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Diversity and Seasonal Variation of Aquatic Hyphomycetes in Nandhaur Wildlife Sanctuary, Uttarakhand, India

Saima Altaf*[1], Saraswati Bisht[2], Ruchi Jalal[3] and Jasvinder Kaur[4]

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A B S T R A C T

Aquatic hyphomycetes, the main fungal decomposers of submerged dead organic matter are known to play an important role in unlocking the energy flow in aquatic ecosystems. These fungi are yet to be explored from different freshwater bodies for their multifarious benefits. The Nandhaur Wildlife Sanctuary, situated at the foothill of Kumaun Himalaya (Uttarakhand) is still untouched for its aquatic biota exploration especially hyphomycetes. Comparatively warm temperature and diverse substrate pool of the site may favour the occurrence and growth of varied forms of aquatic hyphomycetes and make it an interesting matter of investigation. Therefore, the present work was undertaken to reveal the diversity and seasonal variation of these fungi. The samples were collected monthly and taken to the laboratory for further processing and incubation for sporulation. Altogether 19 species belonging to 12 genera were isolated from leaf litter samples, among which only 4 species were recorded from water foam samples. Seasonal variation was noticed in the species composition with the maximum number of species in winter (18 species) followed by autumn (12 species), spring (7 species), rainy (7 species) and least in the summer season (1 species). The temperature range of 11-25°C was found to favour the maximum species diversity.

***Key words*:** Aquatic hyphomycetes, Decomposers, Diversity, Seasonal variation, Nandhaur Wildlife Sanctuary

Aquatic hyphomycetes are the polyphyletic group of fungi that were first described by Ingold [1]. These are also named Ingoldian fungi, water-borne hyphomycetes or freshwater hyphomycetes. These fungi usually occur on submerged plant debris like leaf litter, petioles, bark etc. [2] and complete the entire or portion of their life cycle in clean, flowing and well-oxygenated water. They also reside as aquatic endophytes in the roots of riparian trees [3]. They reproduce asexually by the formation of conidia and are usually identified by their unique conidial shapes i.e., tetraradiate, triradiate, sigmoid, spherical, helical, lunate etc. Aquatic hyphomycetes cause the decomposition of leaf litter and help in unlocking the nutrients in freshwater streams. These play an essential role in the trophic chain and are considered as important intermediaries in the food webs of streams [4].

To date, several workers reported these fungi from different regions of the world [5-11]. In India, the least attention has been paid towards the study of these fungi [12-13]. Aquatic Hyphomycetes are being extensively explored in the Kumaun Himalayan region [14-16], while no such work has been reported from foothill regions. As the foothill region is having a comparatively warm temperature and diverse substrate pool than the Himalayan region, this may favour the occurrence and growth of diverse forms of aquatic hyphomycetes and make it an interesting matter of investigation. Therefore, the present work is undertaken to investigate the diversity and seasonal variation of water-borne conidial fungi from water bodies flowing through the Nandhaur Wildlife Sanctuary.

* **Saima Altaf**
✉ syedsaima143.ss@gmail.com

[1-4] Department of Botany, IPGGPG College of Commerce (Kumaun University, Nainital), Haldwani - 263 139, Uttarakhand, India

MATERIALS AND METHODS

Nandhaur Wildlife Sanctuary is located at Kumaun Himalayan foothill of district Nainital (Uttarakhand), 32 km away from Haldwani city. It is present at the latitude of 29º 1' 25" (29.0236º) North, the longitude of 79º 48' 18.9" (79.8053º) East and an elevation of 221 meters (725 feet). The site is mostly surrounded by *Tectona grandis*, *Mallotus philippensis*, *Haldina cordifolia* and *Shorea robusta* vegetation.

Sample collection and processing

Samples of water foam and partially decomposed submerged leaves of different plant species, accumulated at barriers were collected monthly (5 samples per date) from November 2018 to October 2019. The samples were collected in pre-sterilized plastic vials (50 ml) and zip lock polyethene bags (10 × 14 inches) respectively. The foam samples were kept in 5% FAA (Formaldehyde Alcohol Acetic Acid) on the spot in order to arrest the germination of conidia and examined in the departmental laboratory under the microscope to check the

presence of conidia. The leaf litter samples were washed thoroughly with running tap water for 5-6 hours to remove extraneous material and cut into small portions (1.5 × 2.5cm) and incubated under laboratory conditions in sterilized Petri dishes containing distilled water for the purpose of sporulation of aquatic hyphomycetes. After 3-4 days, the incubated leaf samples were examined daily under the microscope to detect the conidia.

Isolation and identification of species

The semi-permanent slides of detected conidia from water foam were prepared by using a fungal stain (lactophenol cotton blue) for the purpose of further detailed taxonomic studies. The axenic cultures of detected conidia from incubated leaf litter samples were prepared using 2% MEA (Malt Extract Agar) medium supplemented with streptomycin (antibiotic drug) under aseptic conditions and the semi-permanent slides of each isolate were prepared using lactophenol cotton blue. Based on conidial morphology, the identification of fungal species was done with the help of pertinent literature. The sporulation temperature (°C) for each isolate was measured with the help of a thermometer. The sample of occurrence (whether water foam or leaf sample) for each identified species was recorded. The prepared slides were deposited in Government Girls College Mycological Slide (GGCMS) Collection of Department of Botany, Haldwani (Nainital).

RESULTS AND DISCUSSION

Altogether 19 species belonging to 12 genera of aquatic hyphomycetes were isolated and identified from the present study site from the month of November-2018 to October-2019 (12 months). The monthly occurrence of the identified species, their respective sample of occurrence (water foam or leaf) and sporulation temperature were recorded (Table 1). Species diversity was compared according to different seasons and sporulation temperatures (Fig 1). The camera lucida drawings of the identified species and their photomicrographs were also recorded (Fig 2, Plate 1).

Table 1 Monthly species composition, sample and sporulation temperature

Species	Months (Nov. 2018-Oct. 2019)												Sample	ST (°C)
	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct		
Alatospora acuminata	+	+	+	-	-	-	-	-	-	-	-	-	LL	11-22
Anguillospora crassa	+	+	+	+	-	+	+	-	+	-	-	-	LL	11-34
Anguillospora filiformis	+	-	+	-	-	-	-	-	-	+	-	-	LL, WF	11-32
Anguillospora longisima	+	+	+	+	-	+	-	-	-	+	-	-	LL, WF	11-32
Beltrania rhombica	+	+	-	-	-	-	-	-	-	-	-	-	LL	18-22
Campylospora filicladia	+	-	+	-	-	-	-	-	-	-	-	-	LL, WF	11-22
Clavatospora tentacula	+	-	-	-	-	-	-	-	-	-	-	-	LL	22-24
Flagellospora penicilliodes	+	-	-	+	-	-	-	-	-	-	-	-	LL	20-22
Helicomyces roseus	-	+	-	-	+	+	-	-	-	-	-	-	LL	18-27
Lunulospora curvula	+	+	+	+	-	+	-	-	-	-	-	-	LL	11-27
Lunulospora cymbiformis	-	-	+	+	-	-	-	-	-	-	-	-	LL	11-20
Setosynema isthmosporum	+	-	+	+	-	+	-	-	-	+	+	-	LL, WF	11-32
Speiropsis scopiformis	-	-	+	+	+	-	-	-	-	+	-	-	LL	11-32
Tetracladium apiense	-	-	+		-	-	-	-	-	-	-	-	LL	11-14
Tetracladium breve	-	-	+		-	-	-	-	-	-	-	-	LL	11-14
Tetracladium marchalianum	-	-	+	+	+	-	-	-	+	-	-	-	LL	11-34
Tetracladium setigerum	-	-	+	+	-	-	-	-		-	-	-	LL	11-20
Triscelophorus acuminatus	+	+	+		-	-	-	-	+	-	-	-	LL	11-34
Triscelophorus monosporus	+	+	+		-	-	-	-		-	-	-	LL	11-22
Total	12	8	15	9	3	5	1	0	3	4	1	0		

Occurrence: + = Present, - = Absent; Sample: LL= Leaf litter, WF= Water Foam; ST = Sporulation temperature

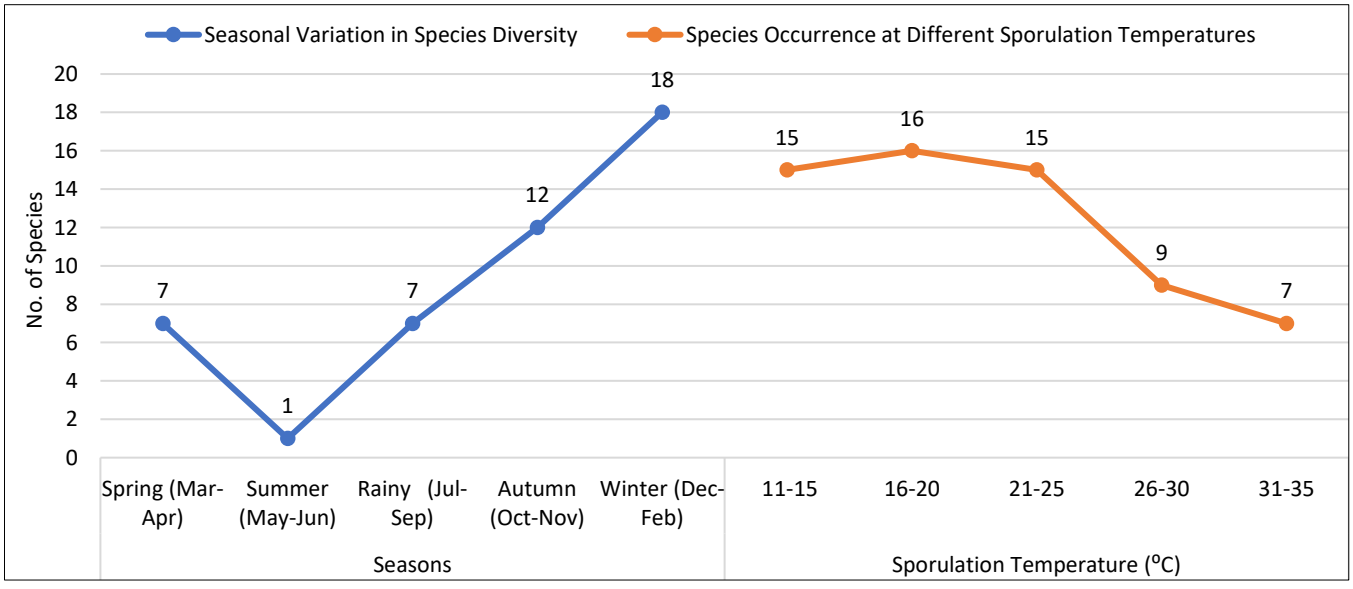

Fig 1 Seasonal variation in species diversity and Species occurrence at different sporulation temperatures

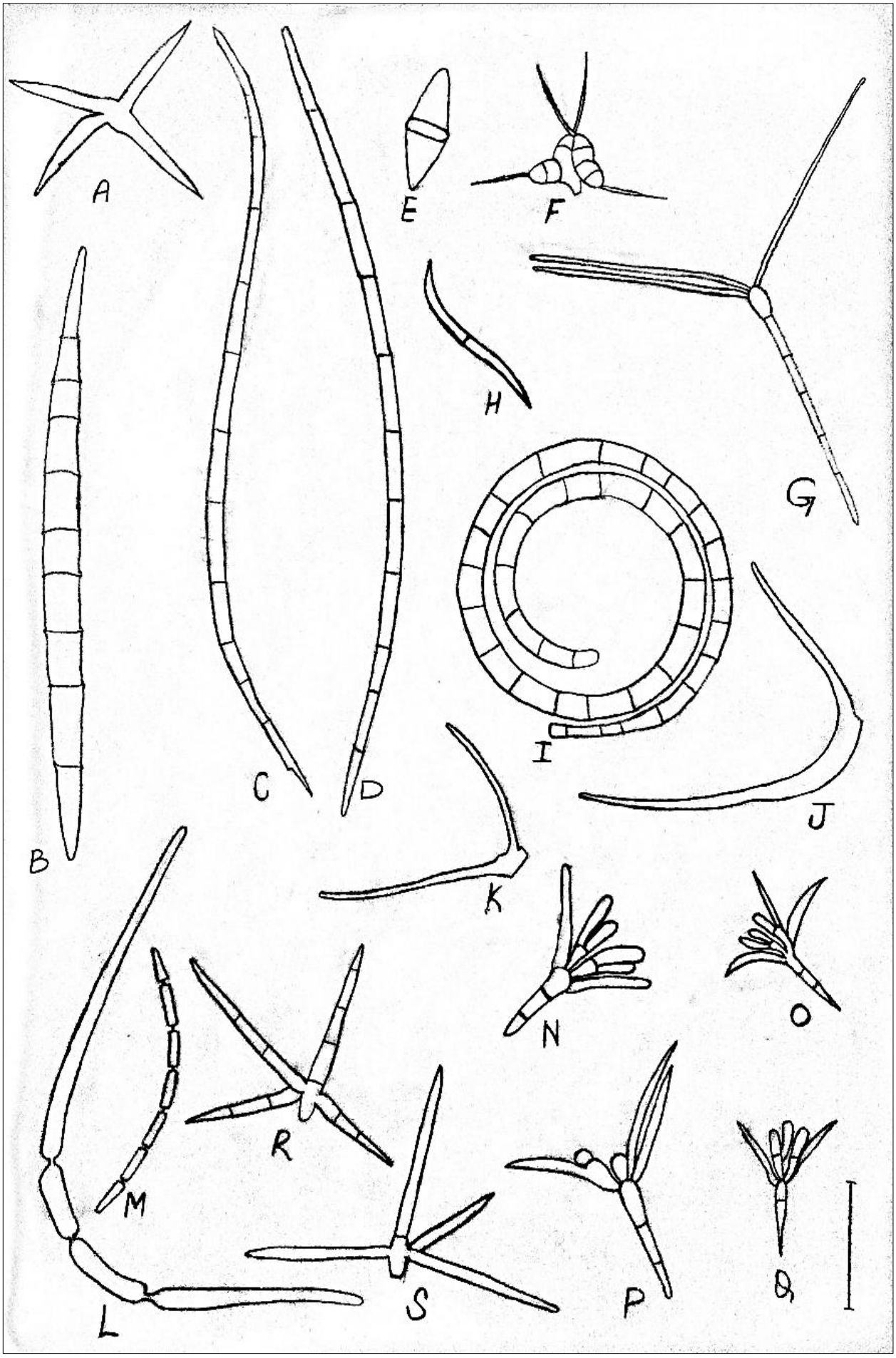

Fig 2 **A.** *Alatospora acuminata;* ***B.*** *Anguillospora crassa;* **C.** *Anguillospora filiformis;* **D.** *Anguillospora longisima;* **E.** *Beltrania rhombica;* **F.** *Campylospora filicladia;* **G.** *Clavatospora tentacula;* **H.** *Flagellospora penicilliodes;* ***I.*** *Helicomyces roseus;* ***J.*** *Lunulospora curvula;* **K.** *Lunulospora cymbiformis;* **L.** *Setosynema isthmosporum;* **M.** *Speiropsis scopiformis;* **N.** *Tetracladium apiense;* **O.** *Tetracladium breve;* **P.** *Tetracladium marchalianum;* **Q.** *Tetracladium setigerum;* **R.** *Triscelophorus acuminatus;* **S.** *Triscelophorus monosporus.* (Scale bar = 25 µm)

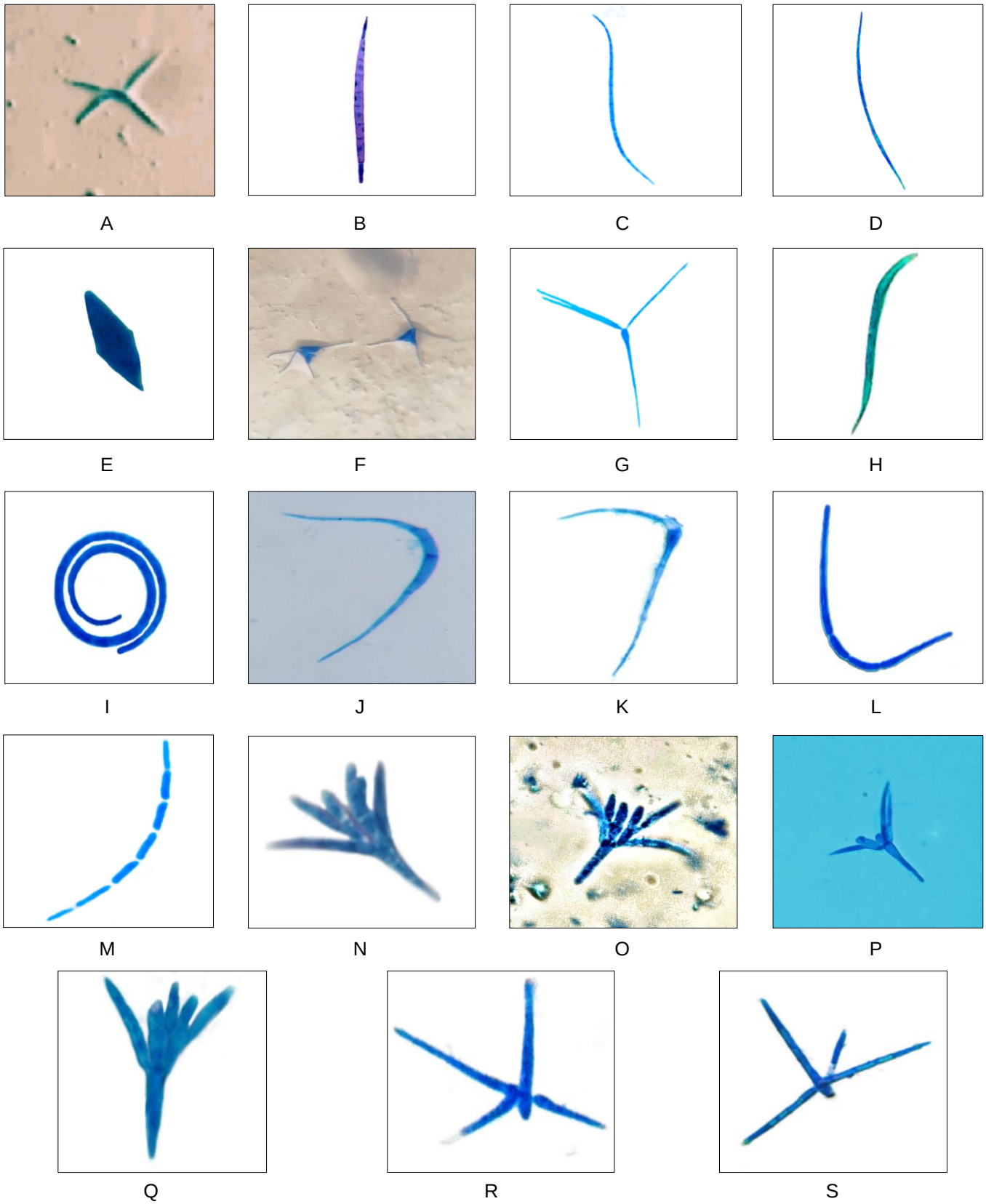

Plate 1 **A.** *Alatospora acuminata;* ***B.*** *Anguillospora crassa;* **C.** *Anguillospora filiformis;* **D.** *Anguillospora longisima;* **E.** *Beltrania rhombica;* **F.** *Campylospora filicladia;* **G.** *Clavatospora tentacula;* **H.** *Flagellospora penicilliodes;* ***I.*** *Helicomyces roseus;* ***J.*** *Lunulospora curvula;* **K.** *Lunulospora cymbiformis;* **L.** *Setosynema isthmosporum;* **M.** *Speiropsis scopiformis;* **N.** *Tetracladium apiense;* **O.** *Tetracladium breve;* **P.** *Tetracladium marchalianum;* **Q.** *Tetracladium setigerum;* **R.** *Triscelophorus acuminatus;* **S.** *Triscelophorus monosporus*

Taxonomic description

1. *Alatospora acuminata* Ingold

Conidia were hyaline, triradiate or tetraradiate, consisting of a smooth, curved main axis having 30-50µm length, 2.5-3.5µm width, tapering towards both ends and two diverging curved appendages, 0-3 septate, 15-25 µm long and 1-2µm wide (Fig 2A, Plate 1A). Isolated from leaf litter samples in the autumn and winter seasons, sporulation temperature 11-22°C.

2. *Anguillospora crassa* Ingold

Conidia were hyaline, vermiform, slightly L or S-shaped having 6-10 septations, 100-180 µm length, 8-14 µm width, tapering towards the ends (Fig 2B, Plate 1B). Isolated from leaf

litter samples in the spring, summer, rainy, autumn and winter seasons, sporulation temperature 11-34°C.

3. *Anguillospora filiformis* Greathead

Conidia were hyaline, filiform, sigmoid having 10-18 septations, 170-300 µm length, 2.5- 3.5 µm width with a basal filiform appendage of 5-20 µm length and 1-2 µm width (Fig 2C, Plate 1C). Isolated from leaf litter and water foam samples in the rainy, autumn and winter seasons, sporulation temperature 11-32°C.

4. *Anguillospora longisima* Ingold

Conidia were hyaline, filiform, curved or sigmoid having 7-14 septations, 160-240 µm length, 4-5 µm width, tapering towards both ends (Fig 2D, Plate 1D). Isolated from leaf litter and water foam samples in the spring, rainy, autumn and winter seasons, sporulation temperature 11-32°C.

5. *Beltrania rhombica* Penzig

Conidia were consisting of a biconic, symmetrical main axis having 20-30 µm length, 9-14 µm width, with a hyaline to sub hyaline transverse band (Fig 2E, Plate 1E). Isolated from leaf litter samples in the autumn and winter seasons, sporulation temperature 18-22°C.

6. *Campylospora filicladia* Nawawi

Conidia were hyaline, tetraradiate, composed of an allantoid part having 10.5 – 13 µm length, 4.5 – 5.5 µm width and a deltoid part having 10.5 – 13 µm length, 6-8 µm width with rounded apical cells and four hair-like branches having 17-25 µm length, 0.5-0.7 µm width with tapered apex and two apical branches usually crossed (Fig 2F, Plate 1F). Isolated from leaf litter and water foam samples in the autumn and winter seasons, sporulation temperature 11-22°C.

7. *Clavatospora tentacula* Nilsson

Conidia were hyaline, tetraradiate having a clavate main axis with 1-5 septations, 35-65 µm length, 1.5-2.5 µm width at base, 4-6 µm width at apex, with three divergent appendages arising from apex having 35-55 µm length and 1-2 µm width (Fig 2G, Plate 1G). Isolated from leaf litter samples in the autumn season, sporulation temperature 22-24°C.

8. *Flagellospora penicilliodes* Ingold

Conidia were hyaline, sigmoid having 1 septation, 30-45 µm length, 2-3 µm width at the middle, tapering to 1.5-2 µm towards the ends (Fig 2H, Plate 1H). Isolated from leaf litter samples in the autumn and winter seasons, sporulation temperature 20-22°C.

9. *Helicomyces roseus* Link

Conidia were hyaline, coiled 2-2.5 times up to 70 µm diameter, having conidial filament with 20-40 septations, 4-6.5 µm width, tapering to a rounded apical cell and an enlarged obliquely flattened basal cell (Fig 2I, Plate 1I). Isolated from leaf litter samples in the spring and winter seasons, sporulation temperature 18-27°C.

10. *Lunulospora curvula* Ingold

Conidia were crescent or lunate, without a prominent central scar, often curved in more than one plane, having 60-125 µm length, 3.5-5.5 µm width, tapering to 1-2 µm towards the ends (Fig 2J, Plate 1J). Isolated from leaf litter samples in the spring, autumn and winter seasons, sporulation temperature 11-27°C.

11. *Lunulospora cymbiformis* Miura (Fig 2K and Plate 1, K).

Conidia were hyaline to light-green, sickle-shaped, bent nearly at a right angle, having 60-102 µm length, 4-4.5 µm at the widest point, with an inflated, rhombic detachment scar in the middle region. Isolated from leaf litter samples in the winter season, sporulation temperature 11-20°C.

12. *Setosynema isthmosporum* Shaw and Sutton

Conidia were sigmoid or helical with 3-7 septations, 150-200 µm length, 3-4 µm width, tapering to 1-2.5 µm towards the ends (Fig 2L, Plate 1L). Isolated from leaf litter and water foam samples in the spring, rainy, autumn and winter seasons, sporulation temperature 11-32°C.

13. *Speiropsis scopiformis* Kuthub and Nawawi

Conidia were hyaline, composed of 5-7 cells connected by a narrow isthmus to form a chain having intermediate cells cylindrical, 6-10 µm long, 2-3.5 µm wide and apical cells conical, 5.5-8 µm long, and 1.5-2.5 µm wide (Fig 2M, Plate 1M). Isolated from leaf litter samples in the spring, rainy and winter seasons, sporulation temperature 11-32°C.

14. *Tetracladium apiense* Sinclair & Eicker

Conidia were hyaline, multiradiate with 0-1 septate, clavate axis giving rise to 8 upper elements composed of 2 subapical, unbranched appendages, 10-24 µm long, 3-4 µm wide, with more or less rounded apices, and 2 apical elements, 7-9 µm long, 3-4.5 µm wide, dichotomously branched at their apex, forming 4 additional, 8-11 long, 2.5-4 µm wide, digitiform appendages appearing like pair of the fork. All elements were non-acicular and delimited by septa (Fig 2N, Plate 1N). Isolated from leaf litter samples in the winter season, sporulation temperature 11-14°C.

15. *Tetracladium breve* Roldan

Conidia were composed of the main axis giving rise to three digitiform elements, 10-13 µm long, 3-3.5 µm wide, two narrow obclavate appendages, 15-20 µm long, 1.5-2.5 µm wide, and one acicular element, 12-30 µm long, 2-3.5 µm wide, arising abaxially at the middle part of one of the 3 digitiform elements (Fig 2O, Plate 1O). Isolated from leaf litter samples in the winter season, sporulation temperature 11-14°C.

16. *Tetracladium marchalianum* De Wildeman

Conidia were hyaline, tetraradiate, composed of the main axis 10-40 µm long, 1-2 µm wide at the base, 3-5 µm wide at apex, bearing an oval or spherical central knob at the apex, 4-9 µm long, 4-6.5 µm wide, three lateral appendages, 20-40 µm long, 1.8-3 µm wide, arising from apex of the main axis just below the central knob, one of the three appendages bears an eccentric knob having 3-4 µm width (Fig 2P, Plate 1P). Isolated from leaf litter samples in the spring, rainy and winter seasons, sporulation temperature 11-34°C.

17. *Tetracladium setigerum* (Grove) Ingold

Conidia were hyaline, multiradiate, composed of 1-2 septate, clavate axis, 8-20 µm long, 2.5-3 µm wide, giving rise to one to two lateral, narrow obclavate appendages, 5-35 µm long, 1-3 µm wide, and one to two apical parts, forming three digitiform, 0-3 septate appendages, 3-12 µm long, 2-5 µm wide, and one acicular appendage at the apex of conidia, 12- 17.5 µm long, 1-2 µm wide. Out of three digitiform appendages, the upper branch was often adaxial (Fig 2Q, Plate 1Q). Isolated from leaf litter samples in the winter season, sporulation temperature 11-20°C.

18. *Triscelophorus acuminatus* Nawawi

Conidia were hyaline with variable size and shape, usually tetraradiate, composed of the main axis having 2-7 non constricted septations, 20-90 µm length, 3-7 µm width, tapering gradually to 0.5 µm towards the tip, and 3-7 appendages having 15-60 µm length, 2-4 µm width with constricted base and tapering apices (Fig 2R, Plate 1R). Isolated from leaf litter samples in the rainy, autumn and winter seasons, sporulation temperature 11-34°C.

19. *Triscelophorus monosporus* Ingold

Conidia were hyaline, tetraradiate, composed of 25-65 µm long, 0-1 septate, subulate to the cylindrical main axis with 3-4 µm wide obpyriform to doliiform basal cell, and 3-5 appendages having 0-2 septations, 14-40 µm length, 1.5-3 µm width, originating from basal cell of the main axis. The appendages were not tapering but were having almost uniform width throughout and their septations were not clearly visible (Fig 2S, Plate 1S). Isolated from leaf litter samples in the autumn and winter seasons, sporulation temperature 11-22°C.

During the present study, all 19 identified species of fresh-water hyphomycetes were isolated from leaf litter samples under aseptic conditions while only 4 species among them, viz: *A. filiformis*, *A. longisimma*, *C. filicladia* and *S. isthamosporum* were recorded from water foam samples. Thus, it was found that foam is not the best sample to use to determine the accurate spora of the water body [17-21].

The species composition and richness were not uniform throughout the year and showed seasonal variation (Fig 1). The comparison of species diversity according to different seasons showed that the number of species was maximum in winter (18 species) followed by autumn (12 species), spring (7 species), rainy (7 species) and least in the summer season (1 species). The highest counts of fungi were reported in the winter season by Goncalves *et al*. [22], Vishwakarma and Srivastava [23], Jalal *et al*. [16] and also in the autumn season by Barlocher [18]. The reason behind the less species diversity in the rainy season may be that the present site is in spate during the rainy season and thus the chances of deposition of leaves on river barriers and their colonization by fresh-water hyphomycetes become less [24].

Maximum growth was noted at the temperature range of 16-20°C (16 species) followed by 11-15°C and 21-25°C (15 species at each temperature range) and then 26-30°C (9 species), while the least growth was recorded at 31-35°C (7 species). Thus, the temperature range of 11-25°C was found to favour the maximum species diversity than 26-35°C and no species were found to exist at the temperature above 35°C. Thus, the optimum temperature for the best growth of these fungi is 10-25°C and there is a noticeable drop in growth at 30°C [25].

A. acuminate, *A. crassa*, *A. filiformis*, *A. longisima*, *C. filicladia*, *L. curvula*, *S. isthamosporum*, *S. scopiformis*, *T. marchalianum*, *T. acuminatus* and *T. monosporus* were commonly found to sporulate at 11-25°C. Although common at 11-25°C, *L. curvula* also tolerated the temperature range of 26-30°C while *A. crassa*, *A. filiformis*, *A. longisima*, *S. isthamosporum*, *S. scopiformis*, *T. marchalianum* and *T. acuminatus* tolerated up to 35°C, thus these 8 species may be considered as temperature tolerant species. *B. rhombica* and *F. penicilliodes* sporulated only at 16-25°C, similarly, *C. tentacula* was observed only at 21-25°C, this may indicate their sensitivity towards extreme temperatures and their specificity towards a narrow range of sporulation temperature. *H. roseus* was observed at 16-30°C, this may reflect its affinity towards high temperature than the lower range, while *L. cymbiformis* and *T. setigerum* were observed only at 11-20°C, showing their affinity towards low temperatures than the higher range. *T. apiense* and *T. breve* may be considered cold-loving species as they preferably sporulated at a low temperature of 11-15°C.

Out of 19 identified species belonging to 12 genera, 4 species were from *Tetracladium* (*T. apiense*, *T. breve*, *T. marchalianum* and *T. setigerum*), 3 from *Anguilospora* (*A. crassa*, *A. filiformis* and *A. longisima*), 2 each from *Lunulospora* and *Trescelophorus* (*L. curvula*, *L. cymbiformis*, *T. acuminatus* and *T. monosporus*) and 1 each from rest of the 8 genera (*Alatospora acuminata*, *Beltrania rhombica*, *Campylospora filicladia*, *Clavatospora tentacula*, *Flagellospora penicilliodes*, *Helicomyces roseus*, *Setosynema isthamosporum* and *Speiropsis scopiformis*). The occurrence of 2 or more species from the same genera may indicate that the present site harbours sufficient substrate and physicochemical conditions that favour the occurrence and growth of the particular genera [26].

Rich species diversity recorded from the present study site may be because of less anthropogenic activities [27] or the type of riparian vegetation of the water body [28].

CONCLUSION

The present study deals with aquatic hyphomycete diversity analysis of foothill water bodies of the Kumaun Himalaya flowing through the Nandhaur Wildlife Sanctuary. The occurrence of 19 species belonging to 12 genera during the year showed species richness of the study site that may be due to the riparian vegetation type or less anthropogenic disturbances. Variation in species composition with changing temperature of winter to summer seasons showed that the temperature is having a direct impact on the growth and sporulation of these fungi. Maximum diversity in the winter season (11-25°C) indicates that these are usually cold-loving fungi while some species viz; *A. crassa*, *A. filiformis*, *A. longisima*, *L. curvula*, *S. isthamosporum*, *S. scopiformis*, *T. marchalianum* and *T. acuminatus* can tolerate temperature up to 35°C.

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