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Biodiversity of asexually produced aquatic conidial fungi as root endophytes from Kumaun Himalaya

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ABSTRACT

Endophytes are the organisms inhabiting inside plant organs during their life cycle or colonizing internal plant tissues without causing apparent harm to the host. Many asexually produced conidial fungi which are also known as aquatic hyphomycetes were collected from fresh water bodies as root endophyte. A total of 24 species of conidial fungi were recovered from root part of plants grown in and around Kumaun Himalaya. Three species namely, *Lemonniera aquatica, Helicomyces torquatus* and *Tetraploa aristata* were recovered as new root endophyte from Kumaun Himalaya. However, *Cylindrocarpon aquaticum, Diplocladiella scalaroides, Pleurophragmium sonam, Setosynnema isthmosporum* and *Tetracladium marchalianum* were recovered with their new host plants.

Keywords : Root Endophytic Fungi, Fungal endophyte, Kumaun Himalaya, New hosts

Majority of fungi produce their spore asexually and in aquatic ecosystem these are also known as freshwater hyphomycetes, Ingoldian fungi or amphibious hyphomycetes (Nilsson 1964, Michaelides and Kendrick 1978, Bärlocher 1982, Wood-Eggenschwiler and Bärlocher 1985). Their occurrence and distribution predominantly occur in running freshwater on submerged partially decomposed leaf litter. These are polyphyletic group of true fungi which are mainly characterized by asexually produced magnificent spore types i.e. spherical or ovoid, tetraradiate or triradiate, long and worm-like structures (Ingold 1975, Bandoni 1975, Webster 1981, Belliveau and Bärlocher 2005). In Indian context, these fungi were studied from various water habitats by different investigators including Subramanian (1983), Manoharachary (1991), Sridhar *et al.* (1992), Kumar and Saha (1992), Sati and Tiwari (1997), Sati *et al.* (2002) and Prasad *et al*. (2009).

The Fungal endophytes describe internal mycota of living plants (Stone *et al.* 2012). Fungal endophytes are also determined as mutualists that protect host plant against insect herbivory (Clay 1990), and many of those fungi are known to produce biologically active secondary metabolites (Stone *et al*. 2012). These are present inside the plant which can not be detected externally from plant morphology. Endophytes occupy a unique niche and have major influence on distribution, physiology, ecology and biochemistry of plants (Sridhar and Raviraja 1995).

Waid (1954) was the first to isolate these fungi as root endophyte, followed by many other workers (Nemec 1969, Park 1974, Fisher and Petrini 1989). Sridhar and Bärlocher (1992) reported some species of aquatic hyphomycetes as root endophyte of spruce, birch and maple respectively. In Kumaun Himalayan region root endophytic aquatic hyphomycetes were also isolated by Sati and his co-workers from roots of different plants grown in and around the freshwater habitats. More than 30 species of conidial fungi are isolated as an endophytes from roots of various angiospermic, gymnospermic and pteridophytic plants of Kumaun Himalaya (Sati and Belwal 2005, Sati *et al*. 2006, 2009, Sati and Pathak 2017, Pant *et al.* 2019).

Root endophytic aquatic fungi are known to produce a wide spectrum of bioactive compounds and play an important role in plant growth promotion and protection from environmental stress. Therefore, the present study was carried out to explore asexually produced conidial fungi associated as root endophyte of ravine area plants in randomly selected Kumaun Himalayan regions.

MATERIALS AND METHODS

Study sites : Present study was carried out in Kumaun Himalaya, Uttarakhand which lies in between the latitudes of 28º-44' to 30º-49'N and longitudes of 77º-48' to 81º-5'E along the western part of Central Himalaya. Root samples were collected from riparian areas around fresh water streams of Almora, Bageshwar, Nainital and Pithoragarh districts of Kumaun Himalaya.

Collection and processing of root samples : Root samples were collected using sterile knife or scissors, rinsed with sterile distilled water and then stored in sterile plastic bags. These sample once bought into laboratory were washed by leaving under running tap water for 4-5 hours and rinsed with sterile water. The washed root samples were immersed in 2% sodium hypochlorite solution for 2-5 minutes to remove attached free living microbes. These samples were further washed with sterilized water and further incubated at room temperature (20±2ºC). The incubated root samples in water

were observed regularly to confirm the emerging mycelia and conidia under microscope using Direct detection method (Sati and Belwal 2005). The thoroughly washed root samples were dipped in 96% ethanol (30-60s) then placed into petridishes containing 2% Malt extract agar (MEA) medium using Agar plating method (Sati and Arya 2010a).

Identification of isolated fungi : The detail conidial development and spore morphology was recorded by preparing aquatic cultures. The sporulated conidia of endophytic aquatic hyphomycetes were studied by preparing semi-permanent slides and further observed under microscope. The isolated root endophytic aquatic fungi were identified with the help of pertinent literature, monographs and concern authorities of this field (Ingold 1975, Descals and Webster 1982, Santos Flores and Lopez 1997, Sati *et al.* 2009). The semi-permanent slides were deposited in Department of Botany, Kumaun University Mycological Slide (KUMS) collection.

RESULTS AND DISCUSSION

During the course of present investigation roots of several plants (herb, shrub or tree) occurring in riparian area of Almora, Bageshwar, Nainital and Pithoragarh districts were examined. A total of 24 species of aquatic hyphomycetes belonging to 18 genera viz., *Alatospora*, *Anguillospora*, *Beltrania*, *Camposporium*, *Campylospora*, *Cylindrocarpon*, *Diplocladiella*, *Flagellospora*, *Helicomyces*, *Lemonniera*, *Lunulospora*, *Pestalotiopsis*, *Pleurophragmium, Setosynnema*, *Tetracladium*, *Tetraploa*, *Tricladium* and *Triscelophorus* were isolated from various host plants (Fig.1). The asexual stage of these root endophytic conidial fungi were observed after their sporulation in incubated root samples in water. The isolated root endophytic freshwater hyphomycetes showed no specificity towards their hosts as single species was recovered from different host plants as well as more than one fungal species were found associated with a common host plants. These fungi have also been recovered as root endophyte from the plants located at a distance from freshwater streams location.

In this study, *A. acuminata, A. longissima, C. pellucidum, C. parvula, C. aquaticum, D. scalaroides, F. penicillioides, H. torquatus, L. aquatica, L. curvula, P. sonam, S. isthmosporum, T. breve, T. marchalianum, T. nainitalense, T. setigerum, T. indicum, T. monosporus* were found moderate to less abundant species as root endophyte. Whereas, *B. rhombica*, *C. chaetocladia*, *L. pseudofloscula, P. submersus, T. aristata,* and *T. marylandicum* were found rare in occurrence (Table 1). It is interesting to note that *Cylindrocarpon aquaticum* was found the most frequently colonizing endophyte as it was recorded from almost all the studied sites (Table 1). These asexually reproduced conidial fungi were also found as endophytes, associated with the roots of various host plants besides with two new host plants (Table 1). Earlier 31 species of aquatic hyphomycetes were recovered as root endophytes from Kumaun Himalaya (Sati and Belwal 2005, Sati *et al*. 2006, Arya and Sati 2008, Sati *et al*. 2009, Sati and Pathak 2017, Pant *et al*. 2019). In addition with these three new root endophytic conidial aquatic fungi i.e. *Helicomyces torquatus*, *Lemonniera aquatica* and *Tetraploa aristata*), the total record from Kumaun Himalaya, reached 34 species. Therefore, it can be concluded that the ravine area of Kumaun Himalaya is a rich source of root endophytic conidial aquatic fungi.

Saikkonen and co-workers (1998) suggested that the ecological role played by these endophytic fungi are diverse and varied. The biodiversity of aquatic fungi as endophyte may be a defensive phenomenon in these fungi to survive during unfavourable conditions inside water. The endophytism of these fungi from their natural habitat to preferred habitat clearly indicates the potentiality to produce bioactive compounds required for host plant. The bio-prospection of root endophytic aquatic fungi as plant growth promoters and anti-microbial potential have also been proved by Sati and Arya 2010a,b, Sati and Singh 2014, Sati and Pant 2019 and Sati and Singh 2019. In future, these root endophytic aquatic fungi may be exploited to develop new drug yielding compounds as well as growth enhancing compounds for plants in eco-friendly way.

Table 1: Asexually produced conidial fungi isolated as root endophytes

S. No.	Endophytes	Host Plant	Locality	Abundance	References
1.	*Alatospora acuminata* Ingold	*AN, EA*	BG, SV, VK	Least	Sati & Belwal 2005, Sati *et al.* 2009, Sati & Pathak 2017
2.	*Anguillospora longissima* (Sacc. & Therry) Ingold	*AN, MI*	GM, K	Least	Sati & Belwal 2005, Sati & Pathak 2017
3.	*Beltrania rhombica* Penzig	*AO, QL*	K	Rare	Sati & Pathak 2017
4.	*Camposporium pellucidum* Hughes	*AO, E, F, QL, EA*	K, P, ST, SV	Moderate	Sati *et al.* 2006

5.	*Campylospora chaetocladia* Ranzoni	*EA*	BG	Rare	Sati & Belwal 2005, Sati & Pathak 2017
6.	*Campylospora parvula* Kuzuha	*AI, G, LO, PS, BV*	BG, GM, P, RT,T	Moderate	Sati & Belwal 2005, Sati *et al.* 2009
7.	*Cylindrocarpon aquaticum* (Nils) Marvanova & Descals	*AO, AI, AN, D, E, EA, F, MI, PC, QF, QL, RE,* SH*, *SR**	K, P, CG, GM, N, RT, SV, VK	Common	Sati & Belwal 2005, Sati & Pathak 2017
8.	*Diplocladiella scalaroides* Arnaud	*AI, LO, SR**	K, CG, RT, ST, SV	Moderate	Sati *et al.* 2006
9.	*Flagellospora penicillioides* Ingold	*QL, AO, EA*	K	Least	Arya & Sati 2010
10.	*Helicomyces torquatus*** Lane & Shearer	*AI, LO*	K, P	Least	-
11.	*Lemonniera aquatica*** De Wild	*AI, AO, D*	K, SV	Least	-
12.	*L. pseudofloscula* Dyko	*MI*	K	Rare	Sati& Belwal 2005
13.	*Lunulospora curvula* Ingold	*AO, EA, QF, QL*	K, GM, ST	Least	Sati & Belwal 2005, Sati *et al.* 2009
14.	*Pestalotiopsis submersus* Sati & Tiwari	*QF*	SV	Rare	Sati & Belwal 2005, Sati & Pathak 2017
15.	*Pleurophragmium sonam* Sati & Tiwari	*AO, QL, SR**	CG, K	Least	Sati & Pathak 2017
16.	*Setosynnema isthmosporum* Shaw & Sutton	*PS, SR**	CG, K	Least	*Ibid.*
17.	*Tetracladium breve* Roldan	*PC*	RG, SV	Least	Arya & Sati 2010
18.	*T. marchalianum* De Wild	*EA, CD, SC, SR*, F, E, AI, BV*	CG, P, RT, ST, SV	Moderate	Sati & Belwal 2005, Sati *et al.* 2009, Sati & Pathak 2017
19.	*T. nainitalense* Sati & Arya	*LC, RE*	GM, RG	Least	Sati *et al.* 2009
20.	*T. setigerum* (Grove) Ingold	*D, BV, AO, RE*	GM, K, P, RT, RG	Moderate	Sati & Belwal 2005, Sati *et al.* 2009
21.	*Tetraploa aristata*** Berkeley & Broome	*G, EA*	T	Rare	-
22.	*Tricladium indicum* Sati & Tiwari	*AP, QF*	K	Least	Pant *et al.* 2019
23.	*T. marylandicum* Crane	*AI*	K	Rare	*Ibid.*
24.	*Triscelophorus monosporus* Ingold	*D, LC, CD, E* RG, RT	BG, GM, K,	Moderate	Sati & Belwal 2005

*New host, **New root endophyte from Kumaun Himalaya

AI- *Aesculus indica*, AN- *Alnus nepalensis*, AO- *Acer oblongum,* AP- *Acer pictum,* BV- *Berberis vulgaris*, CD- *Cedrus deodara*, D- *Debregeasia* sp., E- *Equisetum* sp., EA- *Eupatorium adenophorum*, F- Ferns, G- Grass, LC- *Lantana camara*, LO- *Lyonia ovalifolia*, MI- *Myrica indica*, PC- *Pyracantha crenulata*, PS- *Pilea scripta,* QF- *Quercus floribunda*, QL- *Quercus leucotrichophora*, RE- *Rubus ellipticus*, SC- *Syzygium cumini*, SH- *Sarcococca hookeriana**, SR- *Shorea robusta**

BG- Bageshwar, CG- Chorgalia, GM- Gufa Mahadev, K-Kilbury, N- Niglat, RG- Ramgarh, RT- Ratighat, P- Pangot, ST- Saat Tal, SV-Snow View, T- Tejam

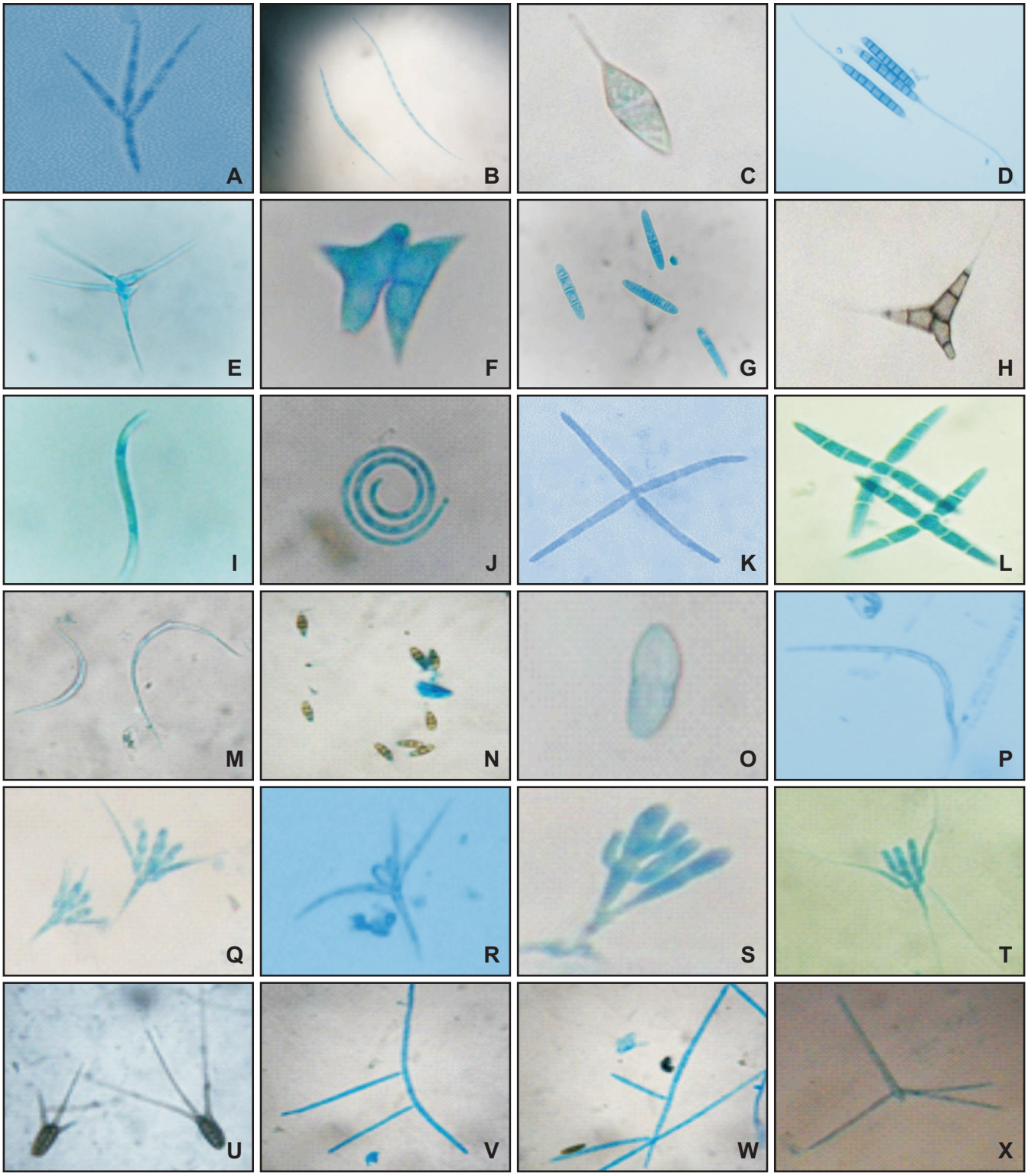

Fig. 1: Anamorphic forms of root endophytic aquatic fungi: A. *Alatospora acuminata,* B.*Anguillospora longissima*, C. *Beltrania rhombica*, D. *Camposporium pellucidum*, E. *Campylospora chaetocladia*, F. *C. parvula*, G. *Cylindrocarpon aquaticum*, H. *Diplocladiella scalaroides*, I. *Flagellospora penicillioides*, J. *Helicomyces torquatus*, K. *Lemonniera aquatica*, L. *L. pseudofloscula*, M. *Lunulospora curvula*, N. *Pestalotiopsis submersus,* O. *Pleurophragmium sonam*, P. *Setosynnema isthmosporum*, Q. *Tetracladium breve*, R. *T. marchalianum*, S. *T. nainitalense*, T. *T. setigerum*, U. *Tetraploa aristata*, V. *Tricladium indicum*, W. *T. marylandicum*, X. *Triscelophorus monosporus*

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