Diversity Of Water Borne Conidial Fungi As Root Endophytes In Temperate Forest Plants Of Western Himalaya

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ABSTRACT*:* Diversity of water borne conidial fungi occurring as root endophytes was studied. Twenty one species were recorded as root endophytes in healthy roots of forest plants of Western Himalaya. Three species viz., *Camposporium pellucidum, Diplocladiella scalaroides* and *Helicomyces roseus* are being reported for the first time as root endophytes whereas 8 species viz., *Acaulopage tetraceros, Alatospora acuminata, Anguillospora longissima, Campylospora purvula, Cylindrocarpon aquaticum, Heliscus lugdunensis, Tetracladium marchalianum* and *Tetracladium setigerum* are recorded with their new host record. Maximum host diversity was represented by *Cylindrocarpon aquaticum* and *Tetracladium setigerum* as the former endophyte was isolated from 14 host plants followed by latter which was isolated from 12 host plants. [Nature and Science. 2008;6(3):59-65]. ISSN: 1545-0740.

Key words: Diversity, Water borne conidial fungi, Endophyte

INTRODUCTION

Microbes living with interior tissues of healthy plants without causing disease symptoms are called endophytes. Wide occurrence of endophytic fungi on many plants including bryophytes and *Botrychium* have been explored from all parts of the world (Clay, 1989; Petrini, 1986; Petrini et. al. 1992; Carroll and Carroll, 1978 and Sati and Belwal, 2005). The role of endophytic hyphomycetes has now been suggested by various workers as promoters of plant growth (Bills and Polishok, 1992; Petrini, 1991and Dreyfuss and Chapela 1992).

The water borne conidial fungi were described previously by Ingold (1942) as these complete their life cycle on submerged substrate in well-aerated waters but Waid (1954) reported some of these water borne conidial fungi from root surface also. The observation of Waid (1954) provided a pavement to various workers who further reported some more water borne conidial fungi from aquatic and terrestrial roots **(**Gourley, 1969; Nemec, 1969 and Watanabe, 1975). Fisher et al (1986) recognized a separate group of water borne conidial fungi as endophytic hyphomycetes and later Fisher et al (1991) confirmed their occurrence on plant roots of aquatic habitats through experimental basis by examining the bark and xylem of aquatic roots of *Alnus gluitinosa*. Similar reports have been made by other mycologists on endophytic aquatic hyphomycetes (Marvanova and Fisher, 1991; Marvanova et. al., 1992 and Sridhar and Barlocher, 1992a).

Endophytic fungi are also known to be a rich source of antibiotic, secondary metabolites and it has been postulated that endophytic fungi may be beneficial to their host plant by antagonizing pathogens and / or by inducing plant defense responses. Webber (1981) was the pioneer to report plant protection by an endophytic fungus *Phomopsis oblonga* protected elm trees against the beetle. This was confirmed by Claydon *et al* (1985) who showed that endophytic fungi synthesize secondary metabolites in host which affect the beetle larvae.

The present study provides an assessment of species diversity of endophytic hyphomycetes associated with the roots of temperate plants growing nearby areas of water bodies of Western Himalaya.

METHODOLOGY

'Three step sterilization' method of Fisher and Petrini (1989) was followed for the systematic study of root endophytic fungi. Living roots of different tree plant species including herbs and shrubs growing in the ravine and wet areas located at Nainital and Almora districts in Western Himalaya were collected in 3 replicates of each. Nearly 10-15 cm long roots were cut off with a sharp knife and washed with sterile water. These root samples were then keep in sterile polythene bags, brought to the lab and processed within 4-5 hours after collection. Root samples were washed under running tap water for about 10 minute to remove extraneous adhering soil particles and cut into 3-4 cm size segments. These were then rinsed with sterile water after surface sterilization with 90% alcohol for 2-3 minutes. The segments were incubated at 20 ± 2^0C for 5-20 days in sterile petri-dishes containing 30 ml of sterile water. Incubated dishes were observed periodically to detect the conidia of endophytic fungi under low power of microscope.

Simultaneously, some of the surface sterilized root segments were placed in 2% Malt Extract Agar, supplemented with streptopenicillin or tetramycin solution (250mg/l) and incubated for a few days depending upon the growth of emerging fungi. Fungal mycelia growing on agar blocks were transferred into another petri dishes containing sterile water for sporulation and identification.

RESULTS

The incubated root segments of different host plants revealed a total of 21 species of water borne conidial fungi belonging to 15 genera as root endophytes (Table 1 and Fig. 1). A perusal of available literature indicates that *Camposporium pellucidum, Diplocladiella scalaroides* and *Helicomyces roseus* are being reported for the first time as root endophytes.

As seen in table 1, *Alatospora acuminata, Campylospora purvula, Cylindrocarpon aquaticum, Heliscus lugdunensis, Tetracladium marchalianum* and *Tetracladium setigerum* were the most commonly occurring endophytes while four species viz., *Alatospora pulchella, Campylospora chaetocladia, Camposporium pellucidum* and *Diplocladiella scalaroides* occurred in only one host species. Similarly all the reported species of *Lemonniera*, as root endophytes, showed their occurrence restricted to *Lyonia ovalifolia*. The maximum host diversity was reported in *Cylindrocarpon aquaticum* as it was colonized on 14 host plants followed by *Tetracladium setigerum* (13), *T. marchalianum* (9), *Heliscus lugdunensis* (8), *Campylospora purvula* (8), *Alatospora acuminata* (6) and *Anguillospora longissima* (6). The remaining species viz., *Acaulopage tetraceros, Anguillospora crassa, Campylospora chaetocladia, Clavariopsis aquatica, Camposporium pellucidum, Diplocladiella scalaroides, Helicomyces roseus, Lemonniera cornuta, L. pseudofloscula, L. terrestris, Lunulospora curvula, Pestalotiopsis submerses* and *Tetrachaetum elegans* showed little host diversity.

Among the 24 host plants studied, the roots of *Lyonia obvalifolia* were colonized by maximum number of endophytes, followed by *Botrychium* (8) and *Machillus duthiei* (6) while *Artemisia vulgaris, Rosa moschata, Debregeasia* species, *Aesculus indica, Valeriana wallichei, Myrseine semiserrata, Nepeta leucophyla* and *Salix tetrasperma* were colonized by less number of root endophytes. As evident from Table-2, the relative contribution to diversity of fungal endophytes in different host plant, maximum relative contribution was found to *Lyonia ovalifolia* i.e. 47.6% and minimum relative contribution of fungal root endophytes was confined to *Aesculus indica, Geranium nepalenses, Murraya koengii* and *Salix tetraspera* (4.7%each).

DISCUSSION

Of all the water borne conidial fungi recorded in this study, 8 species were also reported by earlier mycologists. Two species namely, *Anguillospora longissima* and *Tetracladium marchalianum*, reported earlier by Nemec (1969) from Strawberry plant roots, were recorded here on roots of an Unidentified grass, *Botrychium* sp., *Geranium nepalense, Barberis* sp., *Machilus duthiei, Symplocos chinensis, Eupatorium adenophyllum, Equisetum* sp., *Lyonia ovalifolia, Quercus floribunda, Salix tetrasperma*, unidentified fern and *Viburnum mullaha*. *Campylospora purvula* earlier reported by Fisher and Petrini (1989, 1990) was isolated from *Botrychium* sp., *Lyonia ovalifolia, Barberis* sp., *Acer pictum, Machilus duthiei, Symplocos chinensis, Vibrunum mullah* and *Strobilanthus dalhousianus, . Heliscus lugdunensis* was isolated from *Acer pictum, Barberis* sp., *Botrychium* sp., *Delbergeasia* sp, *Lyonia ovalifolia, Machilus duthiei, Rosa moschata* and *Symplocos chinensis*. It was also reported by Fisher et al (1991) and Sridhar and Barlocher (1992) from the roots of species of *Alnus, Acer* and *Betula*. *Lunulospora curvula*, earlier reported by Sridhar and Barlocher (1992a) on *Alnus* species, in present study it was isolated from *Eupatorium adenophyllum* and

Botrychium sp. *Tetracladium setigerum* recorded earlier from strawberry roots by Watanbe (1975) was isolated from *Alnus nepalensis, Eupatorium adenophyllum, Lyonia ovalifolia***,** *Murraya koenegii,* unidentified fern**,** *Equisetum* sp.**,** *Machilus duthiei, Botrychium* sp.**,** *Quercus floribunda, Artemisia vulgaris, Valeriana wallichii* and *Delbergeasia* sp. In the present study, *Clavariopsis aquatica* was isolated from *Botrychium* sp. and *Quercus floribunda***,** whereas, Sridhar and Barlocher **(**1992a) reported it from roots of *Alnus* and *Picea* sp. Fisher et al (1991) have also found this species as an endophyte. *Cylindrocarpon aquaticum,* found as a most frequently colonizing fungus, was collected from plant roots of *Aesculus indica, Acer pictum, Barberis* sp., *Artemisia vulgaris, Strobilanthus dalhousianus, Valeriana wallichii***,** *Geranium nepalense, Nepta leucophylla, Rosa moschata***,** *Vibrunum mullah***,** *Alnus nepalensis* and *Delbergeasia* sp. It was also earlier reported by Sridhar and Barlocher (1992a, 1992b) from maple and spruce roots.

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Western Himalaya

S. No	Endophyte	Host
1.	*Acaulopage tetraceros* Derchsler	Mk / Lo / Ea
2.	*Alatospora acuminata* Ingold	UG / E / Md / Vm / Id / An
3.	*A. pulchella* Marvanová	UG
4.	*Anguillospora crassa* Ingold	E / B
5.	*A. longissima*(Sacc.and Therry) Ingold	UG / Bm / Gn / B / Md / Sc
6.	*Camposporium pellucidum*(Grove) Hughes	Sd
7.	*Campylospora chaetocladia* Ranzoni	Mk
8.	*C. purvula* Kuzuha	Bm / Lo / B / Ap / Md / Sc / Vm / Sd
9.	*Clavariopsis aquatica* de Wildeman	Bm / Qf
10.	*Cylindrocarpon aquaticum*(Nils.) Marvanova and Descals	Ai / Ea / Md / Ap / B / Av / Sd / Vw / Gn/ Nl / Rm / Vm / An / D
11.	*Diplocladiella scalaroides* Arnaud	Gn
12.	*Helicomyces roseus* Link	Qf / Ap
13.	*Heliscus lugdunensis* Ingold	Ap / B / Bm / D / Lo / Md / Rm / Sc
14.	*Lemonniera cornuta* Ranzoni	Lo
15.	*L. pseudofloscula* Dyko	Lo
16.	*L. terrestris* Tubaki	Lo
17.	*Lunulospora curvula* Ingold	Ea / Bm
18.	*Pestalotiopsis submersus* Sati and Tiwari	E / F/ Lo
19.	*Tetrachaetum elegans* Ingold	Lo / Bm
20.	*Tetracladium marchalianum* de Wildeman	Ea / E / Lo / Ms / Bm / Qf / St / UG / F
21.	*T. setigerum*(Grove) Ingold	An/Ea/LO/ Mk/ F / E / Md / Bm / Qf / Av / Vw / D

Mk = *Murraya koenegii*, **Lo** = *Lyonia ovalifolia*, **UG** = Unidentified grass, **E** = *Equisetum* sp., **B** = *Barberis* sp. , **Bm =** *Botrychium sp.*, **Ai=** *Aesculus indica*, **Ea** = *Eupatorium adenophyllum*, **F** = Unidentified *Fern*, **Md =** *Machilus duthiei*, **Vm** = *Vibrunum mullah*, **Id** = *Ilex* diphyrena, **An** = *Alnus nepalensis*, **Gn** = *Geranium nepalense*, **Sc** = *Symplocos chinensis*, **S** = *Strobilanthes* sp., **Ap** = *Acer pictum*, **Qf** = *Quercus floribunda*, **Av** = *Artemisia vulgaris*, **Vw** = *Valeriana wallichii*, **Nl** = *Nepta leucophylla*, **Rm** = *Rosa moschata*, **D** = *Debregeasia* sp., **St** =*Salix tetrasperma*

* Calculated against 24 studied host species (Table 2)

Table 2: Colonization of endophytes on roots of different host plants

S. No.	Host	Percent contribution to diversity - of fungal endophyte(%) *
1.	*Acer pictum* Thunb	19.0
2.	*Aesculus indica C*olebr	4.7
3.	*Alnus nepalensis* D. Dori	14.3
4.	*Artemisia vulgaris* Linn	9.5
5.	*Berberis* sp. Roxb.	23.8
6.	*Debregeasia* sp Gaud.	14.3
7.	*Equisetum* sp. Linn.	23.8
8.	*Eupatorium haterophyllum* Linn.	23.8
9.	Unidentified *Fern*	14.3
10.	*Geranium nepalense* Sw.	19.0
11.	*Ilex diphyrena* all.	4.7
12.	*Lyonia ovalifolia* Wall	47.6
13.	*Machilus duthiei* King	28.5
14.	*Murraya koenegii* Spreng.	14.3
15.	*Nepeta leucophylla* Benth.	4.7
16.	*Botrychium* sp.	38.1
17.	*Quercus floribunda* Wall.	19.0
18.	*Rosa moschata* J. Herrm.	9.5
19.	*Salix tetrasperma* Roxb.	4.7
20.	*Strobilanthes* sp Blume.	19.0
21.	*Symplocos chinensis* ochinchinewis(Lour)	14.3
22.	Unidentified grass	19.0
23.	*Valeriana wallichii* . DC.	9.5
24.	*Viburnum mullaha* Buch- Ham. Ex . D. Don	14.3

* Calculated against 21root endophytes altogether recorded (Table 1)

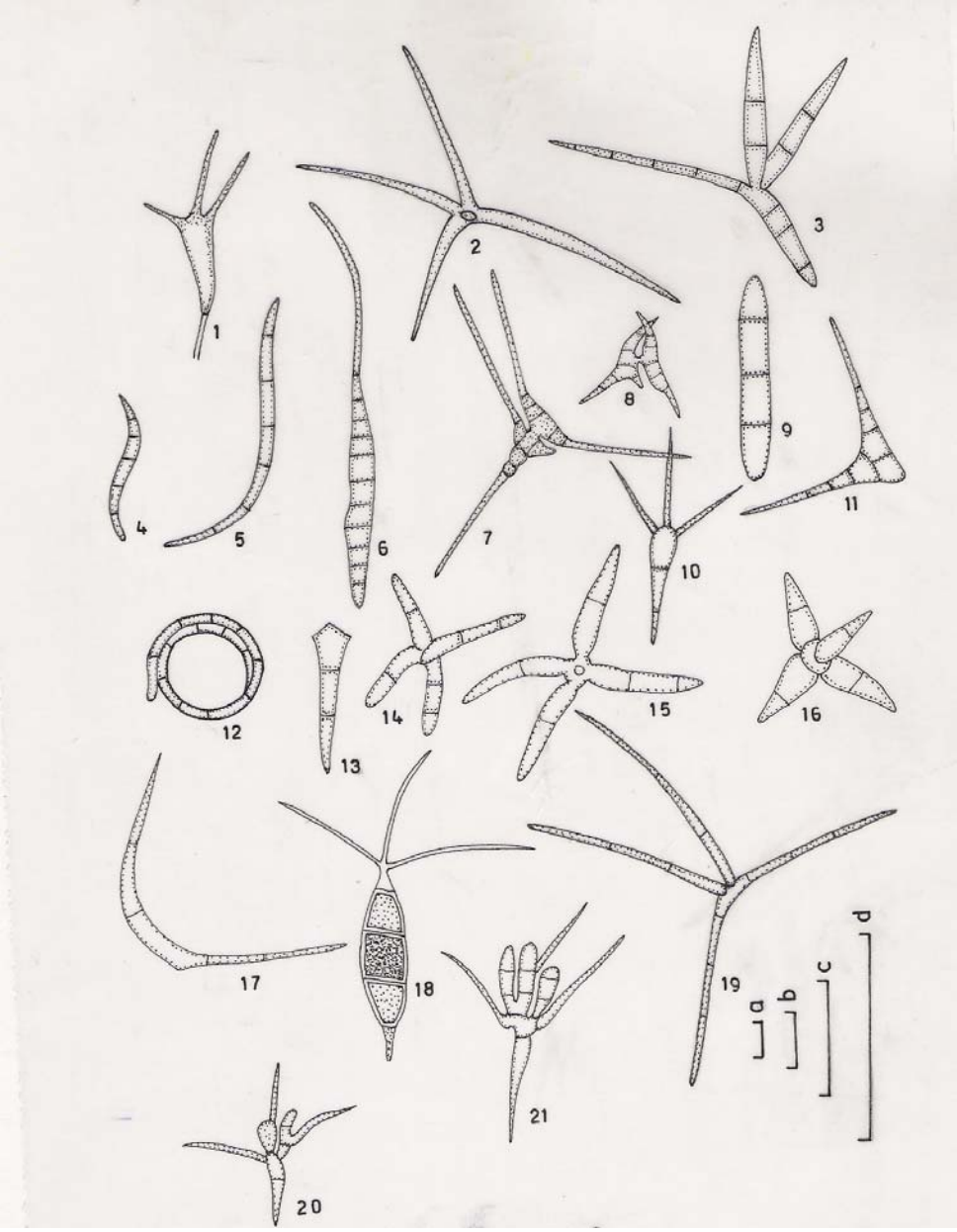

Fig.1

Figs. 1,7, 8, 9,11,13,14-18, 20 and **21** in **scale c**; **4-5** in **scale a**; **6, 10, 12** and **19** in **scale b** and **2-3** in **scale d**. ***(Fig. Nos. are corresponding to Table No. 1)***

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