In vitro Conidial Production of Aquatic Hyphomycetes on Submerged Leaf Litter

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ABSTRACT: Submerged leaf litter of three forest plant species viz., *Acer oblongum*, *Lyonia ovalifolia* and *Pinus roxburghii* were sampled to study the in vitro conidial production of aquatic hyphomycetes. Out of 25 species encountered, only12 species were found common to all host plant but the rate of conidial production was quite variable. Of these 12 species *Acer oblongum* supported maximum conidial production for *Triscelophorus monosporus* and *Lyonia ovalifolia* supported maximum conidial production for *Tetrachaetum elegans* whereas *Pinus roxburghii* supported maximum conidial production for *Flagellospora penicillioides*. Coniferous leaf litter i.e. *Pinus roxburghii* was found with maximum conidial production (92487 conidia/ cm ^{2}/ litre) as compared to the leaf litter of other studied forest plants (51506 and 42144 conidia/cm^2/litre on *Acer oblongum* and *Lyonia ovalifolia* respectively). It is interesting to note that Lyonia ovalfolia was colonized by highest number of species, whereas *Pinus roxburghii* had the least species diversity. The maximum number of conidial production was found during winter and spring months while the maximum number of species variation was observed during rainy and autumn months. [Nature and Science. 2009;7(1):78-83]. (ISSN: 1545-0740).

Keywords: submerged leaf litter, conidial production, aquatic hyphomycetes

INTRODUCTION

Aquatic hyphomycetes, ramifying on decaying leaves especially skeletonized or decorticated petioles occur throughout the year in any fast flowing stream, however, their abundance relates to availability of leaf litter in stream (Webster and Descals, 1981). Though these fungi are being well studied with the reference of their qualitative point of view i.e. occurrence, seasonal periodicity, variation in their species composition by many mycologists (Triska, 1970; Gonczol, 1975; Sander and Webster, 1978), but little is known for their quantitative studies (Willoughby and Archer, 1973; Muller Haeckel and Marvanova, 1979). Iqbal and Webster (1973) took the initial step to understand the rate of conidial concentration in a water body by filtering the water. Recently, Sati and Tiwari (1992, 1995) developed a simple technique to determine the rate of conidial production by modifying the method of Webster and Towfic (1972). In the present investigation an attempt has been made to study the fallen leaves of three forested plant species were studied for the production of conidia in unit area substrate per litre of water in captivity.

METHODOLOGY

To determine the rate of conidial production in per unit area of the substrate in per liter of water, Sati and Tiwari (1995) was followed. Submerged leaf litter of known three forest trees i.e. *Acer oblongum, Lyonia ovalifolia* and *Pinus roxburghii* were incubated in the sterile petri dishes containing 20 ml of sterile water at monthly intervals. Prior placing the leaf litter for incubation, the area of each piece of leaf litter was determined with the help of graph paper. After 2-3 days, the incubated dishes containing leaf litter were gently shaken to homogenize the fungal conidia produced in water. The drops of 0.01 ml conidial suspension were pipetted out on glass slides for screening. The counting of conidia was made directly under the low power of microscope and conidial number was recorded individually to each species. Finally the rate of conidial production for each species occurred and total species in unit area (1 cm^2) were calculated using the following formula –

$$\text{RCP} = \frac{2000\ n}{a}\ \text{Conidia/ cm}^2\text{ / litre}$$

Where, RCP = Rate of conidial production
n = No. of conidia present in .01 ml of conidial suspension used
a = area of leaf litter substrate incubated (cm^2)
(2000 is used if 20 ml sterile water is supplied to the incubated substrate in dish)

RESULTS

The results of monthly variation in conidial production of aquatic hyphomycetes per litre per unit area of substrate i.e. leaf litter are summarized in Table 1-3. Altogether 25 species of water borne conidial fungi were encountered on the incubated leaf litter of *Acer oblongum*, *Lyonia ovalifolia* and *Pinus roxburghii*. The colonization pattern of these species on three different host plants as well as the rate of conidial production per cm^2 area in unit volume of water is tabulated in table 4.

Acer oblongum Wall. ex DC.

19 species of water borne conidial fungi belonging to *Alatospora*, *Anguillospora*, *Articulospora*, *Camposporium*, *Clavariopsis*, *Dimorphospora*, *Flagellospora*, *Heliscus*, *Lemonniera*, *Lunulospora*, *Tetrachaetum*, *Tetracladium*, *Tricladium* and *Triscelophorus* was found colonizing on incubated leaves of *Acer oblongum* (Table 5.1). This substrate was abundantly colonized by *Triscelophorus monosporus.* Remaining species were found as moderately and least abundant. The average conidial production in *Acer oblongum* was 51506 conidia/cm^2/litre. *Triscelophorus monosporus* accounted a maximum number of conidia i.e., 7953 conidia/cm^2/litre where as *Lemonniera terrestris* accounted only 165 conidia/cm^2/litre.

The maximum number of conidial production was analyzed during September while minimum number of conidia were analyzed during April (112060 and 13240 conidia/cm^2/litre respectively).

Lyonia ovalifolia (Wall.)Drude

The leaves of *Lyonia ovalifolia* were colonized by 21 species of water borne conidial fungi (Table 2). The total average conidial production was 42144 conidia in unit area of substrate per litre. *Tetrachaetum elegans* was occurred with maximum number of conidia i.e. 6085 conidia/cm^2/litre in average. A least number of conidia were produced by *Lemonniera terrestris* 188 conidia/cm^2/litre in average.

Maximum conidial production was analyzed during January, which reached upto 74930 conidia/cm^2/litre while the least conidial production (20870 condia/cm^2/litre) was accounted during August.

Pinus roxburghii Sarg.

The submerged needles of *Pinus roxburghii* were colonized by 15 species of water borne conidial fungi (Table 3). Total average conidial production in *Pinus roxburghii* was 92487 conida/cm^2/litre. The maximum contribution was made by *Flagellospora penicillioides*, which reached upto an average of 18060 conida/cm^2/litre whereas least number of conidia were contributed by *Heliscus lugdunensis* (411 conida/cm^2/litre).

As evident from Table 3 January month was found the most favourable for conidial production to have upto 198670 conidia/cm^2/litre while least conidial production was found in the month June i.e. 46720 conidia/cm^2/litre.

Table 1: Monthly variation in conidial production of water borne conidial fungi in per litre/ unit area of *Acer oblongum* Wall. ex DC leaf litter in captivity

S.No.	Fungi	Conidia produced												Average
		June	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	March	Apl	May	
1.	*Alatospora acuminata*	-	-	-	8210	6420	4550	-	-	-	8850	-	-	2336
2.	*A. flagellata*	-	-	-	-	-	-	-	-	14440	-	-	-	1203
3.	*A. pulchella*	-	-	-	4940	4210	-	7770	-	-	5270	-	-	1849
4.	*A. longissima*	4530	-	-	-	7530	-	-	-	-	-	-	2910	1248
5.	*Articulospora tetracladia*	-	-	-	-	-	-	35350	-	-	-	-	-	2946
6.	*Camposporium pellucidum*	-	-	-	-	-	-	-	-	-	7760	-	-	647
7.	*Clavariopsis aquatica*	-	-	-	3140	1150	-	2640	23320	13340	-	-	-	3633
8.	*Dimorphospora foliicola*	-	-	-	-	-	20290	-	15940	-	-	-	-	3019
9.	*Flagellospora penicillioides*	8160	-	2750	-	-	-	-	-	-	16460	-	-	2281
10.	*Heliscus lugdunensis*	-	-	-	-	2780	-	2880	-	-	-	-	-	472
11.	*Lemonniera cornuta*	-	-	-	-	-	-	-	31470	12160	-	-	-	3636
12.	*L. terrestris*	-	-	-	-	-	-	1980	-	-	-	-	-	165
13.	*Lunulospora curvula*	-	13100	2250	-	2820	8930	9240	14600	12260	-	10970	7980	6846
14.	*L. cymbiformis*	-	11710	2900	33990	1640	1140	2500	-	-	-	2270	-	4679
15.	*Tetrachaetum elegans*	-	12250	900	-	1170	2500	10090	-	-	-	-	-	2243
16.	*T. marchalianum*	-	-	-	-	-	-	1580	19410	-	-	-	-	1749
17.	*T. chaetocladium*	-	-	-	11380	3640	9070	11330	-	-	-	-	-	2952
18.	*Triscelophorus acuminatus*	4070	-	-	15750	-	-	-	-	-	-	-	-	1652
19.	*T. monosporus*	8890	22910	7340	34650	3830	7490	-	-	-	-	-	10320	7953
	Total	**25650**	**59970**	**16140**	**112060**	**35190**	**53970**	**85360**	**104740**	**52200**	**38340**	**13240**	**21210**	**51506**

Table 2: Monthly variation in conidial production of water borne conidial fungi in per litre/ unit area of *Lyonia ovalifolia* Wall) Drude leaf litter in captivity

S.No.	Fungi	Conidia produced												Average
		June	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	March	Apl	May	
1.	*Alatospora acuminata*	8810	5070	-	-	-	5220	-	5600	13450	4680	-	11870	4558
2.	*A. pulchella*	-	8460	-	-	-	-	-	-	-	-	-	-	705
3.	*A. longissima*	-	-	1410	-	3790	-	-	-	-	-	-	-	433
4.	*Articulospora tetracladia*	-	-	-	-	-	-	12970	1600	-	-	-	-	1214
5.	*Clavariopsis aquatica*	-	-	-	-	3790	6390	660	-	-	-	-	-	903
6.	*Dimorphospora foliicola*	-	-	-	-	-	6820	-	40820	-	-	-	-	3970
7.	*Flagellospora penicillioides*	-	5010	-	3380	-	-	-	-	-	-	-	3620	1001
8.	*Heliscina campanulata*	-	-	-	-	-	-	-	-	18580	-	-	-	1548
9.	*Lemonniera cornuta*	-	-	-	-	3280	9410	13540	-	-	-	-	-	2186
10.	*L. pseudofloscula*	-	-	-	-	1310	-	9470	-	-	-	-	-	898
11.	*L. terrestris*	-	-	-	-	2250	-	-	-	-	-	-	-	188
12.	*Lunulospora. curvula*	10450	10690	9300	8720	4900	2150	-	-	-	-	2690	11540	5037
13.	*L. cymbiformis*	3950	8550	-	-	5350	-	-	-	-	-	-	-	1488
14.	*Pestalotiopsis submersus*	-	-	-	-	-	-	-	-	8270	-	-	-	689
15.	*Speiropsis scopiformis*	-	4650	-	-	-	-	-	-	-	-	-	-	388
16.	*Tetrachaetum elegans*	-	-	2940	9130	7970	13190	12030	10910	9040	7810	-	-	6085
17.	*T. marchalianum*	-	-	-	-	4420	2250	-	-	-	-	-	-	556
18.	*Tricladium chaetocladium*	-	-	-	-	-	6930	12710	16000	8120	9830	-	-	4466
19.	*Triscelophorus acuminatus*	-	-	5640	1880	2270	-	-	-	-	-	2340	-	1011
20.	*T. monosporus*	8700	7210	1580	6850	3760	-	-	-	-	10560	7050	2490	4017
21.	*T. konajensis*	-	-	-	-	-	-	-	-	-	-	9650	-	804
	Total	**31910**	**49640**	**20870**	**29960**	**43090**	**52360**	**61380**	**74930**	**57460**	**32880**	**21730**	**29520**	**42144**

Table 3: Monthly variation in conidial production of water borne conidial fungi in per litre/ unit area of *Pinus roxburghii* Sarg. leaf litter in captivity

S.No.	Fungi	Conidia produced												Average
		June	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	March	Apl	May	
1.	*Alatospora acuminata*	14510	-	11220	-	26290	-	-	-	-	-	-	-	4335
2.	*A. pulchella*	-	-	7010	-	-	--	-	-	-	-	-	-	584
3.	*Clavariopsis aquatica*	-	-	-	-	-	-	23640	17810	-	-	-	-	3454
4.	*Flagellospora penicillioides*	13410	10400	19530	-	-	-	-	-	-	93260	39740	40380	18060
5.	*Heliscus lugdunensis*	-	-	-	-	-	-	4930	-	-	-	-	-	411
6.	*Lemonniera cornuta*	-	-	-	-	10940	-	-	17810	15920	-	-	-	3723
8.	*Lunulospora curvula*	-	26160	-	-	9730	30890	4450	41270	32230	6580	10050	19730	15091
9.	*L. cymbiformis*	-	-	-	-	-	11470	-	38000	-	-	-	-	4123
10.	*Pestalotiopsis submersus*	-	-	-	-	-	-	-	-	34770	-	-	-	2898
11.	*Setosynnema isthmosporum*	-	5780	-	-	-	-	-	-	-	-	-	-	482
12.	*Tetrachaetum elegans*	-	-	-	10200	13120	17290	46500	29220	-	-	-	7530	10322
13.	*Tetracladium marchalianum*	-	4370	-	3430	-	-	-	-	-	-	-	-	650
14.	*Tricladium chaetocladium*	-	4080	10410	23030	13760	10630	51830	54560	30910	8510	-	-	17310
15.	*Triscelophorus acuminatus*	2730	3820	7620	21780	-	--	-	-	-	-	-	-	2996
16.	*T. monosporus*	16070	19920	6300	-	18350	10410	7980	-	-	-	-	17570	8050
	Total	**46720**	**74530**	**62090**	**58440**	**92190**	**80690**	**139330**	**198670**	**113830**	**108350**	**49790**	**85210**	**92487**

Table 4: Comparative variation in occurrence of fungi and their rate of conidial production on different substrates in captivity

S. No.	Fungi	Average conidial production on different substrate		
		Acer oblongum	*Lyonia ovalifolia*	*Pinus roxburghii*
	Alatospora acuminata	2336	4558	4335
	A. flagellata	1203	-	-
1.	*A. pulchella*	1849	705	584
2.	*A. longissima*	1248	433	-
3.	*Articulospora tetracladia*	2946	1214	-
4.	*Camposporium pellucidum*	647	-	-
5.	*Clavariopsis aquatica*	3633	903	3454
6.	*Dimorphospora foliicola*	3019	3970	-
7.	*Flagellospora penicillioides*	2281	1001	18060
8.	*Heliscella campanulata*	-	1548	-
9.	*Heliscus lugdunensis*	472	-	411
10	*Lemonniera cornuta*	3636	2186	3723
11	*L. pseudofloscula*	-	898	-
12	*L. terrestris*	165	188	-
13	*Lunulospora curvula*	6846	5037	15091
14	*L. cymbiformis*	4679	1488	4123

15	*Pestalotiopsis submersus*	-	689	2898
16	*Setosynnema isthmosporum*	-	-	482
17	*Speiropsis scopiformis*		388	-
18	*Tetrachaetum elegans*	2243	6085	10322
19	*T. marchalianum*	1749	556	650
20	*Tricladium chaetocladium*	2952	4466	17310
21	*Triscelophorus acuminatus*	1652	1011	2996
22	*T. monosporus*	7953	4017	8050
23	*T. konajensis*	-	804	-
	Total Conidial Production	51506	42144	92487
	Total no of species	19	21	16

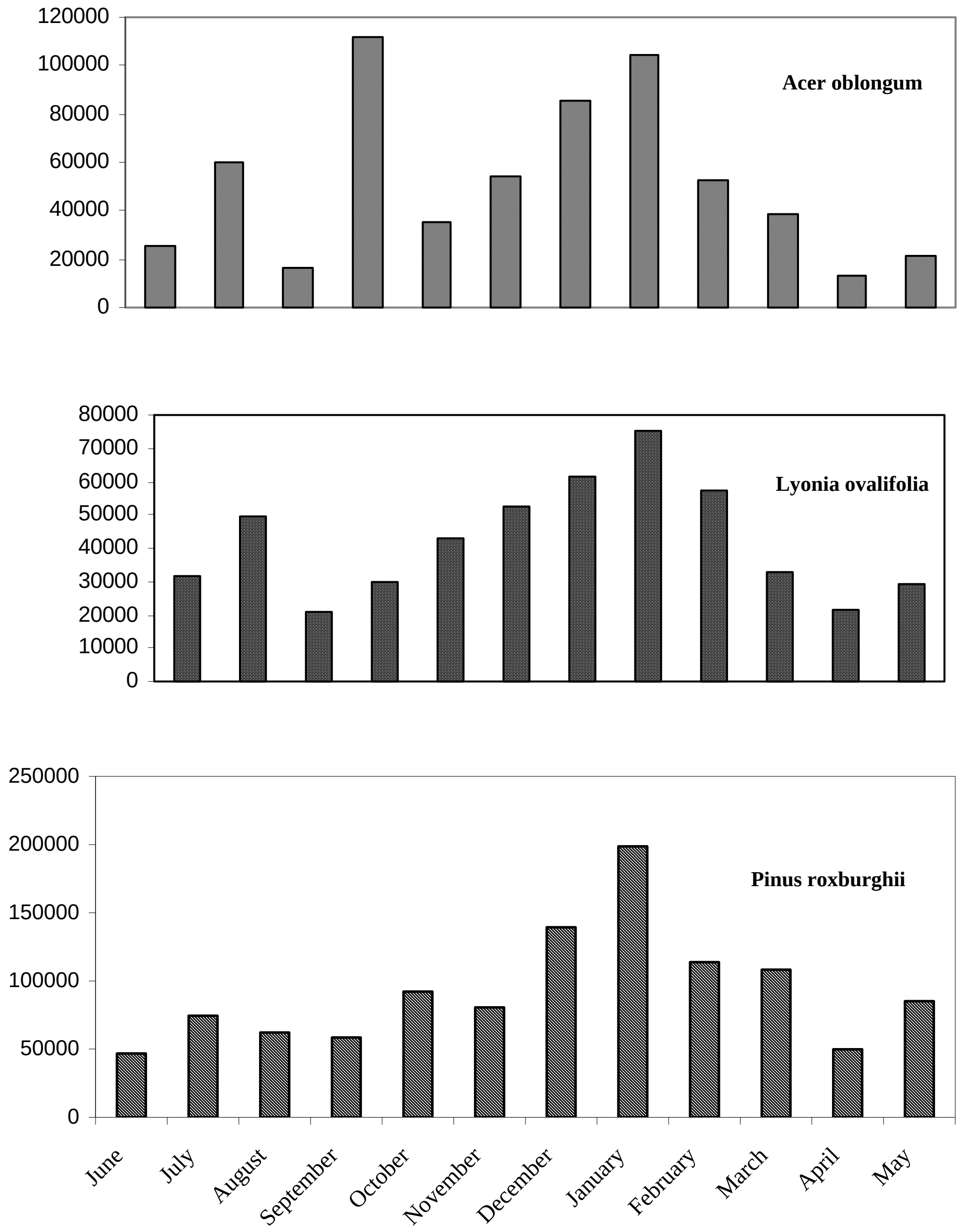

Fig.1: Total conidial production of water borne conidial fungi/litre/cm^2 in different host species

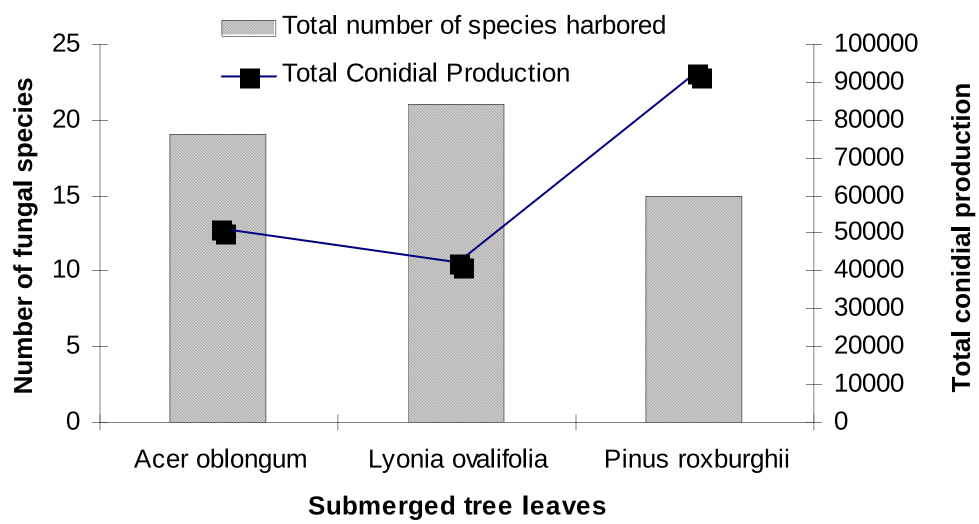

Fig. 2: Total average conidial production/cm^2/liter and total species colonization in different host species

DISCUSSION

On reconnaissance of table 1-3, an inclining trend of conidial production was found from autumn months to winter months i.e. September to February, when temperature remains low. It was interesting to note that during autumn months like September to November a maximum number of species were observed. The similar trend was followed by winter (December to February) and spring (February to April) months (Fig. 1). Relying upon these results it can be said that the maximum number of conidial production take place during winter and spring months while the maximum number of species variation take place during autumn and rainy months (Fig. 1). The result of present investigation confirms the findings of some of the previous workers (Iqbal and Webster 1973, Alasoadura 1968, Barlocher and Rosset 1981, Mer and Sati 1989, Thomas et al 1979).

As evident from fig. 2 *Lyonia ovalifolia*, which was colonized by highest number of water borne conidial fungi (i.e. 21 species) but the total average conidial production for this host remained in low profile (42144 conidia/cm^2/litre respectively). On the other hand, in *Pinus roxburghii* the less species colonization was reported, however the total average conidial production reached in its highest profile (92487 conidia/cm^2/litre). The total average conidial production on unit area of different studied three plant leaf litter in per liter of water is summarized in table 4. On the perusal of table 4 and fig. 2 it could be concluded that the gymnospermous leaf litter have maximum conidial production /cm^2/litre and support the view of Sati and Tiwari (1995).

Thus relying up on these observations it could be visualized that the number of species colonization and rate of conidial production varies species to species of leaf litter or might be depend on the nature of available substrate. On the basis of above said observation it could be concluded that the higher rate of conidial production might depend upon the nature and nutritive value of substrate. The present observation also support the findings of Willoughby and Archer (1973).

As evident from table 4, a total of 25 species of water borne conidial fungi were encountered. A maximum number of species were harbored on the submerged leaves of *Lyonia ovalifolia* (21 species) followed by *Acer oblongum* and *Pinus roxburghii* were colonized by 19 and 15 species of water borne conidial fungi respectively. However each species had different species composition (Table 4). This suggests a preferential occurrence of water borne conidial fungi on the nature of plant substrates. Therefore, it could also be envisaged that the occurrence of water borne conidial fungi much depend on the available substrate provided by the plant leaf litter.

On perusing table 4, 12 species viz., *Alatospora acuminata*, *A. pulchella*, *Clavariopsis aqautica*, *Flagellospora penicillioides*, *Lemonniera cornuta*, *Lunulospora curvula*, *L. cymbiformis*, *Tetrachaetum elegans*, *Tetracladium marchalianum*, *Tricladium chaetocladium*, *Triscelophorus acunminatus* and *T.*

monosporus were found to occur in all the studied plant leaf litter. It shows their habit tolerant nature as appear to be common in occurrence and do not show selective substrate requirement. On the other hand, 4 species of water borne conidial fungi i.e. *Alatospora flagellata*, *Camposporium pellucidum*, *Setosynnema isthmosporum* and *Triscelophorus konajensis* were restricted to only specific leaf litter of plant species showing specific habitat loving nature. Present observation also confirms the findings of Willoughby and Archer (1973).

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