International Journal of Ecology and Environmental Sciences 49: 309-313, 2023
ISSN: 2320-5199 (Online); https://doi.org/10.55863/ijees.2023.2534

An Assessment of Ingoldian Fungi from Hillock Streams of Kumaun Himalaya, India

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ABSTRACT
Ingoldian fungi, the beguiling group of deutromycetous fungi form an indispensable component of the aquatic ecosystems, helping in decomposition of submerged plant material for energy transfer and nutrient cycling. Foam samples, roots of riverine plants and decomposed plant material were collected seasonally from hillock streams of Amritpur region (600 m) situated in Kumaun Himalaya, Uttarakhand, India. The study area is an unexplored region for diversity of aquatic hyphomycetes which is under the grip of anthropogenic stress. Collected samples were processed, incubated and examined for the presence of Ingoldian fungi. 20 species belonging to 12 genera were recorded viz., *Acaulopage dichotoma, A. tetraceros, Alatospora acuminata*, *Anguillospora* sp., *A. crassa*, *A. longissima, A. filiformis, Campylospora* sp., *C. chaetocladia, C. filicladia, C. parvula, Cylindrocarpon aquaticum, Diplocladiella scalaroides, Flagellospora penicillioides, Lunulospora curvula, Setosynnema isthmosporum, Tetracladium marchalianum, Triscelophorus monosporus, T. acuminatus* and *Wiesneriomyces laurinus.* The present study divulges that Ingoldian fungi sporulate best in winter season and leaf litter is the best substrate for their growth.

Key words: Ingoldian fungi, Diversity, Hillock, Kumaun Himalaya, Season, Substrate

INTRODUCTION

Ingoldian fungi are a phylogenetically heterogeneous group occurring mainly in lotic waters. These were initially described and named as 'Aquatic Hyphomycetes' by C.T. Ingold (1942) due to their presence on submerged plant substrate in well-aerated water. These fungi also known as conidial fungi, are adapted to running waters due to the wide array of conidial shapes viz., tetra-radiate, tri-radiate, sigmoid, crescent, helical etc. which facilitate their dispersion as well as adherence to plant substrate (Chauvet et al. 2016, Moro et al. 2018). Aquatic hyphomycetes also play pivotal roles in decomposing the organic matter of aquatic ecosystems (Bai et al. 2018). Waid (1954) reported some of them as root endophytes of riverine plants too. Endophytic aquatic hyphomycetes having distinctive ecological niches play an important role in the distribution, ecology, physiology and biochemistry of plants.

Mycological investigations in aquatic habitats have endured less consideration as compared to terrestrial habitats (Barlocher and Boddy 2016). These fungi have a worldwide distribution, but studies so far point to higher species richness in temperate regions (Duarte et al. 2016, Seena et al. 2019, Raposeiro P. et al. 2020). Geographically, the aquatic hyphomycetous community has been influenced by a narrow spatial scale (Pascoal et al. 2005, Duarte et al. 2016). Thus, there is a wide scope for an extensive inventory of aquatic hyphomycetes from various geographical locations. In India, the first record on aquatic hyphomycetes was *Varicosporium elodeoe,* from submerged leaves by Bhattacharya and Baruah (1953) in Assam. A review by Sridhar (2021) consolidated the studies on five decades of research on freshwater hyphomycetes in India. Nowadays, the researchers are also focusing on the conservation strategies of Ingoldian and other aquatic fungi which is a need of an hour (Barros and Seena 2022).

The Kumaun region of Uttarakhand owing to its strategic geographic location offers a variety of climatic conditions and ecosystems with numerous water bodies. Ingoldian fungi have been reported extensively from high-altitude freshwater bodies of Kumaun Himalaya by many workers (Sati and Arya 2009, Pant and Sati 2018, Pant et al. 2019, Koranga et al. 2021). Yet, very little information is available on their occurrence from hillock streams of Kumaun

Himalaya. Amritpur an anthropogenically stressed foothill region of Kumaun Himalaya (600 m) is being explored first time for the diversity of Ingoldian fungi.

MATERIAL AND METHODS

Study area

The present study was carried out in different streams of the Amritpur in Uttarakhand, India, situated at 29°17'43.6" N latitude, 79°33'28.5" E longitude and H" 600 m asl. The major vegetation nearby the region is *Shorea robusta*, *Cassia fistula* and other small herbs and shrubs. From February 2019 to December 2021, nine expeditions were made seasonally viz., (summer, rainy, winter) to collect foam samples, decomposed leaf litter and root samples of riparian plants. Water temperature was recorded 25-30°C during summer, 20-25°C during rainy and 15-20°C during winter.

Collection and processing

The foam samples were collected in a wide-mouthed jar and fixed in 5% FAA on the spot to prevent conidia germination. These were observed directly under the microscope for the presence of conidia. The collected leaf and root samples were taken to the laboratory and washed in running tap water to remove extracellular debris. The root samples were treated with 0.01% sodium hypochlorite solution for 3-4 minutes followed by 96% ethanol for up to 30 seconds for surface sterilization (Sati and Belwal 2005). The processed leaf litter and surface-sterilized roots were 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, leaf and root samples were regularly examined under a low-power microscope to detect the conidia of aquatic hyphomycetes.

Identification of fungal species

Conidia were picked aseptically and placed on a 2% malt extract agar (MEA) medium supplemented with ciproflaxin 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 were compared with pertinent literature for identification (Santos and Betancourt 1997).

RESULTS AND DISCUSSION

In the present study, 20 species of Ingoldian fungi belonging to 12 genera were isolated. from different rivulets of the Amritpur region viz., *Acaulopage dichotoma, A. tetraceros, A. acuminata*, *Anguillospora* sp., *A. crassa, A. longissima, A. filliformis, Campylospora.* sp., *C. chaetocladia, C. fillicladia, C. parvula, Cylindrocarpon aquaticum, Diplocladiella scalaroides, Flagellospora penicilliodes, Lunulospora curvula, Setosynnema isthmosporum, Tetracladium marchalianum, Triscelophorus monosporus, T. acuminatus* and *Wiesneriomyces laurinus* (Table 1, Fig. 1).

Maximum number of species (15) were recorded during the winter season while minimum (6) during the summer season. The increased temperature and less availability of substrate in summer, prevents fungal growth (Belwal and Sati 2001, Dang et al. 2009). Maximum number of species (17) were recorded from leaf litter, making it the most suitable substrate for the growth of aquatic hyphomycetes. Root samples were also found to be preferable host for some species, such as *C. fillicladia, C. purvula* and *C.* sp. Sati and Pant (2020) also identified *C. purvula* as a root endophyte from *Pilea scripta* in Kumaun Himalaya and tested it as a plant growth promoter. *A. filliformis* showed heavy mycelial growth alongside the margins of leaf samples of *Cassia fistula* in winter season. It was interesting to note that the conidial sporulation in leaf samples is

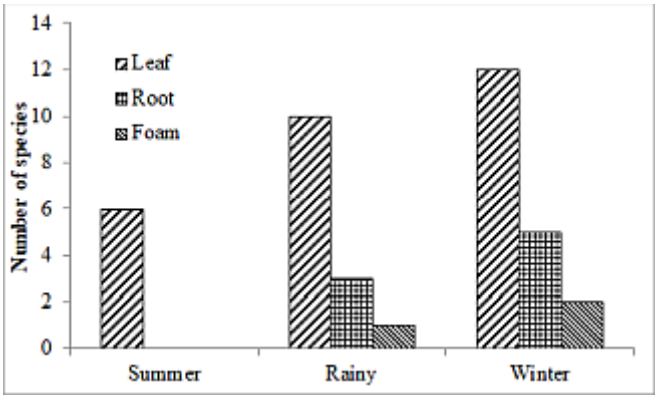

Figure 1. Seasonal occurrence of Ingoldian fungi in relation to different sources

Table 1. Ingoldian fungi isolated from hillock streams in different seasons

	TAXA	SOURCES	SEASON
1.	*Acaulopage dichotoma* Derchsler	Leaf Litter	Summer
2.	*Acaulopage tetraceros* Derchsler	Leaf Litter	Winter
3.	*Alatospora acuminata* Ingold	Leaf Litter	Summer, Rainy, Winter
4.	*Anguillospora* sp. Ingold	Leaf Litter	Summer
5.	*Anguillospora crassa* Ingold	Leaf Litter	Rainy
6.	*Anguillospora longissima* Ingold	Leaf Litter	Summer
7.	*Anguillospora filliformis* Greathead	Leaf Litter	Winter
8.	*Campylospora* sp. Ranzoni	Root	Winter
9.	*Campylospora chaetocladia* Ranzoni	Leaf Liiter, Foam	Rainy, Winter
10.	*Campylospora fillicladia* Nawawi	Root	Winter
11.	*Campylospora parvula* Kuzuha	Root	Winter
12.	*Cylindrocarpon aquaticum* Nilsson	Leaf Litter, Root	Summer, Rainy, Winter
13.	*Diplocladiella scalaroides* Arnaud	Leaf Litter, Root	Rainy
14.	*Flagellospora penicillioides* Ingold	Leaf Litter	Winter
15.	*Lunulospora curvula* Ingold	Leaf Litter	Rainy, Winter
16.	*Setosynnema isthmosporum* Shaw and Sutton	Leaf Litter	Rainy, Winter
17.	*Tetracladium marchalianum* De Wildeman	Leaf Litter, Root, Foam	Summer, Rainy, Winter
18.	*Triscelophorus monosporus* Ingold	Leaf Litter	Rainy, Winter
19.	*Triscelophorus acuminatus* Nawawi	Leaf Litter	Rainy, Winter
20.	*Weisneriomyces laurinus* (Tassi) Kirk	Leaf Litter	Winter

about one week and in root samples it was 25-30 days.

Foam is formed when the physical characteristics of the water are altered by the presence of organic materials. Foam samples are considered to be a good entrapper of conidial fungi (Sati et al. 2014). But it was found to harbor limited number of species (2) due to less formation of foam in the present site.

CONCLUSIONS

The present study reveals, the diversity of Ingoldian fungi varied considerably from season to season, due to temperature alteration and substrate availability. Water temperature was recorded 25-30^{o}C during summer and 15-20^{o}C during winter. Minimum species (6) were isolated during summer, maximum species (15) during winter and the optimum temperature for their growth is estimated as 15-20^{o}C. It is concluded that species occurring in winter season have narrow thermal tolerance as compared to those occurring during all the seasons. Submerged leaf litter was recorded to be the most suitable source for growth of these fungi. The present study, briefly accounts for the diversity of Ingoldian fungi from an anthropogenically stressed water body situated in the foothills of Kumaun Himalaya and focuses on seasonal variation and impact of different sources on their diversity.

ACKNOWLEDGEMENTS

The authors are thankful to the Jawaharlal Nehru Memorial Fund, New Delhi for providing financial support. We also express our gratitude to Prof. Shashi Purohit, Principal, I.P.G.G.P.G. College of Commerce, Haldwani and Dr. S.D. Tewari former HOD, Department of Botany, for providing necessary laboratory facilities.

Author's contribution: The corresponding author has collected, designed and prepared the first draft of the manuscript. The first author has revised and finalized the manuscript. The co-author has helped in revising the manuscript.

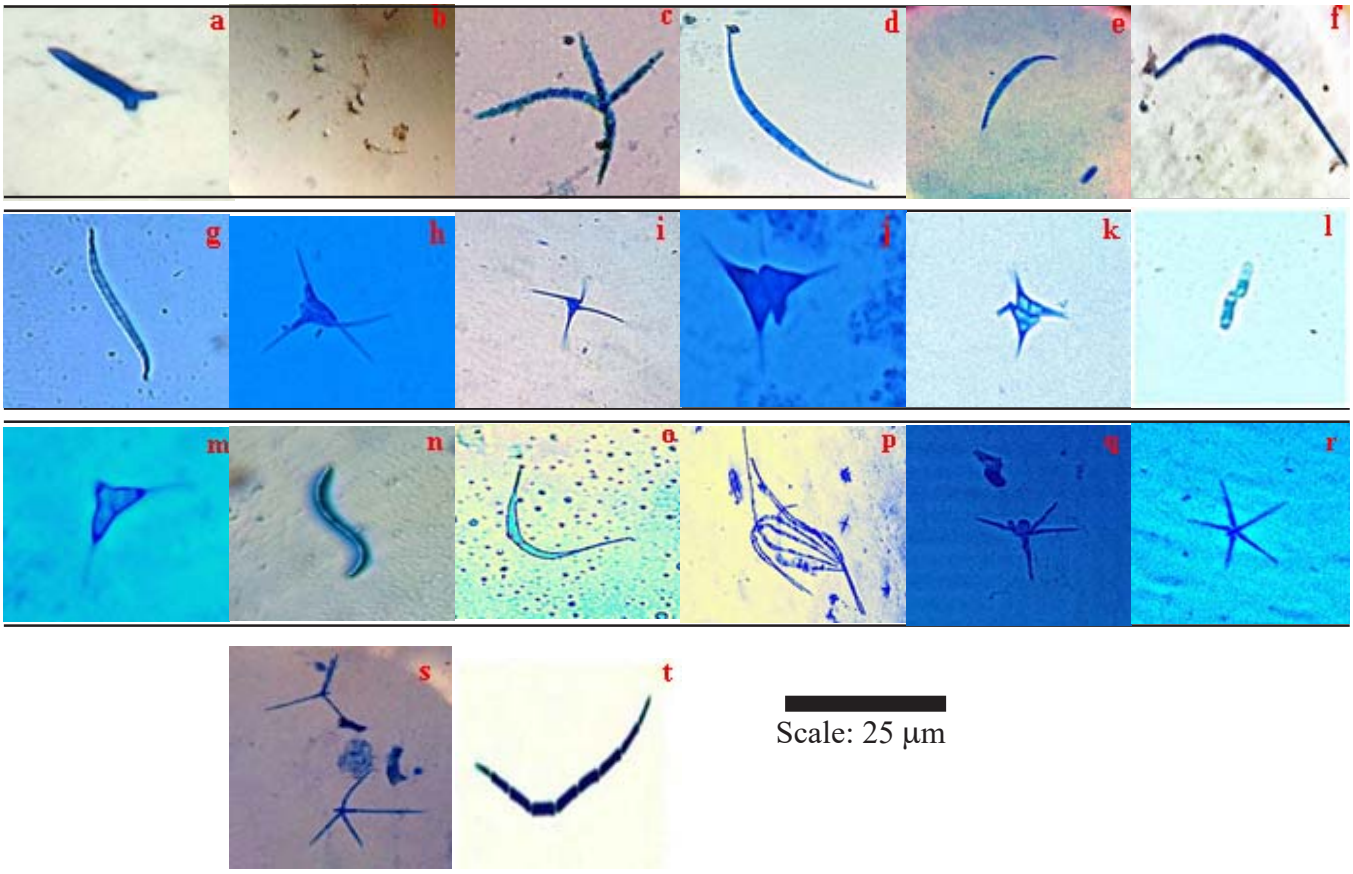

Figure 2. Photomicrographs of fungal strains: (a) *Acaulopage dichotoma*, (b) *A. tetraceros*, (c) *Alatospora acuminata*, (d) *Anguillospora* sp., (e) *A. crassa*, (f) *A. longissima*, (g) *A. filliformis*, (h) *Campylospora* sp., (i) *C. chaetocladia*, (j) *C. fillicladia*, (k) *C. purvula*, (l) *Cylindrocarpon aquaticum*, (m) *Diplocladiella scalaroides*, (n) *Flagellospora penicillioides*, (o) *Lunulospora curvula*, (p) *Setosynnema isthmosporum*, (q) *Tetracladium marchalianum*, (r) *Triscelophorus monosporus*, (s) *T. acuminatus*, (t) *Weisneriomyces laurinus*.

Conflicts of interest: The authors declare no conflicts of interest

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Received:17th May 2022
Accepted:17th August 2022