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Diversity and seasonal appearance of aquatic fungi in three streams of Western Ghat forests of Goa, India

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ABSTRACT In the absence of any detailed and intensive investigation on the mycota of freshwater streams of the northern part of Western Ghats, in the present paper an effort was made to study the diversity and seasonal appearance of aquatic fungi of this region. It has been observed, from the study made among the three seasonal samplings, that monsoon season is the best for recovery of this group of fungi.

KEY WORDS Aquatic fungi; Biodiversity; Freshwater streams; Western Ghats.

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INTRODUCTION

Aquatic fungi in freshwater habitats are represented by two major groups namely Saprolegniales (watermoulds) and Aquatic Hyphomycetes. A number of fungi belonging to Ascomycotina and Mastigomycotina (zoosporic fungi) and a few Basidiomycetes also occur in freshwater habitats but in the dynamics of freshwater stream ecosystem, hyphomycetous fungi are considered as the most significant participants in view of their ability to digest a variety of submerged organic matter (Koushik & Hynes, 1971; Barlocher & Kendrick, 1976; Barlocher, 1992; Graca et al., 1993). Growing on submerged leaves and twigs, these fungi abound in fast flowing tree lined streams and well aerated rivers. The streams contain sufficient runoff organic material which, in the living or dead state, serve as a constant source of nutrients for the fungi living in water. The production of characteristic conidial-shape-like sigmoid, tetraradiate, helicoids and branched spores are the significant feature of this group of fungi.

The aquatic fungi of the streams of Western Ghat forests of Goa (India) have been a subject of study since the publication of a preliminary survey of 11 streams of the region carried out by Subramanian and Bhat in 1981 where *Anguillospora longissima*, *Tetracladium setigerum* (Grove) Ingold and *Triscelophorus monosporus* were reported for the first time from Dudhsagar falls in Goa State. Since then, a number of publications appeared on these fungi from the forest streams of Western Ghats in Karnataka and Kerala and have been reviewed in detail by Sridhar & Barlocher (1992).

The present work aims at the study of occurrence and abundance of aquatic fungi on submerged leaf litter in different seasons ie., the monsoon, post-monsoon and the summer seasons in Goa state. Studies were carried out in one stream each in three wildlife sanctuaries of Goa - ie., Bondla, Cotigao and Molem (Fig. 1). All the three sites have dense riparian canopy and have fast running water streams during monsoons which dry up during the summer seasons. An attempt was made to discover the diversity and number of species present in each season.

MATERIAL AND METHODS

Sampling sites

For the study of aquatic fungi occurring in different seasons, sampling was done at regular intervals in one stream each in three wild life sanctuaries, Bondla, Cotigao and Mollem. Assuming that the tree canopy may play a decisive role on the ecology of aquatic fungi by providing organic matter input, only those streams in the sanctuaries with dense riparian tree cover were chosen for the study. Besides abundance and frequency of occurrence, diversity of aquatic fungi was also studied from these sites. The sampling sites are described below:

Stream-1. A seasonal moderately fast flowing stream originated at a slightly high altitude in the ghats and flown down through Bondla wildlife sanctuary (120 m asl), in Pondataluka was considered as stream 1. The collecting site was about 2 km before the entry gate to the sanctuary and 60 km north-west of Goa University campus. The stream bed at the site was rocky. Banks of the stream on either sides were lined mainly by *Calamus thwaitesii* Becc. and *Dendrocalamus strictus* (Roxb.) Hook. and *Bambusa arundinaceae* (Retz.) Willd, besides tree species such as *Adina cordifolia* (Roxb.) Hook., *Bauhinia tomentosa* L., *Dillenia indica* L., *Grewia hirsuta* Vahl., *Hydnocarpus laurifolia* (Dennst.) Sleumer, *Indigo feradalzelii* Cooke., *Stephania japonica* Thunb Miers. and *Terminalia tomentosa* Wt. et Arn.

Figure 1. Study area: Western Ghat forests of Goa, India.

Stream-2. A fast flowing seasonal stream originated at Anmod Ghat and flown down into Bhagavan Mahavir sanctuary (230 m asl) was chosen as stream 2. The collection site was located about 4 km west of Mollem in Sanguem Taluka, 65 km west of Goa University campus. The stream bed had soft soil at the collecting site. Even though a variety of tree species was present in the sanctuary, riparian vegetation along the stream mainly composed of *Hopea ponga* (Dennst.) Mabb, the roots of which extend into the flowing waters along the sides of the stream. The other dominant tree species in the catchment area included evergreen types such as *Careya arborea* (Roxb.) De Wilde and *Tinospora cardifolia* Miers.

Stream-3. A moderately fast flowing seasonal stream, originated above in the ghat and flown down near Tree-top point of Cotigao wild life sanctuary (280 m asl) in Canacona Taluka, was considered as stream 3 for the study. The stream had lateritic soft soil in the bottom and good run off in monsoon season but gradually dried up during the late post monsoon and summer months. The site was 75 km south-east of Goa University campus. The catchment area was covered by dense vegetation of semi-evergreen and evergreen tree species, and some of the dominant riverine trees found in the area were *Careya arborea* (Roxb.), *Calycopteris floribunda* (Roxb) Lamk., *Dillenea indica* L., *Grewia hirsuta* Vahl., *Kandelia candel* (L.) Druce., *Lagerstoemia lanceolata* Wall. Ex Wt. et Arn., *Terminalia paniculata* Roth. and *Xylia xylocarpa* Taub.

Sampling seasons and intervals

Sampling of aquatic hyphomycetes was done during three sampling seasons namely monsoon, post-monsoon and summer season. During monsoon, from June to September every year, the windward western slopes of the Western Ghats receive a total rainfall of 250–350 cm. The mean annual temperature varies between 22–36 °C, the minimum seldom falling below 18 °C. Humidity ranges between 60–90%. All along the Western Ghats, in

monsoon the streams are usually gorgeous with flowing water. During post-monsoon (October to January), the streams either have little flowing water or mostly at many places exhibit pools of stagnant water bodies. In the summer months (February to May), the streams are practically dry and without water, except those perennial ones where flow of water is very slow.

While raining, fallen leaves from the trees lining the stream and river banks and adjoining forests get washed into the streams, the leaf litter in the stream either remains submerged or gets parked against rock crevices, fallen logs or any obstacles. These samples were carefully collected and brought to the laboratory, assuming that aquatic fungi colonise on submerged leaf litter.

Samples for floristic study

Spores of Deuteromycotina, Ascomycotina and Basidiomycotina get trapped in foam of freshwater streams. If a drop of foam is examined under a light microscope, spores and hyphal fragments of a variety of aquatic fungi can be seen.

Fixed foam. Foam accumulated on the surface of stream water was gathered by gently and repeatedly scooping a wide-mouth glass jar or glass petri plate lid over the foam. The collected foam was fixed by adding few drops of FAA fixative (a mixture of Formaldehyde 40% (5 ml), Glacial Acetic Acid (5 ml) and 70% Ethyl alcohol). The foam bubbles break into a cream coloured or slightly turbid liquid at the bottom of the container. The sample was maintained in 10 ml screw-capped vials. The vials were appropriately labelled in the field indicating the sample number, location and date of collection.

Dried foam. A drop of fresh foam was directly placed and spread over a clean slide and air-dried at the collection site. The slides were appropriately labelled indicating the sample number, location and date of collection and brought to the laboratory by arranging them vertically and in rows in a slide box. These slides were observed under the microscope by placing a drop of lactophenol-cotton blue mountant over the fixed foam dried area. Examination of FAA-fixed or air-dried foam under a microscope revealed the floristics of aquatic fungi of the catchment region. The slides examined were deposited as specimens at the Herbarium of Botany Department, Goa University (GUBH).

Leaf litter. Leaves of trees lining the stream and rivers on senescence fall into water. The fallen leaves act as substrate for growth and colonisation of aquatic fungi (Ingold, 1975). The submerged and partially to fully decayed leaf litter and twigs thus form excellent source for recovery of aquatic fungi. These leaves were hand-picked, thoroughly washed in water and placed in clean polythene bags. These were transported to the laboratory in ice-pack container. Of the decayed and well-skeletonised leaf litter brought to the laboratory, 2–3 leaves were washed thoroughly with de-ionized tap water and placed in large specimen jars containing 1L of sterile distilled water. The jars were aerated continuously for 5–7 days using a fish tank aerator. The aerated water was filtered through a Millipore filter (8 µl pore size) and the aquatic spora on the filter were counted (Iqbal & Webster, 1973).

Statistical Analysis

Data collected during the 24 month study period were subjected to statistical analysis. Margaliff's and Shanon's index were used for analysis of diversity of fungi. Analysis of Variance (ANOVA) Test was carried out to analyse the variations in occurrence of fungi in different seasons. The following formulas were used for statistically analysing the data obtained during the seasonal study of aquatic fungi.

$$\text{Percentage Frequency (\% F)} = \frac{\text{Total no. of quadrats in which species occurred}}{\text{Total no. of quadrants sampled}} \times 100$$

$$\text{Relative Frequency (R.F.)} = \frac{\text{No. of occurrence of a species}}{\text{No. of occurrence of all species}} \times 100$$

$$\text{Density (D)} = \frac{\text{Total number of individuals of the species}}{\text{Total number of quadrants taken}}$$

$$\text{Abundance (A)} = \frac{\text{Total number of individuals of a species in all quadrats}}{\text{Total number of quadrats in which the species occurred}}$$

The density of fungi on leaf litter, as expressed in water by aquatic spora, during the three different seasons and their significance of occurrence was analysed using 'Analysis of Variance' (ANOVA) test.

RESULTS

In all, conidia of 62 species of aquatic fungi were recorded in varying concentration in the aerated water. Fungal taxa seen as conidia in water and their average relative abundance (%) are given in Table 1. The relative abundance of species of fungi in 3 different seasons at three sites, namely Cotigao, Bondla and Molem indicates that during monsoon, highest number of species were at Bondla (54). Followed by Cotigao (51) and Molem (45). During post monsoon, the species richness remained in the same order of abundance in three sites ie., Bondla (41), Cotigao (37) and Molem (31). However, the abundance was in different order in summer, showing highest number of species at Cotigao (19), followed by Bondla (15) and Molem (16).

Analysis of variance test (ANOVA) (Table 2) on seasonal sampling carried out during the year showed a highly significant variation in the species richness in three different seasons at three sites and the order of significance was as follows: Cotigao (4.47), Bondla (3.31) and Molem (2.45). Less significant variation was observed between the sites during the same seasons: Monsoon (1.69), post-monsoon (1.18) and summer (0.92). The significant level tested between the places during same seasons did not show much variation. The F ratio obtained in monsoon, post-monsoon and summer from the three sampling sites was 1.84, 1.37 and 0.44, respectively. This similarity in significance may be attributed to the similar type of vegetation composition seen at the three sites.

Among the three different seasons chosen for the collection of freshwater fungi encountered on randomly sampled leaves, highest number of species were observed during monsoon (June to September) and the lowest during the summer (February to May). The results also indicated that the density and number of species recorded did not show a significant correlation with the pH and temperature, which was noted on the sampling sites. As an overall analysis, it may be said that occurrence and species density of fungi of a given stream ecosystem is largely dependent on factors such as rainfall, substrate availability and leaf deposition.

DISCUSSION AND CONCLUSIONS

The results showed that there is no much difference amongst the 3 sites considered for seasonal study in their fungal wealth (Fig. 2). This is because all the three sites had dense riparian canopy and good flow of water in the streams during monsoon and post monsoon. Of the fungi recovered, 75.5% were foam trapped and 56.3% associated with submerged leaf litter (Fig. 3). This clearly showed that aquatic foam from natural streams will continue to be the best source of diverse fungi as described by Descals (1997).

It may be seen from figure 4 that fungi with blastic type of conidiogenesis were of higher percentage (89.65) than phialidic (10.35) type. It may be said that from the evolutionary stand point (Hawkswort et al., 1995), the fungi of aquatic ecosystem need not to possess phialidic conidiogenesis. This is an instrument largely used for conidiogenesis by fungi in terrestrial environment.

The study also revealed (Fig. 5) that aquatic spora of branched and appendaged type were of higher percentage (29.31) followed by tetraradiate (24.14), sigmoid (15.52) and helicoids (1.72). These are adaptations for aquatic environment where the spora can remain afloat and get disseminated to a much larger distance. It has been realised that fungi that are taxonomically unrelated while converging into water, during the course of evolution exhibited similar morphology as an adaptation to aquatic system (Ingold, 1975; Dix & Webster, 1995). As can be seen from the study, there is no such significance in the abundance of different types of conidia. All the three to four types of conidia, ie., appendaged, branched, sigmoid and tetraradiate were found in abundance in aquatic systems. It is also clear from the study that natural foam accumulated on the surface of water was the best source of aquatic fungi (75.5%) for isolation. However, when aerated, significantly high percentage was recovered from submerged leaves (56.3%). This justified our taking of submerged leaves as a substrate to evaluate the aquatic flora in the stream ecosystem. Twigs with a very low percentage (8.8%) occurrence of aquatic fungi proved to be a poor substrate for isolation.

From the investigation it is clear that monsoon and post monsoon seasons are the best for recovery of freshwater fungi from the streams of forests of Western Ghats, and that those streams with dense riparian tree canopy and abundant substrate availability would yield higher diversity of aquatic fungi.

		BONDLA			COTIGAO			MOLEM		
Sl.	**Name of fungus**	**M**	**PM**	**S**	**M**	**PM**	**S**	**M**	**PM**	**S**
1	*Actinospora megalospora* Ingold	0.46	0.09	-	0.19	0.05	-	0.17	-	-
2	*Alatospora acuminate* Ingold	0.93	0.49	-	0.47	0.39	0.05	1.15	0.39	-
3	*Anguillospora crassa* Ingold	3.17	1.22	0.59	3.12	0.74	0.29	1.82	1.24	-
4	*Cylindrocarpon* sp.	1.05	0.46	-	1.38	0.92	0.42	-	-	-
5	*Cylindrocladium* sp.1	1.75	0.95	0.16	2.30	2.20	0.49	1.02	0.53	0.22
6	*Cylindrocladium* sp.2	3.89	2.24	1.88	3.41	1.36	1.19	11.23	7.73	3.46
7	*Anguillospora longissima* (Sacc. et P. Syd.) Ingold	0.39	-	-	1.09	0.57	-	-	-	-
8	*Ardhachandra solenoides* (de Hoog) Subram et Sudha	0.19	0.03	-	0.29	0.12	-	0.39	0.18	-
9	*Ardhachandra* sp.	0.82	0.63	-	0.17	-	-	-	-	-
10	*Articulospora tetracladia* Ingold	0.19	0.19	-	2.13	1.04	0.59	0.79	0.75	0.35
11	*Bahusutrabeeja angularis* V. Rao et de Hoog	1.45	0.59	0.19	1.83	0.92	0.62	1.24	0.04	-
12	*Beltrania rhombica* Penz.	0.75	0.33	-	3.54	1.86	-	3.24	0.62	-
13	*Scutisporus brunneus* K.Ando et Tubaki	0.93	0.36	-	3.19	1.31	0.42	3.46	1.11	0.44
14	*Articulospora* sp.	0.53	0.06	-	-	-	-	-	-	-
15	*Camposporium pellucidum* (Grove) S. Hughes	0.63	0.29	-	-	-	-	-	-	-
16	*Campylospora chaetocladia* Ranzoni	1.92	0.85	0.29	1.61	1.04	0.29	3.06	0.84	0.13
17	*Lunulospora curvula* Ingold	1.78	0.59	-	3.04	0.64	-	1.28	1.06	-
18	*Centrospora acerina* (R. Hartig) A.G. Newhall	0.56	0.06	-	-	-	-	-	-	-
19	*Lemonniera aquatica* De Wild.	-	-	-	0.47	0.29	0.19	0.53	0.08	-
20	*Chaetendophragmia triseptata* Matsush.	0.53	0.16	-	0.42	0.17	-	0.44	0.13	-
21	*Diplocladiella scalaroides* G. Arnaud ex M.B. Ellis.	-	-	-	-	-	-	1.99	0.79	-
22	*Condylospora spumigia* Nawawi	4.36	-	-	5.25	1.78	1.38	8.21	1.55	0.75
23	*Dactylella ellipsospora* (Preuss) Grov.	2.47	0.76	0.29	4.16	2.03	0.82	2.39	0.88	-
24.	*Dactylaria* sp.	0.49	-	-	0.59	-	-	0.53	0.35	0.26
25	*Dactylaria aquatica* Udaiyan	1.25	0.69	-	1.53	0.67	-	0.84	0.35	0.22
26	*Dendrospora erecta* Ingold	0.92	-	-	2.15	1.06	0.37	0.53	-	-
27	*Ceratosporium* sp.	0.46	0.56	-	0.69	-	0.39	0.75	0.35	0.22
28	*Tripospermum myrti* (Lind) Hughes	0.86	0.69	-	0.29	0.19	0.12	0.48	-	-
29	*Lateriramulosa uni-inflata* Matsush.	0.89	0.06	0.09	0.05	0.19	0.09	0.62	0.22	-
30	*Dendrosporium lobatum* Plakidas et Edgerton ex J.L. Crane	0.16	0.06	-	0.07	-	-	0.39	-	-
31	*Dichotomophthoropsis aquatica* Sreekala et Bhat	0.49	-	-	0.12	0.27	-	-	-	-

Table 1/1. Fungal taxa seen as conidia in water and their average relative abundance (%).

		BONDLA			COTIGAO			MOLEM		
Sl.	**Name of fungus**	**M**	**PM**	**S**	**M**	**PM**	**S**	**M**	**PM**	**S**
32	*Dictyochaeta assamica* (Agnihothr.) Aramb., Cabello et Mengasc	-	-	-	-	-	-	1.19	0.57	0.09
33	*Endophragmia inaequiseptata* Matsush.	-	-	-	0.12	0.09	-	-	-	-
34	*Flabellospora crassa* Alas.	2.84	1.88	-	1.46	0.29	-	1.24	0.66	0.08
35	*Flagellospora curvula* Ingold	0.96	0.33	-	-	-	-	-	-	-
36	*Beltraniella odineae* Subram.	0.86	0.36	0.16	0.32	0.05	-	0.35	0.13	-
37	*Flabellospora verticillata* Alas.	0.43	0.29	0.19	0.22	-	-	0.66	0.35	0.08
38	*Flabellospora multiradiata* Nawawi	0.49	0.39	0.09	0.72	0.12	-	0.35	-	-
39	*Helicomyces roseus* Link	0.56	0.26	-	0.05	-	-	-	-	-
40	*Helocosporium* sp.1	8.45	6.97	0.92	6.23	2.87	-	2.57	-	-
41	*Helicosporium* sp.2	1.75	0.36	0.26	0.77	0.29	-	0.84	0.53	0.18
42	*Helicosporium* sp.3	3.23	1.92	-	2.00	-	-	0.22	-	-
43	*Ingoldiella hamata* Shaw	2.08	1.16	-	1.68	1.26	-	0.93	-	-
44	*Isthmotricladia laeensis* Matsush.	0.56	0.26	-	0.05	0.12	-	1.99	1.15	-
45	*Isthmotricladia britanica* Descals	-	-	-	1.46	-	0.05	-	-	-
46	*Nawawia filiformis* (Nawawi) Marvanová	0.53	0.49	0.36	0.69	0.29	0.19	0.84	0.44	0.13
47	*Phalangispora constricta* Nawawi et J. Webster	0.66	0.36	-	0.29	0.19	-	-	-	-
48.	*Mycoleptodiscus indicus* (V.P. Sahni) B. Sutton	0.19	0.19	-	0.22	0.05	-	0.13	0.04	-
49	*Tetrachaetum elegans* Ingold	0.49	-	-	0.54	0.35		0.26	-	-
50	*Sopagraha sibika* Subram. et Sudha	0.69	0.39	0.26	0.72	0.37	-	0.44	0.04	-
51	*Speiropsis hyalospora* Subram. et Lodha	0.46	0.16	-	0.07	-	-	0.35	0.31	0.08
52	*Speiropsis pedatospora* Tubaki	0.65	-	-	-	-	-	-	-	-
53	*Subulispora* sp.1	0.39	-	-	0.42	-	-	0.84	-	-
54	*Subulispora* sp.2	0.23	-	-	0.05	-	-	0.79	-	-
55	*Tetraploa aristata* Berk. et Broome	0.63	-	-	0.39	-	-	-	-	-
56	*Tetracladium* sp.	0.23	-	-	-	-	-	0.53	-	-
57	*Tetracladium angulatum* Ingold	3.17	0.63	0.36	1.28	0.64	0.17	3.86	0.66	0.26
58	*Triscelophorus acuminatus* Nawawi	-			0.29	-	-	0.62	-	-
59	*Triscelophorus konajensis* K.R. Sridhar et Kaver.	-	-	-	0.39	-	-	-	-	-
60	*Triscelophorus monosporus* Ingold	0.39	-	-	-	-	-	-	-	-
61	*Seimatosporium* sp.	-	-	-	-	-	-	0.22	-	-
62	*Robillarda phragmitis* Cunnell	0.26	-	-	-	-	-	-	-	-
	Total (no. of species)	**54**	**42**	**15**	**51**	**37**	**19**	**45**	**31**	**16**

Table 1/2. Fungal taxa seen as conidia in water and their average relative abundance (%).

Places	Seasons	Experimental Method				
		Sum of squares	Degree of Freedom	Mean square	F ratio	Significance
Bondla	Between seasons	8604.65	2	4302.33	3.13	Yes
	Residual	148591.24	108	1375.84		
Cotigao	Between seasons	16697.25	2	8348.63	4.47	Yes
	Residual	199862.1	107	1867.87		
Molem	Between seasons	6875.37	2	3437.69	2.45	Yes
	Residual		91	1402.71		
Monsoons	Between places	127646.46	2	3989.72	1.69	No
	Residual	361992.46	154	2350.60		
Post-Monsoons	Between places	2281.51	2	1140.79	1.18	No
	Residual	102324.58	106	965.33		
Summer	Between places	469.65	2	234.83	0.92	No
	Residual	11782.76	46	256.15		

Table 2. Analysis of Variance (ANOVA) for the three sampling sites.

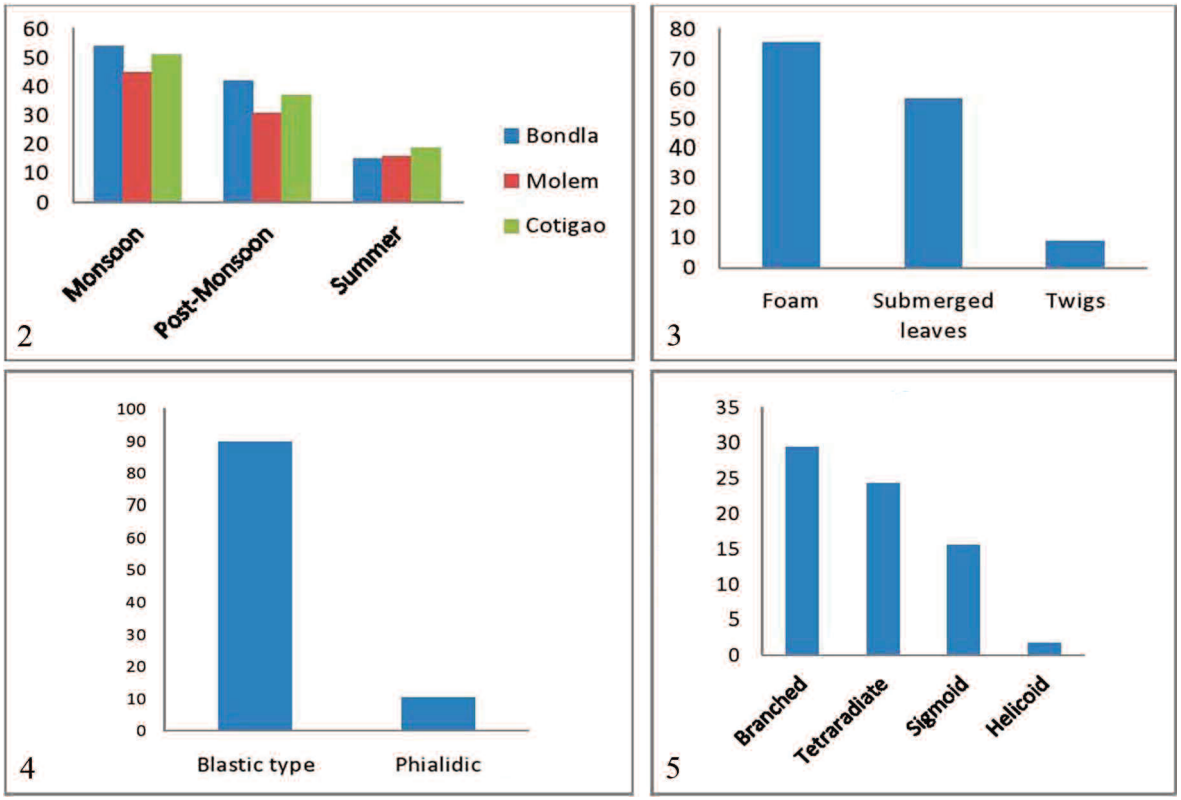

Figure 2. Seasonal occurrence of acquatic fungi in different seasons. Figure 3. Substrate specificity of acquatic fungi. Figure 4. Percentage occurrence of conidia based on its conidiogenesis. Figure 5. Percentage appeareance of different types of conidia.

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