Diversity of water-borne conidial fungi in some freshwater bodies of Kumaun Himalaya in district Nainital (Uttarakhand), India

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INTRODUCTION

Fungi inhabiting the submerged decomposed leaf litter in stream and river waters are referred to as aquatic hyphomycetes, freshwater hyphomycetes, amphibious hyphomycetes, Ingoldian fungi, water-borne conidial fungi etc. The pioneer studies of Professor C.T. Ingold (1942) were the most significant contributions to advert to these fungi. Water-borne conidial fungi are deutromycetous fungi characterized by producing distinctive shapes of conidia colonizing deciduous leaves, decaying in freshwater bodies (Ingold, 1975). The shapes of conidia and their attachment with conidiophores help in identification and characterization of these conidial fungi (Barlocher and Marvano-va, 2010). The altitudinal and seasonal differences in diversity of these fungi may be due to physico-chemical properties of water, nutrient and substrate availability (Pant et al., 2019). Environmental variables such as temperature and pH are also the dominant factors that affect the growth of these fungi (Duarte et al., 2013; Bai et al., 2018). Their occurrence and frequency to extreme temperatures and pH may have a profound effect on fungal community composition and metabolic activities. For example, temperate species are found to survive at freezing temperatures and tropical species may survive at higher temperatures.
(Sridhar and Barlocher, 1993; Krauss et al., 2011). Freshwater ecosystems comprise complex food webs in which each species plays an essential role as producer and consumer. Among those, water-borne conidial fungi are known to fulfill an important and unique function of degrading allochthonous dead plant litter allowing the transfer of resulting energy and nutrients to higher trophic levels (Gulis, 2019). Therefore, the hidden aspects of these fungi are needed to be studied with great pace from unexplored areas.

Kumaun Himalaya, a temperate climatic zone with many water bodies at different elevations having a rich source of substrate pool provides suitable habitat for the growth of diversified forms of water-borne conidial fungi. A substantial contribution on these fungi from this region has been made earlier (Sati et al., 2002; Sati et al., 2009; Pant and Sati, 2018, Pant et al., 2019).

The present study was undertaken to know the species diversity of water-borne conidial fungi from some unexplored areas of Kumaun Himalaya along an altitudinal gradient (1000-1500 m) and to assess species composition of these fungi along different seasons, pH preferences and temperature requirements.

MATERIALS AND METHODS

Study area: The present study was carried out in different unexplored streams flowing along varied localities viz., Ramgaarh, Kulgarh and Devdwar. Ramgaarh region (=1000 m) is situated near Haldwani-Almora highway. A small hydropower project by Uttarakhand Renewable Energy Developmental Agency (UREDRA) is established on the stream to provide electricity to the nearby villages. Kulgarh region (=1100 m) is situated in Nainital district (Uttarakhand) also known for Dho-kaney waterfall, and Devdwar region (=1500 m) is situated in Kosya Kutoli, Nainital.

Collection and processing: Submerged decomposed leaf litter was collected in pre-sterilized polythene bags seasonally from summer (April to June), rainy (July to September), winter (November to December), 2019 and brought to the laboratory. The collected samples were washed thoroughly under running tap water to remove soil particles and other extracellular debris. The leaf litter was then cut into small pieces and placed into pre-sterilized Petri dishes containing distilled water for incubation at room temperature. After 2-3 days of incubation, samples were regularly examined under low power of a compound microscope to detect the conidia of water-borne conidial fungi. Water pH was also measured with the help of portable pH meter on the spot during collection and the sporulating temperatures of different fungal species in different seasons were measured by using room thermometer.

Identification of fungal species: Conidia were picked aseptically and placed on 2% malt extract agar (MEA) supplemented with streptomycin to obtain Axenic cultures. Semi-permanent slides of these conidia were prepared using lactophenol cotton blue stain and deposited in Govt. Girls College Mycological Slide (GGCMS) collection of Department of Botany, Haldwani (Nainital). Photomicrographs and Camera Lucida drawings of conidia were prepared and compared with pertinent literature for identification (Ingold, 1975; Santos and Betancourt, 1997).

RESULTS AND DISCUSSION

During the present study, 18 species of water-borne conidial fungi belonging to 14 genera were isolated and identified from submerged decomposed leaf-litters collected from different streams of Ramgaarh, Kulgarh and Devdwar at an altitudinal range of 1000-1500 m (Fig. 1a, 1b; Plate 1; Table 1). Comparison of species composition along altitudinal gradients in relation to temperature and pH requirements is represented graphically (Fig. 2, 3).

Taxonomic description

**Alatospora acuminata** Ingold (Plate 1. A; Fig. 1a. A): Conidia collected from Ramgaarh, Kulgarh and Devdwar in summer (April to June) and winter (November to December) seasons at water pH 7 and 8, sporulation temperature ranging from 17-30 °C, were hyaline, tetra radiate, with curved main axis 21-40 µm long, and two curved appendages 25-50 µm long having 3-4 septations.

**Anguillospora crassa** Ingold (Plate 1. B; Fig. 1a. B): Conidia collected from Ramgaarh, Kulgarh and Devdwar in summer (April to June) and rainy (July to September) seasons at water pH 6 and 8, sporulation temperature ranging from 30-32 °C, were hyaline, vermiform, 90-200 µm long, 10-20 µm wide, 5-10 septate, tapering towards ends.

**Anguillospora longissima** (Sacc. And Syed) Ingold (Plate 1. C; Fig. 1a. C): Conidia collected from Ramgaarh in summer (April to June) season at water pH 8, sporulation temperature ranging from 30-36 °C, were hyaline, unbranched, filiform, curved with main axis 150-350 µm long, 4-6 µm wide at middle having 5-12 septations, tapering at both the ends.

**Beltrania rhombica** Penzig (Plate 1. D; Fig. 1a. D): Conidia collected from Devdwar in rainy (July to September) season at water pH 6, sporulation temperature ranging from 30-32 °C were light-brown to pale-olive, consisting of a bi-conic, symmetrical, main axis, 16-30 µm long, 6-9 µm wide, with a distinct, hyaline, transverse band and an apical, hyaline 10-15 µm long appendage with a basal septum.

**Campylospora chaetoclada** Ranzoni (Plate 1. E; Fig. 1a. E): Conidia collected from Kulgarh in rainy (July to September) season at water pH 6, sporulation temperature ranging from 20-30 °C were tetra radiate, main axis alantoid, composed of a smaller part 10-15 µm in length and 8-11 µm in width, while arms are 27-38 µm long. Apical cells of axis conoid to bulbous, each end in one of the slender appendages.
Clavariopsis aquaticum (De Wildeman) Ingold (Plate 1. F; Fig. 1a. F): Conidia collected from Ramgaarh and Devdwar in summer (April to June) and winter (November to December) seasons at water pH 7 and 8, sporulation temperature ranging from 17-30 °C were hyaline, 2-3 celled obconical main axis, 25-60 µm long, 10-16 µm wide, lateral arms arising from the broad end of the 1st arm are 20-100 µm long. Usually, appendages or arms are longer than the axis.

Clavatospora tentacula Nilsson (Plate 1. G; Fig. 1a. G): Conidia collected from Kulgarh in winter (November to December) season at water pH 7, sporulation temperature ranging from 15-17 °C were tetra-radiate, main axis 30-75 µm long and 1.5-2.5 µm wide at the base, 4-7 µm wide at apex, with 3-equidistant, divergent 30-55 µm long and 1-2 µm wide appendages arising from the apex and constricted at the base.

Cylindrocarpon aquaticum Nilsson (Plate 1. H; Fig. 1a. H): Conidia collected from Ramgaarh, Kulgarh and Devdwar in summer (April to June) and rainy (July to September) seasons at water pH 6 and 8 sporulation temperature ranging from 27-30 °C were brown, cylindrical, small, septate and measured up-to 15-18 µm in length and 3-4 µm in width.

Helicomyces roseus Link (Plate 1. I; Fig. 1a. I): Conidia collected from Ramgaarh and Devdwar in rainy (July to September) season at water pH 6 sporulation temperature ranging from 30-32 °C were helical, coiled up to 2.5-3 times with rounded ends, 20-30 µm in diameter with 10-15 septa, tapering to an enlarge, obliquely flattened basal cell.

Leptonnia pseudoofloscula Dyko (Plate 1. J; Fig. 1b. A): Conidia collected from Kulgarh and Devdwar in winter (November to December) season at water pH 7 sporulation temperature ranging from 17-20 °C were hyaline, tetra-radiate, main axil cell spherical 3-6 µm in diameter, 1-6 septations, consisting of 3-4 appendages, 13-72 µm long and 3-4 µm wide.

Leptonnia terrestris Tubaki (Plate 1. K; Fig. 1b. B): Conidia collected from Kulgarh in winter (November to December) season at water pH 7, sporulation temperature ranging from 17-20 °C were hyaline, tetra-radiate, main axil cell spherical 4-7 µm in diameter, appendages 9-13 µm long and 3-6 µm wide.

Lunulospora curvula Ingold (Plate 1. L; Fig. 1b. C): Conidia collected from Ramgaarh, Kulgarh and Devdwar in rainy (July to September) and winter (November to December) seasons at water pH 6 and 7, sporulation temperature ranging from 17-28 °C were hyaline, lunate, unbranched, elongated, aseptate, dematiaceous conidia 40-50 µm long and 4-6 µm wide.

Lunulospora cymbiformis Miura (Plate 1. M; Fig. 1b. D): Conidia collected from and Devdwar in rainy (July to September) and winter (November to December) seasons at water pH 6 and 7, sporulation temper-

<table>
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Place- D- Devdwar (=1500 m), Ku- Kulgarh (=1100 m), R- Ramgaarh (=1000 m)
Seasons- S- Summer (April to June), R- Rainy (July to September), W- Winter (November to December)


ature ranging from 17-28 °C were hyaline to light green, sickle-shaped, aseptate dematiaceous conidia bent at right angle, 40-50 µm long and 3-5 µm wide with a characteristic scar in the middle.

Setosynnema isthmosporum Shaw and Sutton (Plate 1. N; Fig. 1b. E): Conidia collected from Ramgaarh, Kulgarh and Devdwar in rainy (July to September) and winter (November to December) seasons at water pH 6 and 7, sporulation temperature ranging from 17-25 °C were sigmoid 150-200 µm long, 3-4 µm wide, tapering towards the tips, 6-7 septate.

Tetrachaetum elegans Ingold (Plate 1. O; Fig. 1b. F): Conidia collected from Devdwar in winter (November to December) season at water pH 7, sporulation temperature ranging from 15-20 °C were hyaline, tetra-radiate, main axis bent at the insertion of 2 appendages, main axis 130-200 µm long, arms 50-150 µm long and uniform in width with 6-8 septations.

Tetracladium marchalianum de Wild (Plate 1. P; Fig. 1b. G): Conidia collected from Ramgaarh, Kulgarh and Devdwar in summer (April to June), rainy (July to September) and winter (November to December) seasons at water pH 6-8, sporulation temperature ranging from 15-30 °C were tetra-radiate, with two spherical knobs (one central and one eccentric), the main axis is 15-20 µm long, while the central knob is 9-12 µm long, septations not prominent.

Tetracladium setigerum Grove (Plate 1. Q; Fig. 1b. H): Conidia collected from Kulgarh in rainy (July to September) season at water pH 6, sporulation temperature ranging from 17-20 °C were hyaline, 12-15 µm long and 3-4 µm wide with 3-6 septations, conidial axis.
distally digit form. The knobs are 5-12 µm long with 1-3 septations.

**Triscelophorus acuminatus** Nawawi (Plate 1. R; Fig. 1b. I): Conidia collected from Ramgaarh, Kulgarh and Devdwar in rainy (July to September) and winter (November to December) seasons at water pH 6 and 7, sporulation temperature ranging from 20-32 °C were hyaline, variable in size and shape, the main axis is 30-50 µm long, gradually tapering at tip; the lateral arms are 27-36 µm in length.

Thus, in the present study, out of 14 genera, 10 genera were recorded with single species and the rest 4 genera namely, *Anguillospora, Lemonniera, Lunulospora,* and *Tetracladium* were found with 2 species each. A perusal of seasonal occurrence of different species in the habitat indicates that most of the water-borne conidial fungi showed a marked fluctuation in their occurrence (Sati and Arya, 2009). In the present study, maximum species (11 species) were recorded in both rainy (July to September) and winter seasons (November to December). *A. crassa, B. rhombica, C. chaetocladia, C. aquaticum, H. Roseus* and *T. setigerum* were isolated only during rainy season; *A. acuminata, A. aquatica, C. tentacula, L. pseudofloscula, L. terrestris,* and *T. elegans* were isolated only during winter season while *L. curvula, L. cymbiformis, S. isthmosporum, T. elegans,* and *T. acuminatus* were isolated in both rainy and winter seasons. Least species (6 species) viz., *A. acuminata, A. crassa, A. longissima, C. aquatica, C. aquaticum* and *T. marchalianum* were recorded during summer season (April to June). This may be due to high temperature (> 30 °C) and high pH (8) in summers and moderate to low temperature (15-30 °C) and acidic to neutral pH (6-7) in rainy and winter seasons. The pH of water bodies differed seasonally from 6-8, being maximum during summer and minimum during the rainy season. The pH condition between 6-7 favoured maximum

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growth of these fungi and the sporulation temperature between 15-20°C was found to be optimal (Fig. 2 and 3). Many investigators have also observed similar results that the water-borne conidial fungi show maximum growth in rainy and winter seasons due to low temperature and pH (6-7) (Barlocher, 1987; Belwal and Sati, 2001; Dang et al., 2009; Krauss et al. 2011).

It was interesting to note that altitudinal variations considerably influenced the species composition of water-borne conidial fungi in all the selected sites. A. longissima was isolated only from Ramgaarh stream (=1000 m); C. chaetocladia, C. tentacula, L. terrestris and T. setigerum were isolated only from Kulgarh stream (=1100 m); while B. rhombica, L. cymbiformis and T. elegans were isolated only from Devdwar stream (=1500 m). This may be because of the temperature and altitudinal preferences of different species that they are linked to geographical distribution, and some species are common in temperate regions while some in tropical regions. Some temperate species are found to survive at freezing temperatures, and tropical species may survive at higher temperatures as reported by Sridhar and Barlocher, (1993) and Krauss et al. (2011).

Further, out of the 18 species isolated from different streams, T. marchalianum was found to occur throughout the year from all the sites. This may be regarded as temperature tolerant and common species of all the streams. These fungi were best sporulated at 15-20 °C and preferred pH (6-7) slightly acidic to neutral. This study gives a brief account of the diversity of Hyphomycetous fungi from some unexplored water bodies flowing through different altitudinal ranges of Kumaun Himalaya and also provided a database for altitudinal impact on species composition of water-borne conidial fungi.

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Conflict of interest

The authors declare that they have no conflict of interest.

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