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Research Journal of Agricultural Sciences
Volume 14; Issue 02 (Mar-Apr 2023); pp 611–615

Open Access
ISSN: 0976-1675 (P)
ISSN: 2249-4538 (E)

Full Length Research Article

Species Composition of Aquatic Hyphomycetes in Machilivan- A Piedmont Region of Kumaun Himalaya, India

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Abstract

Aquatic hyphomycetes are the mycota, playing an imperative role in maintaining ecological services viz. food web and energy flow in lotic aquatic ecosystems. The assessment of these fungi was done by surveying the leaf litter, water foam and riparian root samples of the Machilivan area of district Nainital, Uttarakhand (India). Machilivan constitutes a part of the Nandhaur River, flowing through the piedmont region of Kumaun Himalaya which is still unexplored for its mycofloral diversity. The samples were collected and processed for incubation, isolation and identification of aquatic hyphomycetes. Twelve species belonging to eight genera namely *Acaulopage, Anguillospora, Flagellospora, Lunulospora, Setosynema, Tetracladium, Trescelophorus,* and *Wiesneriomyces* were encountered from the present study site. *Acaulopage dichotoma* and *Anguillospora crassa* were recorded only in the form of riparian root endophytes. *Wiesneriomyces laurinus* was the first record from the foothill region of Kumaun Himalaya. Species richness was recorded as maximum in the leaf litter and minimum in the water foam samples. Winter season was noticeably most favourable for the growth of these fungi.

***Key words*:** Aquatic hyphomycetes, Machilivan, Kumaun Himalayan Piedmont, Diversity, Endophytes, Seasonal occurrence

Aquatic hyphomycetes are a polyphyletic group of fungi. Studies on these fungi were initiated more than a century ago [1], while C.T. Ingold described them for the first time in 1942 [2]. Usually dwelling in lotic aquatic ecosystems on submerged decaying plant parts, these fungi have reportedly extended their habitat to the riparian plant roots [3]. Reproducing asexually by the formation of morphologically unique conidia (crescent-shaped, elongated, helical, spherical, triradiate, tetraradiate, multiradiate etc.), forms the basis of identification of these fungi. Though the concept regarding the significance of conidial shape was given by Ingold [2], it was experimentally proved by Webster [4], who established their role in dispersal, anchorage and resistance against downward stream flow [5-6]. These fungi occupy the base of the decomposer food chain, owing to their intermediary role between decaying leaves and stream invertebrates [7]. Their role is undoubtedly substantial in the processing of leaves, cycling of elements, energy flow and productivity of an aquatic ecosystem. To investigate their multifarious benefits, these fungi need to be explored at a great pace from different water bodies.

The pioneering work of Ingold [2] attracted the consideration of mycologists that resulted in the discovery of the first aquatic hyphomycetes i.e., *Varicosporium elodeae* from Assam [8], followed by *Speiropsis hyalospora* from Uttar Pradesh [9]. Later, worldwide researchers contributed to the study of different aspects of these fungi and the brief contribution of India is reported by Sridhar [6]. The main source of organic matter and energy of the water bodies is plant detritus [10-11], which is primarily colonized and decomposed by these fungi [12]. The need of the hour is to explore this fascinating group of mycota from different climate zones to contribute to the science of the environment and its conservation. Kumaun Himalaya, having a temperate climate is being explored extensively [13-14], while the foothill region with its comparatively warm temperature and diverse vegetation, has been of least concern regarding the mycofloral diversity exploration [15]. In the present study, Machilivan is being explored for the aquatic hyphomycetous diversity for the first time. The study intended to enhance the existing knowledge of aquatic hyphomycetes from India, especially Kumaun Himalaya.

MATERIALS AND METHODS

Received: 27 Dec 2022; Revised accepted: 12 Apr 2023; Published online: 29 Apr 2023

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Citation: Bisht S, Altaf S, Jalal R. 2023. Species composition of aquatic hyphomycetes in Machilivan- A piedmont region of Kumaun Himalaya, India. *Res. Jr. Agril. Sci*. 14(2): 611-615.

Machilivan (221 m asl) area of district Nainital, situated in the piedmont of Kumaun Himalaya, Uttarakhand (India) was visited for the seasonal sample collection of submerged leaf litter, water foam and riparian roots from November 2018 to October 2021. The pH of the water was recorded as 7.9-8.9 (alkaline) and the temperature as 11-20 °C during winter, 20-28 °C during rainy and 28-35 °C during the summer season. Water foam was collected in plastic vials (50 ml), fixed on the spot with 5% FAA (Formaldehyde Alcohol Acetic Acid) to stop the conidial germination and examined microscopically in the laboratory for the occurrence of conidia. Leaf litter and riparian root samples were collected in sterile polyethene bags (10-14 inches), taken to the laboratory, washed with running tap water to remove inessential material and then cut into small segments. In the laboratory, root samples were surface sterilized by treating them with 0.01% sodium hypochlorite solution for 3-6 minutes and then with 96% ethanol for about 30 seconds [16]. Afterwards, the leaf litter and root samples were incubated at room temperature in sterilized Petri dishes containing distilled water for the sporulation of aquatic hyphomycetes and the incubated samples were examined regularly under a microscope to check and isolate the conidia. Under aseptic conditions, the axenic cultures of isolated conidia were prepared on a 2% MEA (Malt Extract Agar) medium supplemented with Ciprofloxacin (antibiotic drug). By using Lacto phenol cotton blue, the semi-permanent slides of isolated conidia were prepared and deposited in Govt. Girls College Mycological Slide (GGCMS) collection of the Department of Botany, Haldwani, Nainital. Identification was done by matching the photomicrographs with the pertinent literature [17-18]. The methodology for the assessment of leaf litter, riparian roots and water foam for the occurrence of aquatic hyphomycetes is represented schematically in (Fig 1).

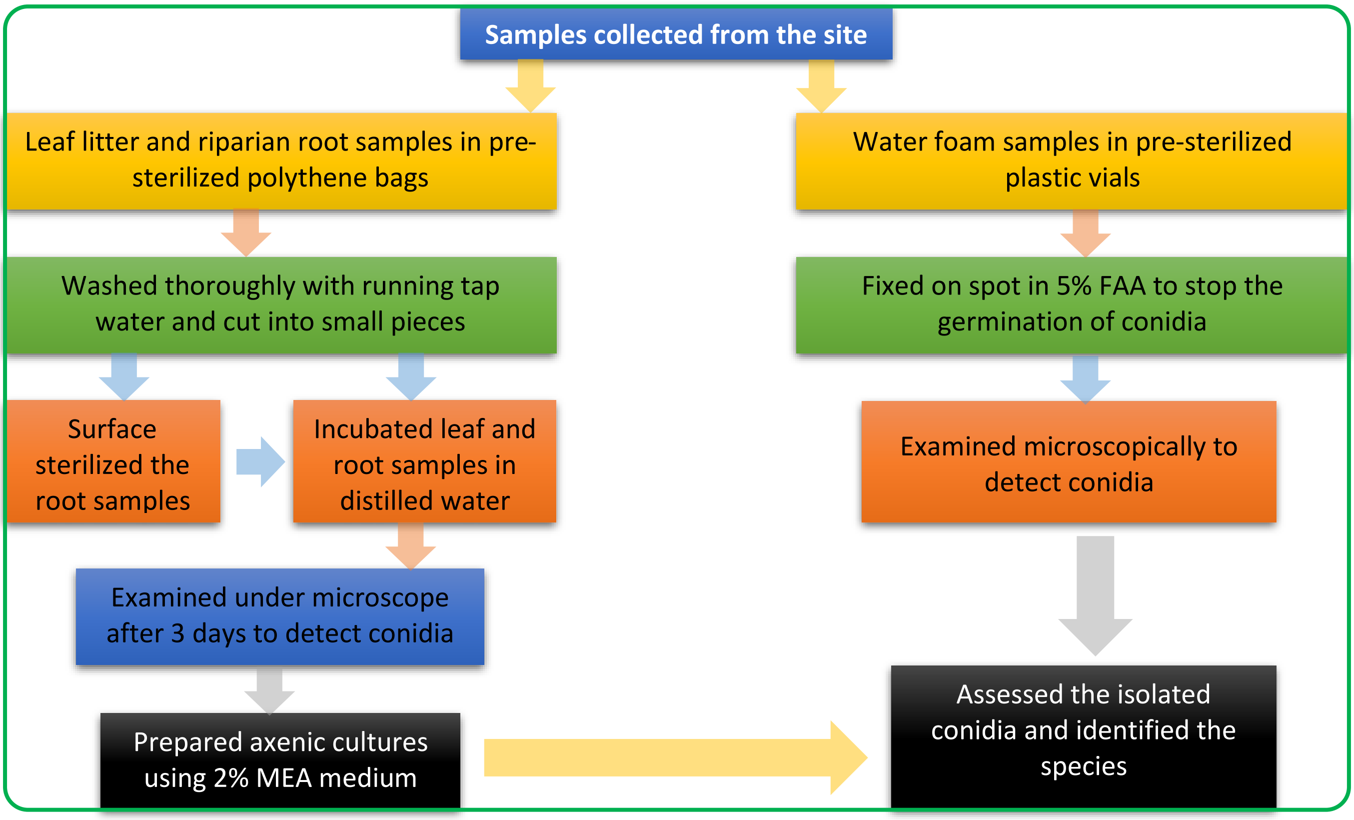

Fig 1 Schematic representation of assessment of leaf litter, riparian roots and water foam for aquatic hyphomycetes

RESULTS AND DISCUSSION

In the present study, twelve species belonging to eight genera were identified and illustrated in detail (Fig 2). Ten species namely *Acaulopage tetraceros*, *Anguillospora filiformis*, *Flagellospora penicilloides*, *Lunulospora curvula*, *Lunulospora symbiformis*, *Setosynemma isthamosporum*, *Tetracladium marchalianum*, *Tetracladium setigerum*, *Trescelophorus acuminatus* and *Wiesneriomyces laurinus* were isolated from leaf litter samples. Four species namely *Anguillospora filiformis*, *Setosynemma isthamosporum*, *Tetracladium marchalianum* and *Trescelophorus acuminatus* were isolated from water foam samples. Six species namely *Acaulopage dichotoma*, *Acaulopage tetraceros*, *Anguillospora crassa*, *Flagellospora penicilloides*, *Lunulospora curvula* and *Tetracladium marchalianum* were isolated as endophytes of riparian roots. Incubation time for sporulation was recorded as 3-8 days for leaf litter and 10-35 days for riparian root samples and the optimum temperature was recorded as 10-25 °C. The season and substrate of occurrence were recorded (Table 1) and compared with species composition (Fig 3).

Interestingly, *Wiesneriomyces laurinus* has been reported for the first time from the foothill region of Kumaun Himalaya. This may be due to the availability of required nutrients and physicochemical properties of the site, showing good agreement with Sati and Bisht [19] that fungal growth is affected by the nutrient availability and physicochemical parameters of the water body.

Noteworthy was to isolate *A. dichotoma* as a root endophyte from the foothill region of Kumaun Himalaya for the first time, while Sati *et al*. [20] reported it from leaf litter in the Nainital hills. Occurrence of *A. dichotoma* and *A. crassa* only in the form of riparian root endophytes, shows their preference towards the living roots than the plant detritus, supported by Sati and Pathak [3] that the aquatic hyphomycetes preferably occur as root endophytes. The presence of *A. dichotoma*, *A. tetraceros*, *A. crassa*, *F. penicilloides*, *L. curvula* and *T. marchalianum* as root endophytes of healthy riparian plants depicts their contribution to the health of the host plants. Sati and Pant [21] also reported the significant effect of *Campylospora parvula* and *Tetracladium setigerum* on the enhancement of plant growth.

Table 1 Seasonal occurrence of aquatic hyphomycetes in different substrates

S. No.	Species	Seasons			Substrate		
		Winter	Summer	Rainy	Leaf litter	Riparian root	Water foam
1	*Acaulopage dichotoma* Drechsler (Fig 2A)	-	+	-	-	+	-
2	*Acaulopage tetraceros* Drechsler (Fig 2B)	-	+	-	+	+	-
3	*Anguillospora crassa* Ingold (Fig 2C)	+	+	-	-	+	-
4	*Anguillospora filiformis* Greathead (Fig 2D)	+	-	+	+	-	+
5	*Flagellospora penicilloides* Ingold (Fig 2E)	+	-	-	+	+	-
6	*Lunulospora curvula* Ingold (Fig 2F)	+	+	-	+	+	-
7	*Lunulospora symbiformis* Miura (Fig 2G)	+	-	-	+	-	-
8	*Setosynemma isthamosporum* Shaw and Sutton (Fig 2H)	+	+	+	+	-	+
9	*Tetracladium marchalianum* De Wildeman (Fig 2I)	+	+	-	+	+	+
10	*Tetracladium setigerum* (Grove) Ingold (Fig 2J)	+	-	-	+	-	-
11	*Trescelophorus acuminatus* Nawawi (Fig 2K)	+	+	-	+	-	+
12	*Wiesneriomyces laurinus* (Tassi) Kirk (Fig 2L)	+	-	-	+	-	-
	Total	10	7	2	10	6	4

Occurrence: '+' = Present; '-' = Absent

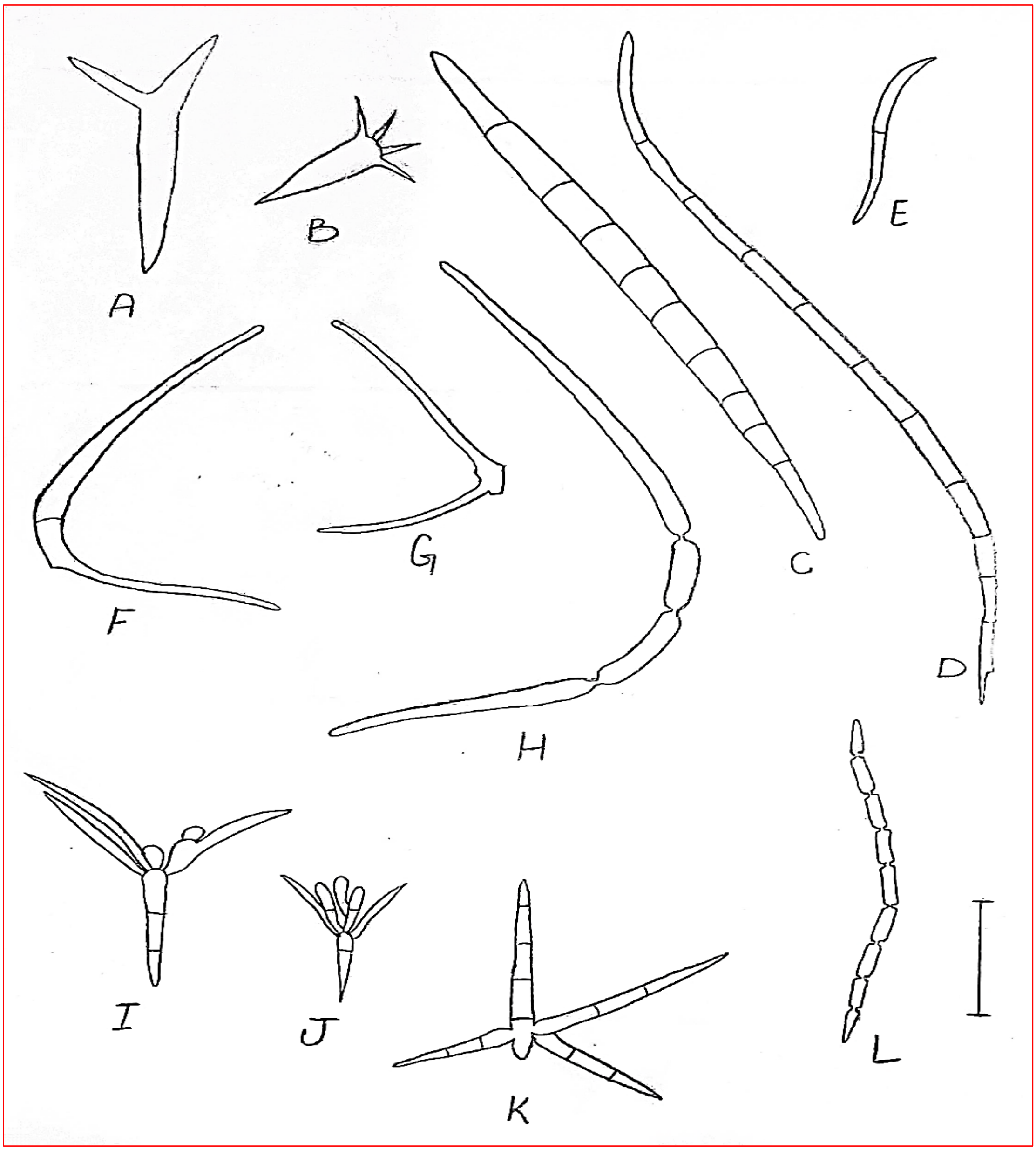

Fig 2 (A) *Acaulopage dichotoma*; (B) *Acaulopage tetraceros*; (C) *Anguillospora crassa;* (D) *Anguillospora filiformis*; (E) *Flagellospora penicilloides;* (F) *Lunulospora curvula*; (G) *Lunulospora symbiformis*; (H) *Setosynemma isthamosporum*; (I) *Tetracladium marchalianum*; (J) *Tetracladium setigerum*; (K) *Trescelophorus acuminatus*; (L) *Wiesneriomyces laurinus*. (Scale Bar = 25µm)

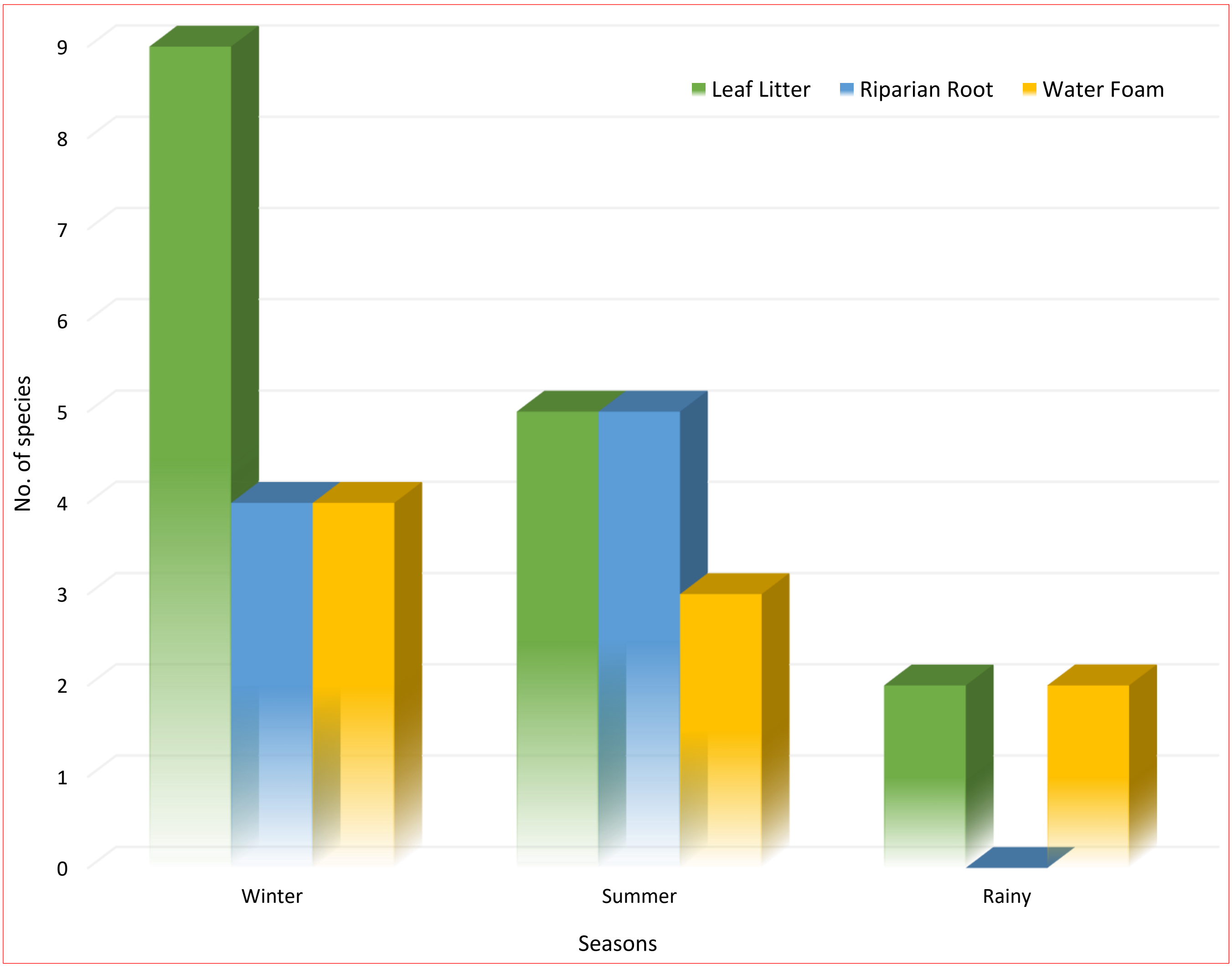

Fig 3 Occurrence of aquatic hyphomycetes in relation to seasons and substrate

Rich species diversity was recorded in leaf litter (10) than the water foam (4), showing leaf litter provides the best environment and can be considered the best substrate for the growth and sporulation of these fungi. In accordance with our observations, Sharathchandra and Sridhar [22] also reported the highest number of species in the leaf litter samples. The occurrence of diverse forms of aquatic hyphomycetes coincides with less anthropogenic disturbances in the study site, as also reported by Kshirsagar and Gunale [23] that anthropogenic activities influence the species richness of the water body.

The highest number of species were recorded in the winter season (10) than in the summer season (7) and the lowest in the rainy season (2), favoured by the result of Vishwakarma and Srivastava [24] that winter is the best season for the growth of aquatic hyphomycetes. On the contrary, a few researchers [25-26] reported the rainy (monsoon) as the best season to recover these fungi. Seasonal variation was quite noticeable in the species composition (Fig 3), proposing that the temperature of changing seasons affects the fungal growth, which was supported by Chandrashekar *et al*. [27] and Altaf *et al*. [15]. For the best growth of aquatic hyphomycetes, the optimum temperature was recorded as 10-25 °C, coinciding with the report of Sridhar and Barlocher [28]. *A. crassa*, *Lunulospora curvula*, *Tetracladium marchalianum* and *Trescelophorus acuminatus* occurred in winter as well as summer seasons and *Setosynemma isthamosporum* in all the three seasons, signifying the temperature tolerance of these species.

CONCLUSION

The study focused on the species composition of aquatic hyphomycetes from Machilivan, which harboured 12 species belonging to 8 genera. *W. laurinus* was the first record and *A. dichotoma* was recorded as root endophytes for the first time from the piedmont region of Kumaun Himalaya. Species composition varied seasonally with the maximum number recorded in the winter season, on the leaf litter samples. The occurrence of diverse aquatic hyphomycetes depicts that the study site is anthropogenically less stressed and provides a rich source of substrate due to the presence of the surrounding forest. Despite their affinity for cold water, these fungi were isolated in warm temperatures, owing to their tolerance for a wide range of temperatures. The occurrence of these fungi as endophytes in healthy riparian roots exhibits their benefaction in the health and growth of these plants and this area of research needs further investigation to utilize these fungi for enhancing crop production. The study was undertaken with the sole purpose to contribute to the diversity of aquatic hyphomycetes in India, particularly in the Kumaun Himalaya (Uttarakhand), and to lend a hand to the aquatic biology in the exploration and conservation of this multifarious group of fungi.

Acknowledgements

The authors are grateful to Prof. Shashi Purohit, Principal, I.P.G.G. (P.G) College of Commerce, Haldwani (Kumaun University, Nainital, Uttarakhand) for providing the necessary laboratory facilities. All the members of the Botany department are sincerely acknowledged for their timely support.

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