyphomycetes have been recorded in a canal near University Campus , Botany department, and the canal has been described as a new niche for these fungi (Firdaus-e-Bareen and Iqbal, 1994, 2003). It seemed worth investigating their occurrence in a very large body of water such as a river from which these irrigation channels have been drawn out in various directions.

Detail of the sampling site

The sampling site in the River Ravi is located at the old bridge of Ravi, near Jehangir's tomb. This site was selected because of thick riparian vegetation very close to the bank of river and also because of lesser chances of mixing polluted water from the metropolitan areas.

The river at this site is lined by trees like *Acacia nilotica* (L.) Del., *Dalbergia sissoo* Roxb., *Ficus relegiosa* L., *Tamarix articulata* Yahl. and *Zizyphus jujuba* Mill. Shrubs and tall statured plants like, *Calatropis procera* (Willd.) R. Br., *Malva sylvestris* L., *Parthenium hysterophorus* L., *Phragmites australis* (Cav.) Trin ex Stend., *Saccharum munja* Roxb. were observed. The herbs included a variety like *Achyranthes aspera* L. *Cenchrus pennisetiformis* Hochst., *Chenopodium murale* L., *Coronopus didymus* (L.) Sin., *Datura alba* Nees., *Euphorbia helioscopia* L., *Imperata cylindrica* (L.) P. Beauv., *Launea nudicaulis* Hk. F., *Lippia nodiflora* (L.) Rich. *Melilotus albus* Desr., *Polygonum barbatum* L., *Saussurea candicans* Clarke, *Sissymbrium irio* L., *Solanum nigrum* L., *Vicia faba* L. etc.

Direction of flow of the river Ravi at the site is southwest. Water level of the river Ravi shows daily fluctuations depending upon the prevailing conditions. It faces many disturbances like the cows and buffaloes entering and leaving the water daily thus adding their excretory materials and fodder into the water.

The physico-chemical characterstics of the river are given in Table I. Usually the water level stoops down during the months of January and February because of less water supply from mountain streams in winter. It keeps on increasing with the approaching summer and becomes maximum in the rainy season during July and August. However, during the study period, the lowest water level was recorded in March and April.

Table I: Some physico-chemical characteristics of water in the River Ravi during January to June, 1999.

Parameter	Jan	Feb	Mar	Apr	May	Jun
Water level	Med. To V. High	V.Low to V. High	V. Low	V. Low	V. Low to V. High	V. High
Prevailing Conditions	Mostly Dry	Mostly Dry	V. Dry	V. Dry	Rainy & Dry	Dry
Temperature (°C)	12-14	13-15	10-18	20-28	28-35	30-32
pH	7.86	7.95	7.75	7.85	7.75	7.70
E.C. (μS/cm)	340	360	330	340	290	250
K (milliequivalents/L)	0.07	0.09	0.05	0.07	0.04	0.04
HCO (milliequivalents/L)	2.50	2.20	1.60	2.10	1.90	1.40
Cl (milliequivalents/L)	0.40	0.40	0.30	0.30	0.40	0.60
SO (milliequivalents/L)	0.60	1.20	1.50	1.10	0.60	0.60
NO (milliequivalents/L)	0.02	0.02	0.02	0.02	0.01	0.01
Total cations	3.57	3.78	3.46	3.58	3.01	2.65
Total anions	3.52	3.82	3.42	3.52	2.91	2.61
Dissolved solutes (mg)	196	212	200	196	164	144

The chemical analysis of water was done in the laboratory of SCARPS (WAPDA), Lahore. The maximum amount of cations and anions was recorded in the month of February and minimum in June but with insignificant difference. Dissolved solutes showed a decreasing trend towards June. The temperature showed an increasing trend with the shift of season from winter to summer while pH and E.C. showed a decreasing trend during this time period of six months. Only temperature and E.C. showed some significant change.

Materials and Methods

Freshwater hyphomycete river spora was sampled from January to June, 1999. The water was filtered through Millipore membrane filters of 8 μm size (Iqbal and Webster, 1973). River water was sucked up at the spot in 100 ml syringes adjustable onto filter assemblies of 1 cm diameter. At least 10 replicate filters were prepared daily. They were fixed in Trypan Blue stain in small petridishes. They were transported to the laboratory and processed by flooding in the same stain and heating in an oven at 60°C for half an hour. Variable quantities of water were filtered daily depending upon the variation in turbidity of water. Thus more water could be filtered during the days when water was clear. The total number of conidia per litre of water was calculated daily.

For preparation of artificial foam trap (Iqbal, 1993) a spot in the river was selected where the water passed in relatively higher speed. A pool was prepared by the addition of branches and logs and enough detergent was added to form a luxurious amount of foam. It was allowed to persist for 10 minutes and then scooped up in a McCartney bottle. It was fixed in F.A.A. and filtered in a way similar to water.

Natural foam when present was scooped, fixed and filtered in a similar way as artificial foam. The submerged material present in the river in the form of leaves and twigs was collected daily and brought to the laboratory where it was incubated at 20°C for twenty -four hours. Sporulation of any hyphomycete conidial species was recorded.

Results

A total of 43 conidial species was observed during six months in the River Ravi (Table II). The maximum species were observed in natural foam and the least number was recorded on submerged plant material.

About ten species were recorded in water. The number of species fluctuated between 3 to 5. The maximum number of conidia was observed in February and a decreasing trend was observed from February to June. The conidial numbers decreased significantly after March conforming to the temperature changes. The number of species appearing on submerged material did not exceed 4.The data obtained by artificial foam trapping was quite similar to that obtained by water filtration. The species of hyphomycetes were almost the same.

The richest spora appeared in natural foam. A total of 42 species was observed. Some of the species could belong to other types of aquatic fungi. The species observed throughout the six months of sampling include *Articulospora proliferata* and *Tetracladium marchalianum*. Certain species appeared in the first quarter, indicating the preference for low temperature e.g.

Alatospora acuminata, Anguillospora crassa, Campylospora chaetocladia, Mycocentrospora iqbalii, T. elegans, while others appeared later during the warm span like *Anguillospora gigantea* and *A. curvula.*

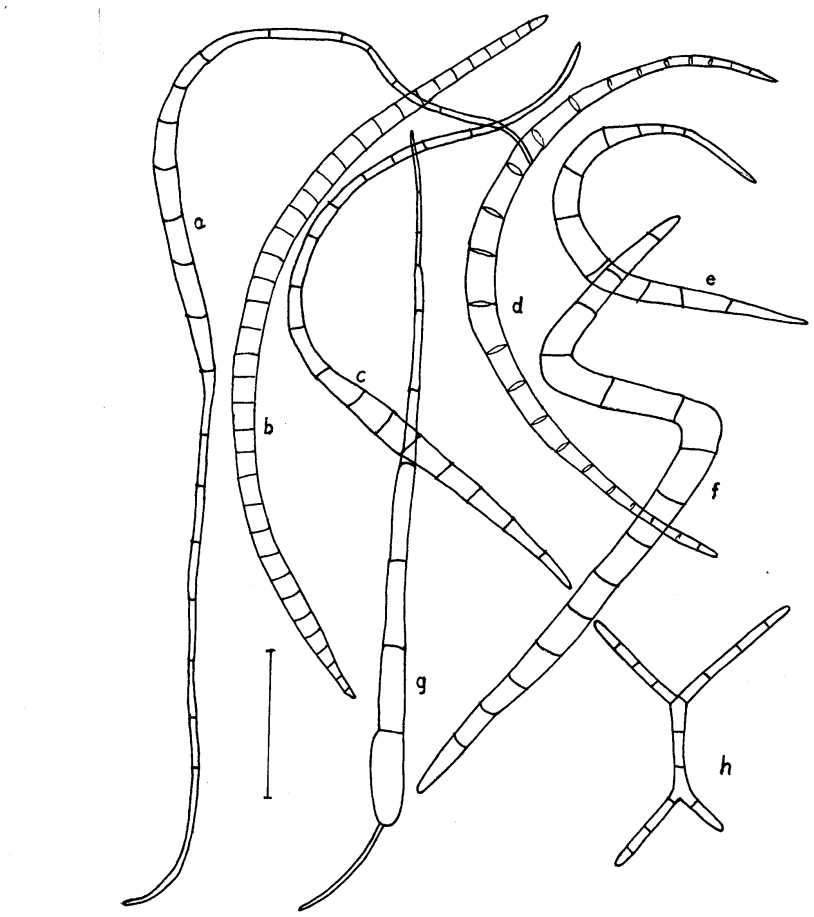

Fig. 1: Some unknown species of Freshwater hyphomycetes from the river Ravi. a: *Anguillospora* sp. I; b: *Anguillospora* sp. II; c: *Anguillospora* sp. III; d: *Anguillospora* sp. IV; e: *Anguillospora* sp. V; f: *Anguillospora* sp. VI; g: *Mycocentrospora* sp. I; h: *Scorpiosporium* sp. I. Scale line= 50 µm

Table II: Conidial Species in the River Ravi as observed by four sampling techniques during January to June (J: Jan, F: Feb, M: Mar, A: Apr, Ma: May ,Ju: Jun), 1999.

Conidial Species	**Sampling Method**			
	Water filtration	**Leaf sampling**	**Artificial Foam**	**Natural foam**
Alatospora acuminata Ingold	-	-	-	(J, Ma)
Alatospora pulchella Marv.	-	-	-	(J)
Anguillospora crassa Ingold	-	-	-	(J, A)
Anguillospora curvula Iqbal	-	-	-	(Ju)
Anguillospora gigantean Ranzoni	-	-	-	(A, Ma)
Anguillospora longissima (Sacc.& Syd.) Ingold	(A)	-	(J,Ma)	(A)
Anguillospora sp.I	-	-	(A)	(J)
Anguillospora sp.II	-	-	-	(Ma)

Anguillospora sp.III	-	-	-	(Ma)
Anguillospora sp.IV	-	-	-	(Ju)
Anguillospora sp.V	-	-	-	(Ju)
Anguillospora sp.VI	-	-	-	(Ju)
Anguillospora sp.VI	-	-	-	(Ju)
Articulospora proliferata Jooste *et al.*	(J, F, M, A, Ma, Ju)	(F, M, A, Ma, Ju)	(J, F, M, A, Ma, Ju)	(J, F, M, A, Ma, Ju)
Bacillispora inflata Iqbal	-	-	-	(Ma)
Campylospora chaetocladia Ranzoni	-	-	(F, M)	(J, F, M)
Cylindrocarpon aquaticum (Nilss.) Marv. &Descals	(Ma)	-	-	(Ma)
Dimorphospora foliicola Tubaki	-	(Ju)	-	-
Dwayaangam cornuta Descals	(J)	-	(F)	(F)
Flagellospora curvula Ingold	(J)	-	-	(J, A)
Flagellospora penicillioides Ingold	-	-	-	(Ma)
Fusarium sp.	(J, F, M, A)	(F, M, A, Ma)	(J, F, A)	(J, F, M, A, Ma, Ju)
Lunulospora curvula Ingold	(Ma, Ju)	-	(Ma, Ju)	(M, A, Ma, Ju)
Mycocentrospora acerina (Hartig)Deighton	(J)	-	-	(J)
Mycocentrospora iqbalii sp. Ined.	(J, F)	-	(J)	(J, F, M)
Scorpiosporium sp.	-	-	-	(Ju)
Tetrachaetum elegans Ingold	-	-	-	(J, F, M)
Tetracladium marchalianum de Wild.	(M, A, Ma, Ju)	(F, M, A, Ma, Ju)	(J, F, M, A, Ma)	(J, F, M, A, Ma, Ju)
Triscelophorus monosporus Ingold	-	(F, M)	(J, F, Ju)	(J, Ju)
Species 1	-	-	-	(J, A, Ju)
Species 2	-	-	-	(F)
Species 3	-	-	-	(F)
Species 4	-	-	-	(F, Ma)
Species 5	-	-	-	(F, Ju)
Species 6	-	-	-	(A)
Species 7	-	-	-	(A, Ma)
Species 8	-	-	-	(Ma)
Species 9	-	-	-	(Ma)
Species 10				(Ma)
Species 11	-	-	-	(Ma, Ju)
Species 12	-	-		(Ju)
Species 13	-	-	-	(Ju)
Species 14	-	-	-	(Ju)
Total no. of species	10	5	10	42
Total no. of species (Total no. of conidia/L of water) in different months	**J: 6 (1093.93) F:3 (1204.56) M:3 (1111.11) A:4 (284.2) Ma:4 (403.33) Ju:3 (245.37)**	**F:4 M:4 A:3 Ma:3 Ju:3**	**J:6 F:6 M:3 A:4 Ma:4 Ju:3**	**J:14 F:11 M:7 A:11 Ma:17 Ju:17**

Most of the unknown species were recorded in May and June in natural foam. A brief description of the species follows:

Species 1 (Fig.2 a): This species is nearest to *Tricladium eccentricum* .

Species 2 Fig 2 b): These conidia found in natural foam have also been found in the Lahore Branch of the BRB canal.(Firdaus-e-Bareen & Iqbal, 2003).

Species 3 (Fig.2 c): These four-armed conidia have some similarity with spp. of *Lemmoniera.*

Species 4 (Fig.2 d,d'): These flower like conidia had many arms directed in different directions.
Species 5 (Fig.2 e): This conidium showed some superficial resemblence with *Articulospora proliferata.*
Species 6 (Fig.2 f): This flower shaped conidium had three arms directed in the forward direction and the other three in the backward.
Species 7 (Fig.2 g g'): These conidia had attachment of six arms in a very peculiar manner. Similar conidia have been observed from the Lahore Branch of the BRB canal (Firdaus-e-Bareen & Iqbal, 2003).
Species 8 (Fig.2 h,h'): These tetraradiate conidia had four bulbous arms uniting at a common point.
Species 9(Fig.3 i): This triradiate conidium had one basal arm narrower in morphology.
Species 10 (Fig.3 j): These conidia with a bulbous center had a number of curved arms projecting into various directions. Similar conidia with arms have been observed at Kargah, Gilgit in the northern areas. Iqbal & Firdaus-e-Bareen, 1991)
Species 11 (Fig.3 k, k', k", k'"): These star like conidia of small size have also been commonly observed in the Lahore Branch of the BRB Canal. (Firdaus-e-Bareen & Iqbal, 2003).
Species 12 (Fig.3 l): These conidia belong to the genus Articulospora but different in morphology to *Articulospora proliferata* Jooste *et al*.
Species 13 (Fig.3 m): These tetraradiate conidia do not conform in morphology to any known species.
Species 14 (Fig.3 n): These biappendiculate conidia were found in natural foam.

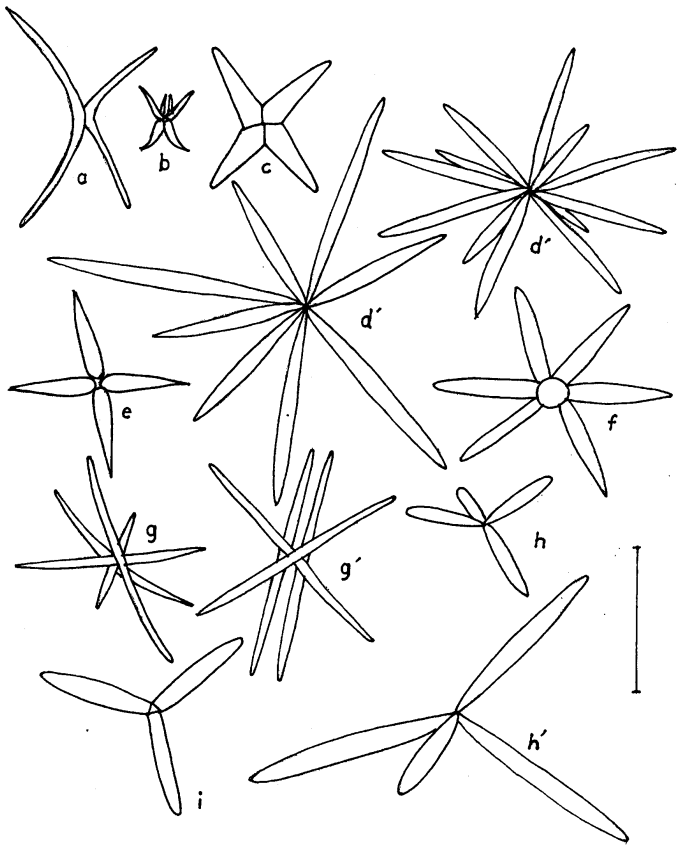

Fig. 2: Some unknown conidial species from the River Ravi. a: Species 1; b: Species 2; c: Species 3; d,d': Species 4; e: Species 5; f: Species 6; g,g': Species 7; h,h': Species 8; i: Species 9. Scale line= 50 μm

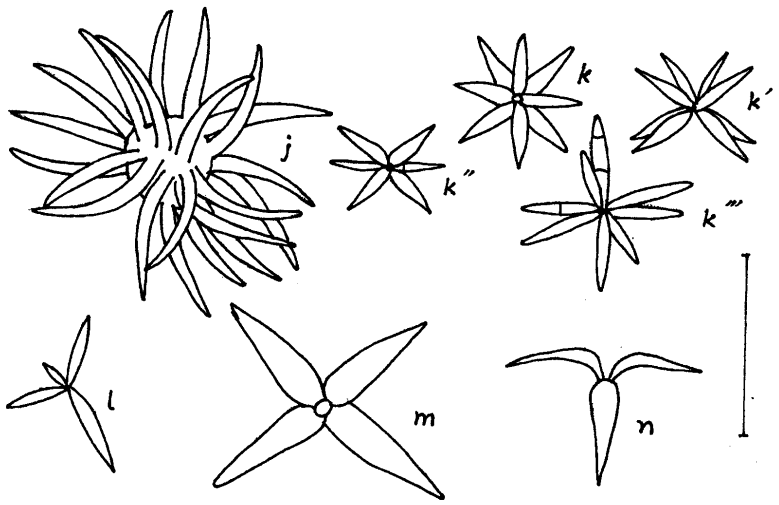

Fig. 3: Some unknown conidial species from the River Ravi. j: Species 10; k,k', k'',k''': Species 11; l: Species 12; m: Species 13; n: Species 14. Scale line= 50 μm.

Discussion

The rivers in the plains of the Punjab are very large and massive bodies of water. They may extend to about 200 m at places in width. There is a fluctuation in the supply of water all the time depending upon the water in streams from melting glaciers in the summer in the high mountain regions. The water flowing in the rivers represents a long course from the temperate clear water streams to flat plains in the sub-tropical regions. During traveling the water gathers enormous eroded soil and many dissolved chemicals. The presence of freshwater hyphomycetes and other conidia in the river Ravi adds to the variety of habitats found for these fungi.

The larger and more massive any body of water, the greater is the turbidity and the lesser is the number of conidia observed. So, as compared to the clear water small streams in Murree Hills and in the northern areas, the water is turbid with greater amount of dissolved and suspended particulate matter. There is thus a greater tendency of the conidia to get settled.

The artificial foam spora did not show any significant species richness as compared to species and conidia present in water. This is because of two obvious reasons. The artificial foam is not persistent for a long time at one particular place and a rich foaming lather could not be formed because of a relatively faster and constant flow rate of water. The time period from ten to fifteen minutes was not enough to trap more species than were really present in the water at that time. Efforts had also failed to form an artificial foam trap in the canal system (Firdaus-e-Bareen and Iqbal, 2003). Obviously, this technique failed in the river too because of its large size. The artificial foam trapping can only be possible in small narrow streams where the water can be pooled in cup like depressions.

The natural foam however, since it could have persisted for several months, was found to be the most species rich, containing conidia of probably diverse groups of aquatic fungi. Thus the importance of natural foam cannot be denied in situations where it is naturally present. In the river Ravi the formation of foam could be because of the detergents being mixed up with water as a biotic factor or certain algae having mucilaginous walls. Several of the conidial types were similar to

that of the canal in Lahore because of a similar climatic situation and also similarity of habitat to some extent.

Like any other semitropical warm climate, the dominant species here were *Articulospora proliferata* and *Tetracladium marchalianum*. *Tetracladium marchalianum* has already been shown to have an affinity for warmer temperature, but *A. proliferata* can be considered to be new ecological variants of the existing types having affinity for higher temperature (Firdaus-e-Bareen and Iqbal, 2003).

The greater number of conidia in the first quarter as compared to the second quarter conforms to the period of low temperature of water. In the river, deposition of organic matter seems meaningless because of its uniform flow rate and massive size. Therefore the temperature factor seems to be the main factor governing change in species composition.

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