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Assessment of water-borne hyphomycete conidia in stemflow in two tree species of southwestern India – a preliminary account

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Abstract

Tree bark is one of the potential niches in the tree canopy that supports a variety of fungi, including water-borne hyphomycetes. Surveys have been carried out to assess the conidia of water-borne hyphomycetes in stemflow and throughfall of several tree canopies in different geographic zones. This study assessed the assemblage and diversity of conidia of water-borne hyphomycetes in stemflow by filtration and latex-trap (*Ficus* latex smear on the microscopic slides) methods for *Acacia* and *Ficus* tree species for up to four days during the monsoon season. Air temperature, humidity and physicochemical features of stemflow between the tree species did not differ drastically. Conidia of 52 species were recorded in stemflow of two tree species using both methods with 21 common species, while 8 and 23 species were confined to filtration and latex-trap, respectively. The species richness and diversity observed were higher in *Ficus* than *Acacia* in the filtration method, while it was the opposite for the latex-trap method. Although filtration showed lower species richness than the latex-trap method, the conidial count was higher in the filtration method than in the latex-trap method. Stemflow filtration as well as the latex-trap method showed a higher number of staurosporous conidia than other conidial types. The conidia of the top five species found in the filtration and latex-trap methods were *Anguillospora longissima, Cylindrocarpon aquaticum, Flagellospora curvula, Triscelophorus acuminatus* and *T. konajensis*. This study demonstrated about 50% uniqueness in species of water-borne hyphomycetes between the tree species as well as among the methods.

Keywords – Abiotic factors – *Acacia* – aquatic fungi – conidia – diversity – *Ficus* – latex-trap – techniques

Introduction

Water-borne hyphomycetes have distribution in aquatic ecosystems throughout the world and are responsible for plant detritus processing to transfer the energy across the food webs (Bärlocher 1992, Duarte et al. 2016). They are successful in aquatic habitats owing to their ability to produce morphologically distinct conidia for buoyancy and dissemination (scolecospores, staurosporous and helicosporous) (Webster 1987, Marvanová 1997, Gulis et al. 2020, Zhao et al. 2007). Many conventional and molecular techniques have been adapted to assess the assemblage, diversity and distribution of water-borne hyphomycetes (Descals 1997, 2020, Gessner et al. 2003, Duarte et al. 2013, Bärlocher et al. 2020). Some of the techniques employed to evaluate the structural and functional roles of water-borne hyphomycetes include detritus incubation (in damp chambers with

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low moisture, shaking chambers with water and aeration in bubble chambers), water filtration and foam observation (Gessner et al. 2003). Conventional methods like the assessment of foam, leaf litter and water filtration using light microscopy provide quick and reliable evidence about the morphological diversity of water-borne hyphomycetes in different geographic locations (Ingold 1975, Descals 1997, 2020).

One of the principal adaptations of water-borne hyphomycetes is the capability of impaction or adherence or trapping or impaction of conidia to the surface of organic matter or inert surfaces in turbulent conditions in lotic waters (Webster 1987, Read et al. 1991, Dang et al. 2007, Kearns & Bärlocher 2008). Such impaction is mediated by the secretion of mucilage at the tips of the conidia (Read et al. 1991). Conidial initial impaction on the organic matter leads to a series of subsequent events such as the formation of appressoria/germ tube, growth, colonization and sporulation (Read et al. 1992). The impaction of conidia is an important feature used to evaluate the conidial richness as well as diversity in aquatic bodies (Webster 1959, Bärlocher et al. 1977, Ghate & Sridhar 2015). Considering the ability of conidial impaction on the surfaces, several materials were employed as conidial traps like cellophane, collodion, latex, plexiglass and rosin (Webster 1959, Lindsey & Glover 1976, Müller-Haeckel & Marvanová 1976, Bärlocher et al. 1977, Ghate & Sridhar 2015). Webster (1959) demonstrated the impaction of conidia on the collodion-coated glass rods to follow the strength of conidia to withstand the stress of water speed. Bärlocher et al. (1977) studied the impaction of conidia on slides coated with rosin (pine resin) as a method of choice for quick evaluation. Sridhar and Kaveriappa (1987a) demonstrated that the plant's latex-coated slides serve as a conidial trap as well as a nutrient for the growth of some water-borne hyphomycetes. Based on these observations, Ghate and Sridhar (2015) developed an inexpensive technique using latex-coated slides for rapid monitoring of water-borne hyphomycetes in streams. Among the six latexes studied, the impaction efficiency of banyan latex (*Ficus benghalensis*) was the highest and impaction efficiency was significantly higher than the plain slides in a coastal stream of southwest India. They extended their study to assess the diurnal periodicity of conidia on the latex-trap in coastal (Konaje) and Western Ghats (Sampaje) streams of southwest India (Ghate & Sridhar 2016).

Water-borne hyphomycetes are not restricted exclusively to the lotic habitats; instead, their territories extend beyond their usual submerged habitats to semi-aquatic to terrestrial habitats (Sridhar 2009, Chauvet et al. 2016, Magyar et al. 2021). They become common inhabitants of the terrestrial litter as well as tree canopy (as endophytes in throughfall and stemflow) (Sridhar & Kaveriappa 1987b, Sridhar & Bärlocher 1993, Sridhar 2009, Sharathchandra & Sridhar 2020, Sridhar et al. 2020, Magyar et al. 2021). Besides the host plants (leaves, bark and tree holes) in tree canopy, other dependent plants also support water-borne hyphomycetes (e.g. epiphytic ferns, lichens and mosses). Phytothelms, stemflow and throughfall of more than 57 plant species revealed the occurrence of morphologically diverse conidia of aquatic hyphomycetes and aquatic hyphomycete-like conidia (Chauvet et al. 2016, Magyar et al. 2021). A rough estimate of conidial fluctuations in tree species ranges from $4–278 \times 10^9$ conidia/ha/annum (Sridhar & Karamchand 2009, Magyar et al. 2021). Similar to freshwater streams, stemflow and throughfall of tree canopies offer a variety of conidia of water-borne hyphomycetes, which can be studied using several conventional techniques (Sridhar 2009). Among different habitats in the tree canopy, tree bark is one of the potential sites occupied by several mycota termed dendronatant fungi, and their conidia drain down in stemflow or barkflow during the rainy season (Magyar et al. 2021). The specific aims of the current study were: 1) to assess the diversity of conidia of water-borne hyphomycetes in stemflow by filtration and impaction of conidia on the banyan latex-coated slides (*Ficus benghalensis*) during the monsoon season in southwest India; 2) to assess the efficiency of filtration and latex-trap methods to monitor the conidia of water-borne hyphomycetes in stemflow.

Materials & Methods

Tree species

One each of two apparently healthy tree species *Acacia auriculiformis* A. Cunn. Ex Benth.

and *Ficus benghalensis* L. (banyan tree) were selected for the study in a mixed scrub jungle stand in the coastal region in the village Konaje (Dakshina Kannada, Karnataka) of southwest India (12°48' N, 74°55' E; 104−112 m asl) (Fig. 1a–c). The trees were devoid of any tree holes. Assessment of stemflow was carried out during the rainy season for four days during August 11–14, 2021. The rainfall was intermittent and ranged between 26 and 41 mm per day. The tree species chosen were about 1 km away from the nearest water source, the Konaje stream. Trees are ~500 m away from the agricultural lands, roads and wood industry.

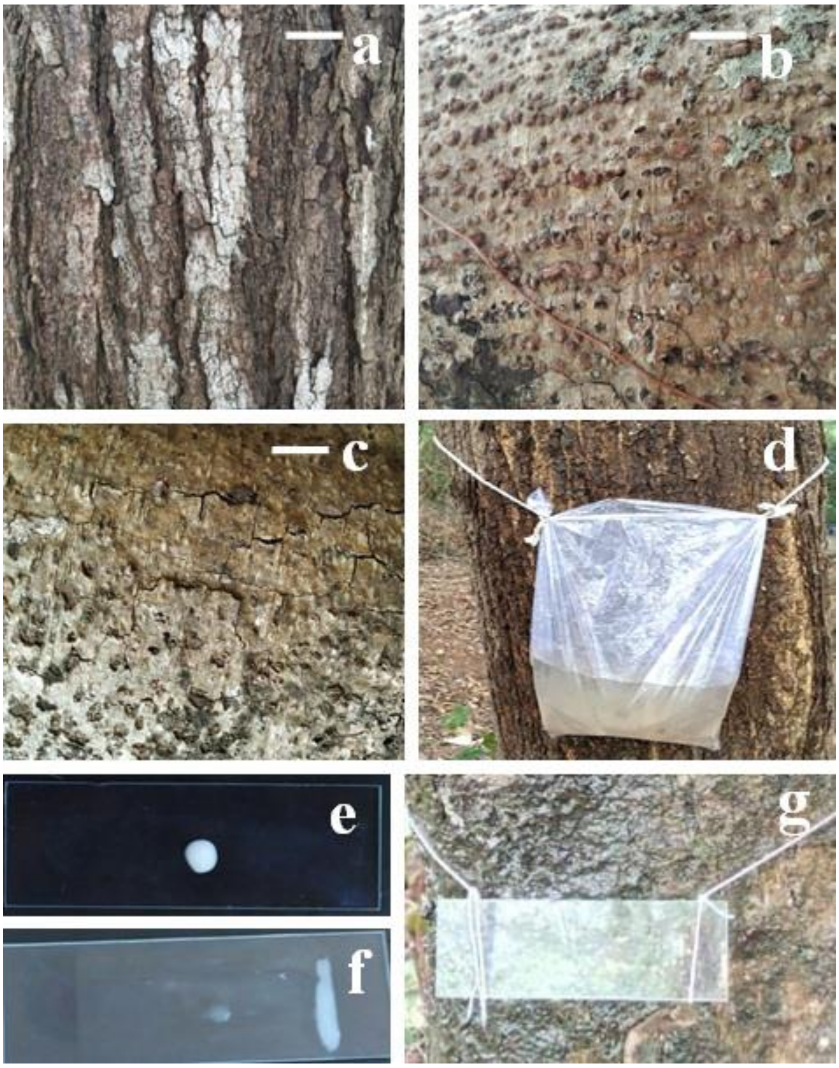

Fig. 1 – a Bark surface texture of *Acacia* tree. b and c Bark surface texture of *Ficus benghalensis* tree (banyan). d Stemflow collection bag fastened to *Acacia* trunk. e A drop of banyan latex on a microscope slide. f Latex smear on microscope slide. g latex smeared slide fastened to banyan trunk to trap the fungal conidia in stemflow. Scale bar, a to c: 1 cm.

Stemflow

Stemflow from the tree species (~200 ml) at an interval of 8 hr per day up to four days was collected into polyethylene bags fastened to the tree trunk to assess the physicochemical features as well as the drift conidia (Fig. 1d). Fresh polyethylene bags were fastened to tree trunks at every 8 hr interval after sampling. The temperature of stemflow was assessed using a mercury thermometer (Model # 17873, N.S. Dimple Thermometer, New Delhi, India). The stemflow pH, conductivity and total dissolved solids were measured using a water analyzer (Water Analyzer # 371, Systronics India Ltd., Ahmedabad, Gujarat, India). The samples were fixed on the collection spot and taken to the laboratory (within 30 min.) to assess the dissolved oxygen by Winkler’s method (APHA 1995).

The air temperature and humidity in the experimental site were also assessed (Mexitech Digital Thermohyrometer M288CTHW, Mumbai, India).

Aliquots of 25 ml of stemflow from three replicate samples were filtered through filters (MF-Millipore™ Membrane filter #SMWP02500, mixed cellulose esters; diameter, 25 mm; pore size, 5 µm). The filters were immediately stained with lactophenol cotton blue (1%) and preserved in dark until screening using a microscope (Nikon, Model: ECLIPSE Ni-U 941966, Nikon Corporation, Tokyo, Japan) for quantitative and qualitative assessment of water-borne hyphomycete conidia by mounting with a few drops of lactic acid.

Latex-trap

One drop of the latex of banyan obtained by excising the petiole of the green leaf was placed on a clean microscope slide and smeared using another slide like a blood smear (Fig. 1e–g). It was kept for air-drying at the laboratory temperature overnight (22–24°C, 12 hr). Three latex-coated slides were fastened to each tree trunk at ~1.5 m above the tree base, avoiding the path of the stemflow collecting bag to trap the conidia. They were collected once for 8 hr per day for up to four days. The fastened slides were brought to the laboratory within 1 hr. A small drop of lactophenol cotton blue stain (1%) was added, and a cover glass (40×22 mm = 880 mm^2) was placed on the slide to shield the latex smear. The slides were examined microscopically for identification and quantification in the area of the cover glass. The water-borne hyphomycetes were identified to the generic or species level based on the monographs (Ingold 1975, Subramanian & Bhat 1981, Nawawi 1985, Marvanová 1997, Santos-Flores & Betancourt-Lopez 1997, Zhao et al. 2007, Gulis et al. 2020).

Data analysis

The *t*-test was employed to assess the variation in physicochemical parameters of stemflow of two tree species, air temperature and humidity (n=4) (StatSoft Inc. 2008). The average number of species and an average number of conidia by filtration (25 ml) (n=12), as well as latex-trap (in 880 mm^2 area), were compared (n=12) for two trees species with the *t*-test. Cumulative species and cumulative conidia were plotted from day 1 to day 4 for the two tree species. Percentage contribution of water-borne hyphomycetes in the filtration and latex-trap methods were calculated by pooling the data of 12 replicates. Simpson's and Shannon's diversity indices along with Pielou's equitability were calculated for water-borne hyphomycetes identified in the filtration and the latex-trap methods (n=12) (Pielou 1975, Magurran 1988). Variation in species richness, diversities and equitability between the tree species (n=4) was assessed by the *t*-test.

Results

Abiotic features

Temperature, pH, total dissolved solids and dissolved oxygen did not significantly differ in stemflow between *Acacia* and *Ficus* (Table 1). The conductivity of stemflow of *Ficus* was significantly higher than *Acacia* ($p<0.05$). Air temperature and humidity in the sampling region did not differ significantly.

Table 1 Physicochemical features of stemflow in two tree species (n=12, mean±SD; *, *t*-test, $p<0.05$).

	Acacia	*Ficus*
Temperature (°C)	24.6±0.5	24.3±0.4
pH	7.0±0.4	7.2±0.9
Conductivity (µS/cm)	31.8±1.4	41.4±3.1*
Total dissolved solids (mg/l)	12.6±0.1	15.1±1.5
Dissolved oxygen (mg/l)	7.5±0.2	8.0±0.3
Air temperature (°C)	25.9±0.7	25.5±0.5
Humidity (%)	88.3±0.5	88.6±0.7

Conidia in filtration technique

The average number of species of water-borne hyphomycetes (~11 species/25 ml) in the filtration did not significantly differ between the stemflow of *Acacia* and *Ficus* ($p>0.05$) similar to the average conidia (79–84 conidia/25 ml) ($p>0.05$) (Fig. 2). The cumulative species among the two tree species in stemflow (25 ml) from day 1 to day 4 followed an almost similar pattern with a higher number of species on *Ficus* than *Acacia* on all occasions and the same pattern shown by the cumulative conidia (Fig. 2).

Filtration of stemflow of both tree species consists of conidia of 29 species of water-borne hyphomycetes with 18 species in *Acacia* and 20 species in *Ficus* (Table 2). Nine species were common to both trees, while nine and 11 species were confined to *Acacia* and *Ficus*, respectively. Species overlap in stemflow of *Acacia* and *Ficus* was 50% and 45%, respectively. Filtering 75 ml stemflow in thee replicate per day (for four days) yielded up to 11 average species with 79–84 average conidia. The cumulative species (18–20 spp.) as well as conidia (314–336) steeply raised in four days. Among the 29 total species in stemflow, staurosporous conidia were dominant (16 spp.), followed by helicosporous (7 spp.), scolecosporus (5 spp.) and cylindroid (1 sp.) conidia.

In this study, the conidial contribution of *Anguillospora longissima* (23.7%) in stemflow of *Acacia* was the highest, followed by *Condylospora spumigena*, *Anguillospora crassa*, *Cylindrocarpon aquaticum* and *Flagellospora curvula* (range, 9–18.2%). In *Ficus*, *Helicomyces hyderabadensis* (19.4%) was the top contributor of conidia, followed by *Triscelophorus acuminatus*, *Flagellospora curvula and Triscelophorus konajensis* (range, 11.3–18.9%).

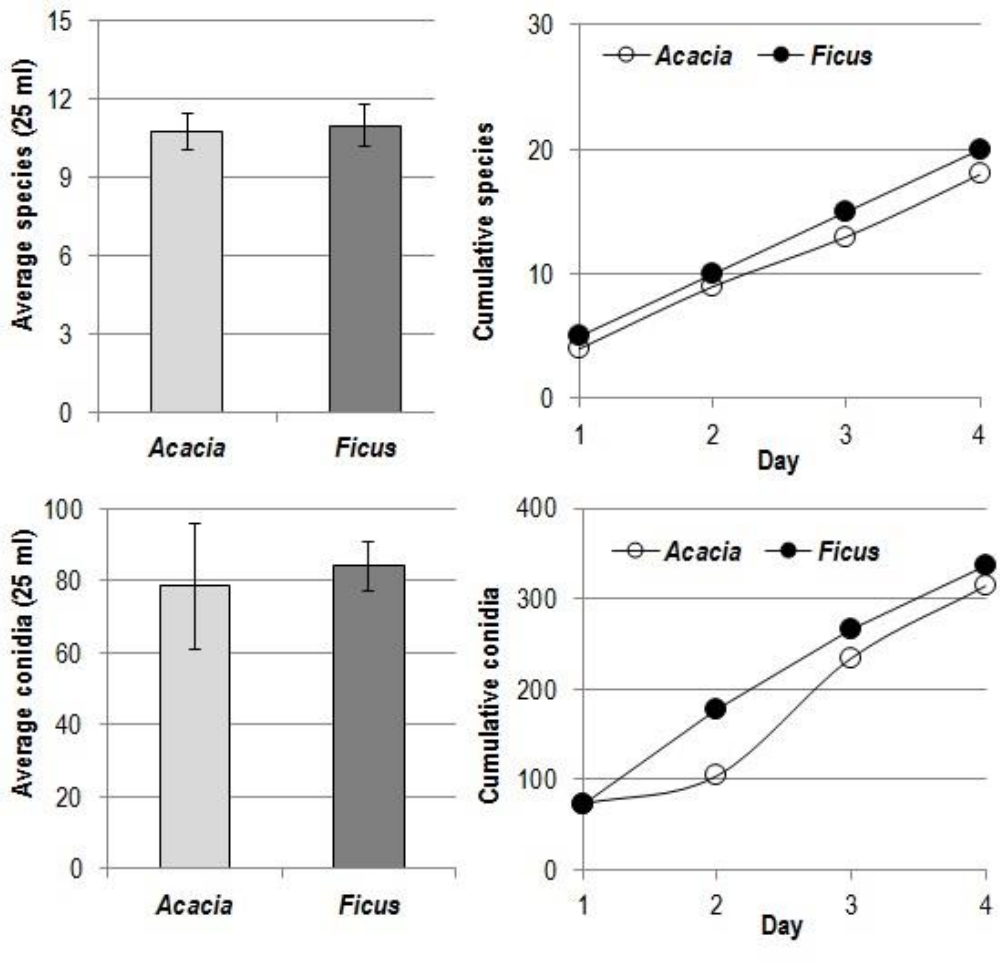

Fig. 2 – Average (n=12±SD) and cumulative species of water-borne hyphomycetes in stemflow filtration (25 ml) of two tree species.

Table 2 Percent contribution of conidia of water-borne hyphomycetes in filtration of stemflow in two tree species (from pooled data of 4 days; n=12).

	Acacia	***Ficus***
Anguillospora longissima (Sacc. & P. Syd.) Ingold (Fig. 4b)	23.7	5.8
Triscelophorus acuminatus Nawawi (Fig. 6d)	9.0	18.9
Flagellospora curvula Ingold (Fig. 4l)	10.2	13.9
Condylospora spumigena Nawawi (Fig. 4d)	18.2	1.2
Helicomyces hyderabadensis P. Rag. Rao & D. Rao (Fig. 5d)	–	19.4
Anguillospora crassa Ingold (Fig. 4 a)	12.2	4.6
Triscelophorus konajensis K.R. Sridhar & Kaver. (Fig. 6e)	1.3	11.3
Cylindrocarpon aquaticum (Sv. Nilsson) Marvanová & Descals	10.4	–
Anguillospora sp.	–	7.5
Trisulcosporium sp. 2 (Fig. 6h)	–	4.9
Diplocladiella scalaroides G. Arnaud (Fig. 4e)	4.5	–
Helicosporium dentophorum G.Z. Zhao, Xing Z. Liu & W.P. Wu (Fig. 5g)	–	2.9
Helicomyces sp. (Fig. 5e)	–	2.3
Helicosporium aureum (Corda) Linder (Fig. 5f)	1.9	–
Helicosporium sp. (Fig. 5i)	–	1.7
Retiarius sp. 1 (Fig. 5 k)	1.0	0.6
Retiarius sp. 2 (Fig. 5l)	1.6	–
Alatospora acuminata Ingold	–	1.5
Isthmotricladia laeensis Matsush.	1.0	0.3
Helicosporium gracile (Morgan) Linder (Fig. 5h)	1.3	–
Helicoma atroseptatum Linder (Fig. 5a)	–	1.2
Dwayaangam sp. 1 (Fig. 4f)	1.0	–
Actinospora megalospora Ingold	0.6	0.3
Dwayaangam sp. 3 (Fig. 4h)	0.3	–
Flabellospora sp. (Fig. 4k)	0.3	–
Flabellospora verticillata Alas. (Fig. 4j)	–	0.3
Speiropsis pedatospora Tubaki	0.3	–
Trinacrium sp. (Fig. 6c)	–	0.3
Trisulcosporium sp. 1 (Fig. 6g)	–	0.3
Number of staurosporous species	11	10
Number of scolecosporus species	4	5
Number of helicosporous species	2	5
Number of cylindroid species	1	0
Total number of species	18	20
Total number of exclusive species	9	11

Conidia in latex-trap

Average species in latex-trap (880 mm^2) was higher in *Acacia* than *Ficus* (19 vs. 16 spp.) without any significant variation (p>0.05), while the average conidia were higher in *Ficus* than *Acacia* (27 vs. 25) without any significant variation (p>0.05) (Fig. 3). The cumulative species were higher from day 1 to day 4 in *Acacia* than in *Ficus* and consistently raised from day 1 to day 3 with a slight plateau on day 4. The cumulative conidia also showed a similar trend (Fig. 3).

The latex-trap of both the tree species showed conidia of 44 water-borne hyphomycetes with 35 and 30 species in *Acacia* and *Ficus*, respectively (Table 3). Twenty-one species were found in both trees, while 14 and nine species were confined to *Acacia* and *Ficus*, respectively. Species overlap in latex-trap of *Acacia* and *Ficus* was 54% and 63%, respectively. Exposing 12 latex-coated slides (for four days) resulted in the impaction of 12–16 average species with 25–27 conidia. The cumulative species raised from 14–16 (day 1) to 27–32 (day 3) spp., and attained up to 30–35 spp. on day four. The cumulative conidia from 74–115 (day 1) increased to 203–209 (day 3) and attained 241–245 conidia on day four. Among the 44 species in latex-trap, staurosporous conidia dominated (28 spp.), followed by an equal number of scolecospores, helicosporous (7 spp. each) and one each of cylindroid conidia.

Table 3 Percentage contribution of fungal conidia of water-borne hyphomycetes in latex-trap in two tree species (from pooled data of 4 days; n=12).

	Acacia	***Ficus***
Triscelophorus acuminatus Nawawi (Fig. 6d)	19.7	32.0
Flagellospora curvula Ingold (Fig. 4l)	19.8	5.7
Triscelophorus konajensis K.R. Sridhar & Kaver. (Fig. 6e)	12.0	11.4
Cylindrocarpon aquaticum (Sv. Nilsson) Marvanová & Descals	7.4	15.6
Anguillospora longissima (Sacc. & P. Syd.) Ingold (Fig. 4b)	9.6	10.2
Condylospora spumigena Nawawi (Fig. 4d)	6.8	–
Lemonniera sp. (Fig. 5j)	1.1	5.3
Helicomyces denticulatus G.Z. Zhao, Xing Z. Liu & W.P. Wu (Fig. 5c)	6.0	–
Triscelophorus monosporus Ingold (Fig. 6f)	0.2	5.0
Helicosporium aureum (Corda) Linder (Fig. 5f)	0.8	4.3
Flabellospora crassa Alas. (Fig. 4i)	3.9	0.1
Tricladium sp. 2 (Fig. 6b)	–	3.5
Helicosporium gracile (Morgan) Linder (Fig. 5h)	2.3	0.3
Diplocladiella scalaroides G. Arnaud (Fig. 4e)	2.0	0.3
Isthmotricladia laeensis Matsush.	1.3	0.1
Lunulospora curvula Ingold	–	1.4
Tumularia aquatica (Ingold) Descals & Marvanová	0.3	1.0
Campylospora chaetocladia Ranzoni (Fig. 4c)	0.3	0.6
Flagellospora penicillioides Ingold	0.4	0.4
Trinacrium sp. (Fig. 6c)	0.8	–
Unknown species 1 (triradiate conidia 1) (Fig. 6j)	0.8	–
Campylospora filicladia Nawawi	0.1	0.6
Anguillospora crassa Ingold (Fig. 4a)	–	0.7
Flabellospora verticillata Alas. (Fig. 4j)	0.1	0.5
Unknown species 2 (triradiate conidia 2) (Fig. 6k)	0.4	0.2
Retiarius sp. 2 (Fig. 5l)	0.5	–
Alatospora acuminata Ingold	0.3	0.1
Lateriramulosa uniinflata Matsush.	0.2	0.2
Retiarius sp. 1 (Fig. 5k)	0.4	–
Dwayaangam sp. 2 (Fig. 4g)	0.4	–
Helicomyces hyderabadensis P. Rag. Rao & D. Rao (Fig. 5d)	0.3	–
Arborispora sp.	0.3	–
Ypsilina graminea (Ingold, P.J. McDougall & Dann) Descals, J. Webster & Marvanová (Fig. 6i)	0.2	–
Helicosporium sp. (Fig. 5i)	0.1	0.1
Isthmotricladia gombakiensis Nawawi	–	0.2
Tricladium sp. 1 (Fig. 6a)	–	0.2
Clavariopsis aquatica De Wild.	0.1	–
Trisulcosporium sp. 2 (Fig. 6h)	0.1	–
Wiesneriomyces laurinus (Tasso) P.M. Kirk	0.1	–
Unknown species 3 (tetraradiate conidia) (Fig. 6l)	0.1	–
Actinospora megalospora Ingold	–	0.1
Helicoma sp. (Fig. 5b)	–	0.1
Helicosporium dentophorum G.Z. Zhao, Xing Z. Liu & W.P. Wu (Fig. 5g)	–	0.1
Tripospermum myrti (Lind) S. Hughes	–	0.1
Number of staurosporous species	22	17
Number of scolecosporus species	6	6
Number of helicosporous species	5	5
Number of cylindroid and ovoid species	2	2
Total number of species	35	30
Total number of exclusive species	14	9

The percent contribution of conidia in *Acacia* was highest in *Flagellospora curvula* (19.8%), followed by *Triscelophorus acuminatus*, *T. konajensis* and *Anguillospora longissima* (ranges 9.6–19.7%). In *Ficus*, *T. acuminatus* was the top contributor of conidia (32%), followed by *Cylindrocarpon aquaticum*, *T. konajensis* and *A. longissima* (ranges 10.2–15.6%).

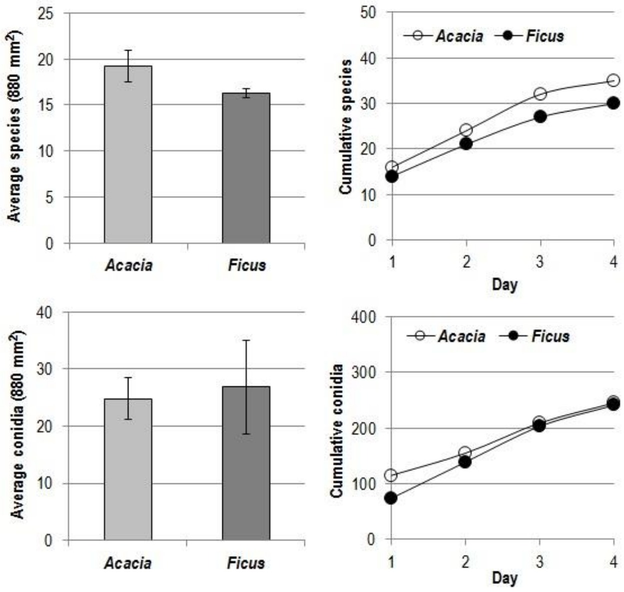

Fig 3 – Average (n=12±SD) and cumulative species of water-borne hyphomycetes in latex-trap (880 mm^2) of two tree species.

Species richness and diversity

The species richness was higher in *Ficus* than *Acacia* in the filtration (20 vs. 18 spp.), while it was the opposite in latex-trap (30 vs. 35 spp.; $p<0.05$). The overlap of water-borne hyphomycetes between the *Acacia* and *Ficus* ranged from 50–54% and 45–63% in the filtration and latex-trap techniques, respectively. The Simpson's and Shannon diversity indices were significantly higher in *Ficus* than *Acacia* in the filtration ($p<0.05$), while in latex-trap, they were significantly higher in *Acacia* than *Ficus* ($p<0.05$) (Table 4). The Pielou's equitability was the lowest in latex-trap in *Acacia*, while it was the highest in its filtration. Figs 4–6 display representative conidia of 36 water-borne hyphomycete species.

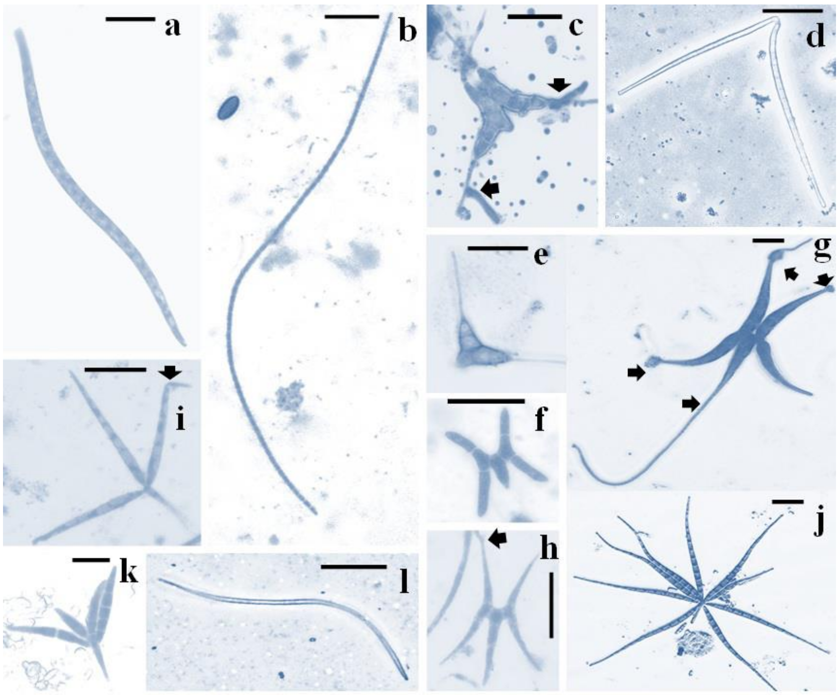

Fig 4 – Conidia of different hyphomycetes: a *Anguillospora crassa*. b *Anguillospora longissima*. c

Campylospora chaetocladia (arrows, germ tubes). d, *Condylospora spumigena*. e *Diplocladiella scalaroides*. f *Dwayaangam* species 1. g *Dwayaangam* species 2 (arrows, germinated main arms, appressoria and germ tubes). h *Dwayaangam* species 3 (arrow, germinated lateral arm). i *Flabellospora crassa* (arrow, germinated lateral arm). j *Flabellospora verticillata*. k *Flabellospora* species l *Flagellospora curvula*. Scale bar, a to l: 20 µm.

Table 4 Species richness, diversity and equitability of water-borne hyphomycetes in filtration and latex-trap in two tree species (n=4, mean; *t*-test: *, p<0.05; **, p<0.01; ***, p<0.001).

	Filtration		**Latex-trap**	
	Acacia	***Ficus***	***Acacia***	***Ficus***
Species richness	18	20	35*	30
Simpson diversity	0.932	0.937*	0.936*	0.931
Shannon diversity	4.004	4.142**	4.457***	4.262
Pielou's equitability	0.960	0.958	0.867	0.869

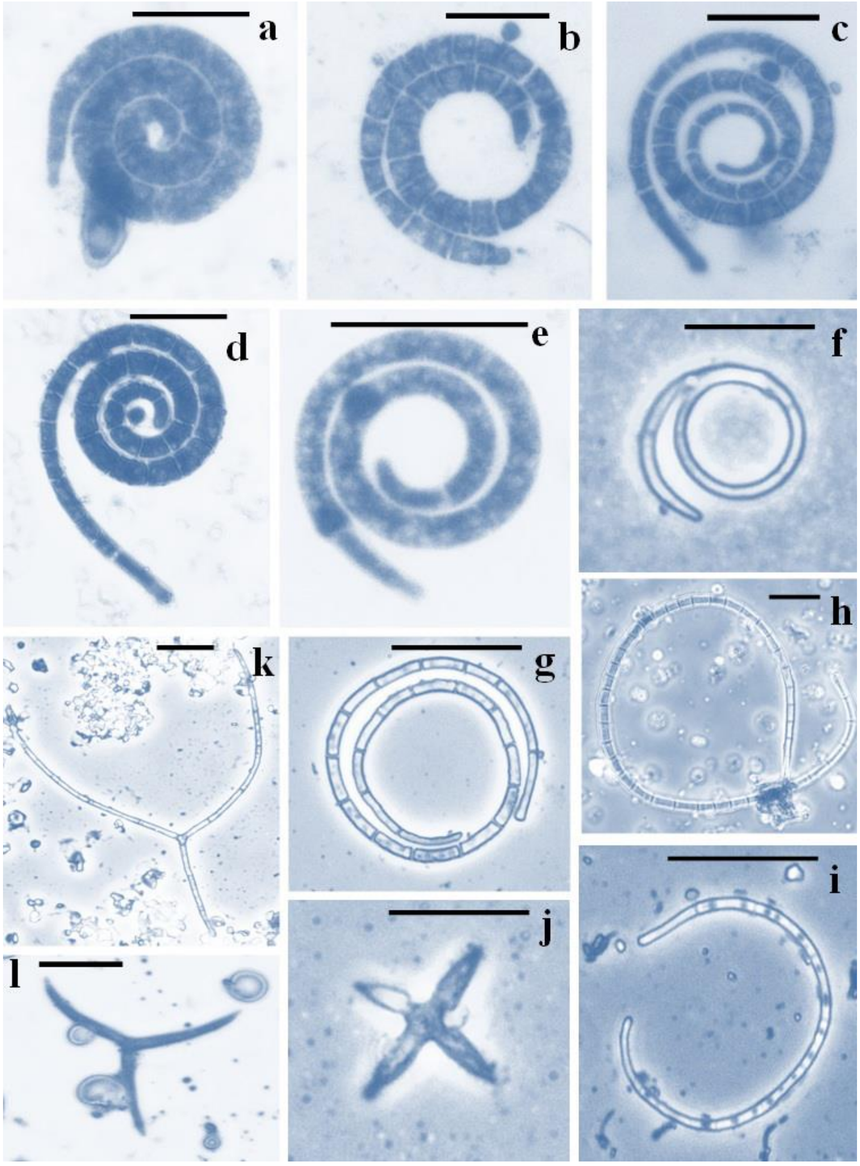

Fig. 5 – Conidia of different hyphomycetes: a *Helicoma atroseptatum*. b *Helicoma* species. c *Helicomyces denticulatus*. d *Helicomyces hyderabadensis*. e *Helicomyces* species. f *Helicosporium aureum*. g *Helicosporium dentophorum*. h *Helicosporium gracile*. i *Helicosporium* species. j *Lemonniera* species. k *Retiarius* species. 1 l *Retiarius* species 2. Scale bar, a to l: 20 µm.

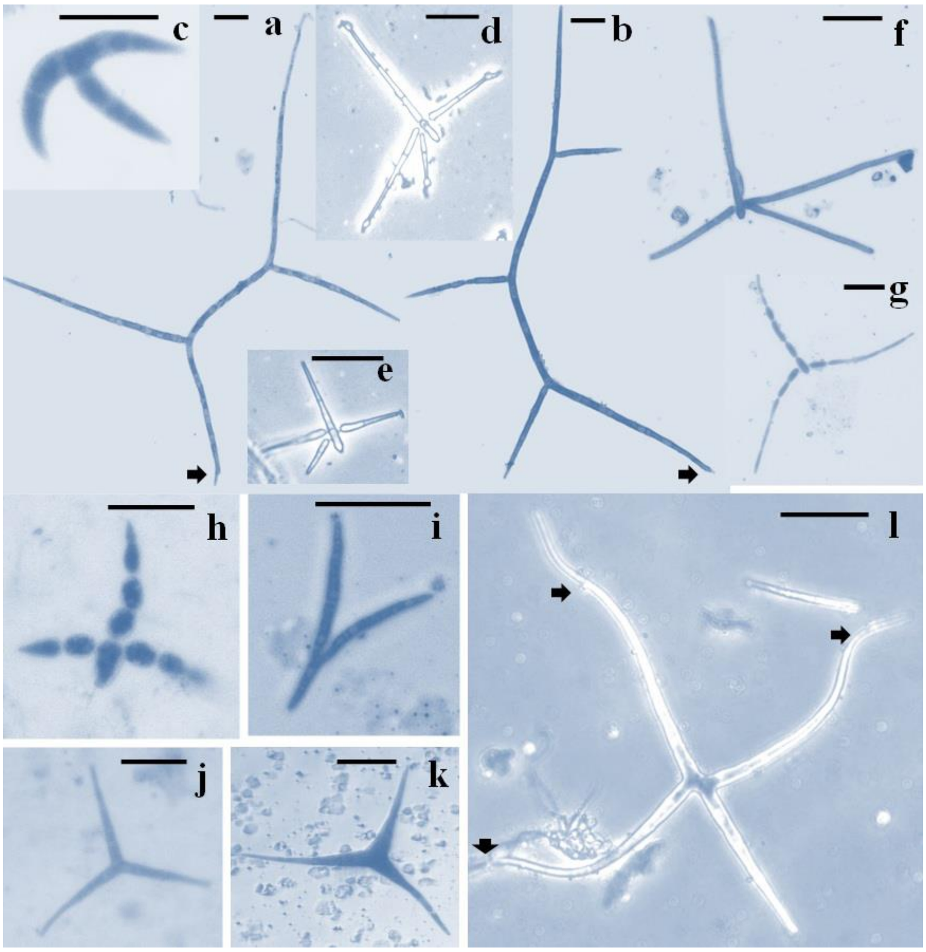

Fig. 6 – Conidia of different hyphomycetes: a *Tricladium* species. 1 (arrow, point of attachment showing incipient germ tube). b *Tricladium* species. 2 (arrow, point of attachment showing incipient germ tube). c *Trinacrium* species. d *Triscelophorus acuminatus* (tips of arms show appressoria and germ tube). e *Triscelophorus konajensis*. f *Triscelophorus monosporus*. g *Trisulcosporium* species. 1. h *Trisulcosporium* species. 2. i *Ypsilina graminea*. j Unknown species 1 (triradiate conidia). k Unknown species 2 (triradiate conidia). l Unknown species 3 (tetraradiate conidia) (arrows, germinated lateral arms). Scale bar: a to l: 20 µm.

Discussion

Throughfall and stemflow of tree canopies possess a variety of conidia of water-borne hyphomycetes in tropical and temperate regions almost similar to lotic habitats (Sridhar 2009, Magyar et al. 2021). In our study, 52 species were recorded in stemflow of two tree species by the filtration and latex-trap methods. Among 29 (isolated by filtration) and 35 (isolated by latex-trap) species, 21 were common, while 8 and 23 were found exclusively in filtration and latex-trap methods, respectively. The overlap of species between the methods (filtration and latex-trap) ranged between 47.5 and 58.5%, while between the trees (*Acacia* and *Ficus*), it ranges 52 and 54%. Thus, this study revealed at least 50% unique water-borne hyphomycetes between the methods and tree species. Earlier studies (Ghate et al. 2015, 2016), as well as the present study, propose that filtration and latex-trap methods serve as prospective techniques to assess the conidia of aquatic hyphomycetes in the stemflow of different tree species.

The term water-borne hyphomycetes have been used in this study to accommodate aquatic, aero-aquatic and dendronatant (bark-dwelling) hyphomycetes. Based on the earlier studies, it is possible to identify the conidia of some species as dendronatant fungi (Magyar et al. 2021). Eight species each in filtration (*Dwayaangam* sp. 1, *Dwayaangam* sp. 3, *Retiarius* sp. 1, *Retiarius* sp. 2, *Speiropsis pedatospora*, *Trinacrium* sp., *Trisulcosporium* sp. 1 and *Trisulcosporium* sp. 2) and in latex-trap (*Dwayaangam* sp. 2, *Retiarius* sp. 1, *Retiarius* sp. 2, *Trinacrium* sp., *Tripospermum myrti* and *Trisulcosporium* sp. 2) could be considered as dendronatant fungi.

Conidia of five species were common (*Anguillospora longissima*, *Cylindrocarpon aquaticum*, *Flagellospora curvula*, *Triscelophorus acuminatus* and *T. konajensis*) (□10%) in filtration as well as latex-trap in *Acacia* and *Ficus* trees. Among them, three species (*A. longissima*, *F. curvula* and *T. konajensis*) were dominantly found in water and the latex-trap in the Konaje stream (Ghate & Sridhar 2015) as well as in the Sampaje stream (Ghate & Sridhar, 2016). These three species were reported as the major producers of conidia in the Konaje stream in earlier studies based on water, leaf litter and foam observations (Sridhar & Kaveriappa 1984, 1989, Sridhar et al. 1992, 2013).

On average, the Konaje stream possesses up to 18–20 water-borne hyphomycete species based on water filtration, foam observation and leaf litter screening (Sridhar & Kaveriappa 1984, Sridhar et al. 1992, 2013). Such species richness matches the stemflow filtration of two tree species in the present study (18–20 spp.). However, the latex-trap added several additional species that were not found in several tree species (stemflow and throughfall) and in the Konaje stream (water, form and leaf litter) (Sridhar et al. 1992, Sridhar 2009). In the Konaje and Sampaje streams, the recovery of conidia in the order of efficiency was latex-trap > water filtration > plain slide-trap (Ghate & Sridhar 2016). Such difference was also seen in stemflow of *Acacia* and *Ficus* in the present study (latex-trap > water filtration). However, the quantity of stemflow passing through the latex-trap may be more than the quantity of stemflow collected or filtered to assess the conidia.

The average and the total number of species found in the filtration were relatively lower than the latex-trap method, while the average and the total number of conidia in the filtration was higher compared to the latex-trap. This observation corroborates an earlier study using the latex traps prepared from the latex of *Artocarpus heterophyllus* and *Ficus benghalensis* to assess the conidia in streams (Ghate et al. 2015, 2016). However, other latex traps (*Artocarpus hirsutus*, *Calotropis gigantea*, *Manilkara zapota* and *Plumeria rubra*) were not as efficient as latex of *A. heterophyllus* and *F. benghalensis* (Ghate & Sridhar 2015). Similar to the latex-trap of *Ficus*, baiting of *Ficus* leaf litter in the Konaje stream also resulted in the maximum recovery of water-borne hyphomycetes (Sridhar & Kaveriappa 1989, Sridhar et al. 1992). The impaction efficiency of staurosporous conidia is relatively higher than scolecosporus or cylindroid/ovoid conidia (Iqbal & Webster 1973, Webster & Davey 1984, Webster 1959). These observations are similar to our study, and the highest number of species with staurosporous conidia are present in the latex-trap compared to the filtration (17–22 vs. 10–11 spp.). However, such bias was not seen in latex traps exposed to Konaje and Sampaje streams (Ghate et al. 2015, 2016).

Similar to the report by Bärlocher et al. (1977) on the rosin-coated slides, the latex-trap method in our study showed germinated conidia (some produced appressoria) (e.g. *Campylospora chaetocladia*, *Dwayaangam* sp. 2, *Flabellospora crassa*, *Triscelophorus acuminatus and* unknown tetraradiate conidia) (see Figs 4, 6) within 24 hr. This depicts that in addition to conidial anchorage, banyan latex serves as a nutrient source for growth as reported by Sridhar and Kaveriappa (1987a) in a laboratory study. The bark texture between the two trees varied; *Acacia* has vertical, deep furrows with scales, while the *Ficus* has horizontal cracks with flakes, depressions and globules. Such textural differences in the bark may also result in differential conidial accumulation in stemflow bags or impaction on the latex trap. Fungal conidia trapped in the bark will fetch the nutrients from dead tissues and accumulated organic matter. Mycelia and conidia produced on the bark may be a potential source of nutrition to many of the bark-dwelling fauna.

The latex trap in this study did not attract the spores of terrestrial fungi similar to the studies in the streams (Ghate & Sridhar 2015, 2016). This supports the opinion of Bärlocher et al. (1977) based on the use of rosin-coated slides. However, in addition to staurospores and scolecospores, the latex-trap attracted one species each producing cylindroid (*Cylindrocarpon aquaticum*) and ovoid (*Tumularia aquatica*) conidia, hence they seem to produce mucilage for anchorage. Besides, the latex trap attracted as many as seven species of helicosporous fungi. Opposing to the latex-trap, stemflow filtration of two tree species besides water-borne hyphomycetes several conidia of terrestrial fungi with cylindroid or ovoid shapes were recovered. Thus, the latex-trap seems to attract mainly the staurosporous, scolecosporus and helicosporous conidia. None or fewer terrestrial fungal spores passively attached to the latex trap may be due to a lack of mucilage in those spores. Further studies need molecular methods to develop precise conclusions about the species richness, which has been overlooked based on only morphological approaches. As the stemflow and throughfall drain conidia of several water-borne hyphomycetes to the canopy floor, their occurrence in leaf litter, woody litter and soil below the canopy is not surprising (e.g. Sridhar et al. 2020, Sharathchandra & Sridhar 2020).

Conclusions

The convergent evolution of conidial morphology of dendronatant fungi (bark-dwelling) seems to facilitate trapping their conidia on the bark surfaces for their survival and perpetuation, similar to the Ingoldian hyphomycetes in streams. Fifty-two species were found in stemflow of *Acacia* and *Ficus* from 12 samples by filtration and latex-trap. Filtration and latex-trap techniques will be advantageous to study the population dynamics of water-borne hyphomycetes in tree canopies. Surprisingly, these techniques revealed the occurrence of water-borne hyphomycetes equivalent to or more than the nearby stream. There seems to be a significant difference between different tree species to generate stemflow and transport of fungal conidia. Further studies in the forest canopy (different tree species) and diurnal studies during the rainy season will disclose more about the adaptation of water-borne hyphomycetes to the niches of the tree canopy. It is interesting to study the efficiency of latex-trap in other aquatic habitats (e.g. lakes, groundwaters and hyporheic zones). The latex-trap technique is also valuable to study the efficiency of impaction of conidia at different ranges of water currents in natural habitats. A question remains when the conidia drain down in a unidirectional pattern, (top to bottom), how the water-borne fungal conidia produced or transported to the top portion of the canopy? There must be a strong mechanism for water-borne hyphomycetes to transport to the canopy by arboreal fauna or water-borne hyphomycetes surviving in the canopy in their teleomorphic states. Water-borne hyphomycetes transport from the stemflow to the tree floors (soil, leaf, wood, bark and others), and they seem to have several functions, especially decomposition and disseminating their propagules to other habitats (forest floors and streamlets) during the wet season.

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