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Endophytic Fungal Community of Seasonal Plant of Bryophytes

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Abstract

Bryophytes are prevalent constituents of both terrestrial and aquatic ecosystems, contributing significantly to the overall biomass in certain environments. This investigation has specifically focused on endophytic fungi within terrestrial bryophytes that grew in winter conditions on surfaces, such as house walls, bricks, or soil. During these periods, species such as *Bryum capillare*, *Polytrichum commune*, and *Riccia bifurca* are commonly observed. These kinds of bryophyte plants during the monsoons do come alive again. These examine the distribution of fungi associated with these bryophyte species. We collected three bryophyte samples from the delta region. Twenty-seven fungal species and 108 endophyte isolates were obtained from 450 tissue segments from three plants. Endophyte isolates belonging to Ascomycetes, Coeleomycetes, Hyphomycetes, Sterile forms, Yeast-like strains and Zygomycetes were recorded. Thus, its diversity was found to be based on the fungi it isolated. The endophyte species were calculated using the method of diversity index, CF%, RPO, species index, and species richness index. We have suggested that even in a relatively small region, the diversity of fungal species can be found in desiccation-tolerant environments.

Keywords: Bryophytes, Endophytes fungi, seasonal plant. Terrestrial and aquatic ecosystems.

INTRODUCTION

One of the planet's diverse life forms is fungi, and mycologists believe it's crucial to estimate the number of fungal species. Hawksworth predicted that there are 1.5 million species of fungi; of these, about 74,000 are currently known. Many tropical mycologists consider 1.5 million to be a rough estimate, and recent research from tropical forests reveals that fungal diversity is greater in the tropics than in the temperate climates. Some researchers, however, feel that the figure of 1.5 million is too high (Hawksworth, 2001). Fungi are eukaryotic micro- and macro organisms that are heterotrophs and have cell walls composed of chitin and glucan substances (Dismukes *et al*. 2003). Heinrich Link, a German botanist, first used the term "endophyte" in 1809. "Entophytae," a different category of harmful fungi that reside in plant tissues, was the term used at the time. According to Southworth (2012), endophytic fungi were related to terrestrial plants that appear to lack structurally similar organs for shoots, leaves, and roots. The diversity of endophytic fungi in a plant is also influenced by its environment.

Endophytes are microbes, bacteria, fungi, or actinomycetes that live inside the tissues of plants for the entire or part of their life cycle without causing any disease to the host (Arpita-Tripathi *et al*., 2022). These microbes are found in practically every higher plants and bryophytes that has been studied (Hardoim *et al*., 2015; Venkatesan and Mahalakshmi, 2022). This study aimed to investigate the diversity, distribution, and adaptation of the endophyte fungi associated with three seasonal bryophyte species in the Delta region of Tamil Nadu.

MATERIALS AND METHODS

Study area

These plants were studied in the Delta region of Tamil Nadu every year from October to January months of rainy seasons. This period receives rainfall under the effect of the northeast monsoon. We investigated time and again in the years 2022, 2023 and 2024, the seasonal bryophyte plants commonly occur of *Bryum*, *Polytrichum*, and *Riccia* species were collected from Mannargudi in the Thiruvarur district (10.6651'-10° 39' 54N, 79.4525-79° 27' 9E), Tamil Nadu, India. This study is reported in 2024.

Sterilization Methods and Observation

Bryophyte plant thalloids from each individual were processed separately within 12 hours of collection. The leaves were washed thoroughly in running water and three segments of 0.5 cm^2 were cut from the piece of each leaf and surface sterilized by immersing in 70% ethanol for 5 seconds, followed by 2% NaOCl for 90 seconds, and finally washed in sterile water for 10 seconds. One fifty segments from each individual were randomly chosen and placed in Petri dishes containing potato dextrose agar (with chloramphenicol 150 mg 1^1). Each Petri dish was plated with ten leaf segments, sealed with Parafilm, and incubated for 21 days at 20 to 30°C in a light chamber. Darkness and light were the light regimens offered (Bills and Polishook, 1992; Suryanarayanan, 1992). At regular intervals, the fungi that emerged from the segments were separated and identified. Those fungi that failed to sporulate were given codes using culture characteristics such as colony surface, texture and hyphal pigmentation, and categorized as 'sterile forms".

STATISTICAL ANALYSIS

Colonization Frequency

Number of colonies divided by the number of totals X100 is the colonization frequency (CF%). Hata and Futai (1995) defined the number of segment colonies as the number of segments colonized by each endophyte and the number of totals as the total number of segments observed.

Relative Parentage of Occurrence of Each Group of Fungi (RPO)

The relative percentage of occurrence (RPO) of each group (viz., Ascomycetes, Basidiomycetes, Coelomycetes, Hyphomycetes, Sterile-like Forms, and Zygomycetes) of fungal species in each plant species was calculated as follows: Tedersoo *et al*. (2018).
RPO = Total colonization frequency of one group / Total colonization frequency for all the groups of fungi X 100.

Diversity Index (Fisher's α)

The diversity index was calculated using the method of Fisher *et al*. (1943).

Species evenness index and species richness index (E5, R1)

The species evenness (E5, modified Hill's ratio) and species (R1, Margalefs index) were calculated as described by Ludwig and Reynolds (1998) using the software provided by John Wiley and Sons, SPDIVERS.BAS.

Plant details

Bryum capillare (mosses) is a very common species. It generally forms dense cushions on hard surfaces such as rocks and tree bases and manmade habitats such as walls, roofs, concrete and tarmac. It is not generally found on soil. *Polytrichum commune* (mosses) is one of the largest mosses, with individual stems. Thus grow favourable habitats of wet meadows and aquatic ecosystems. This species is referred to as the *Sphagnum* species. It is easily recognized (Table 1). *Riccia bifurca* (liverworts) species' rain season grows in a wide variety of habitats, including fertile and productive soil fields and gardens, soil on rocky banks, gravel tracks, footpaths and reservoir margins, however is commonly less viewed.

Table 1: Details plants list of mosses and liverwort groups of Bryophytes studied for the presence of endophytes.

Sl. No.	Name of the Host	Host Code	Family
1.	*Bryum capillare* (Hedw.) J.R. Spence	BC	Bryaceae
2.	*Polytrichum commune* Hedw.	PC	Polytrichaceae
3.	*Riccia bifurca* Warnst. ex Croz.	RB	Ricciaceae

RESULTS AND DISCUSSION

A survey of three bryophyte plants from the delta region of Tamil Nadu showed that the leaves of all these hosts harboured fungal endophytes from a desiccation-tolerant environmental region. We recovered 108 endophyte isolates obtained from 450 tissue segments from three plants. These fungi were taxonomically found in 27 species belonging to 18 genera, and sterile, yeast-like forms of fungi were isolated from seasonal bryophyte plants. The endophyte fungal species were isolated from *Bryum capillare*, *Polytrichum commune*, and *Riccia bifurca*. Four species of ascomycetes, one species coleomycetes, and 16 hyphomycetes, each with two strains of sterile forms, yeast-like forms, and Zygomycetes, remain isolated from three bryophyte plants.

Thirty-nine isolates belonging to 25 species were recorded. Ascomycetes (04), Coleomycete (01), Hyphomycetes (14), Sterile forms (02), Yeast-like forms (02), and two species of Zygomycetes from *Bryum capillare* and *Polytrichum commune* from forty-six isolates and 24 species belonging to Ascomycetes (04), Coleomycete (01), Hyphomycetes (14), and Sterile forms (02), Yeast-like forms (02), and Zygomycetes (01) fungal species, and *Riccia bifurca* were found out of twenty-three isolates belonging to 16 species. Ascomycetes (04), Coleomycetes (01), and Hyphomycetes (08), and each fungal species of sterile form, yeast-like and zygomycete, were recorded. In these results, a small number of fungal colonies are observed, but some fungal species like *Collectotrichum* sp., *Sordaria fimicola*, *Sporormiella intermedia*, *Cheatomium* sp., *Aspergillus niger*, *Cladosporium herbarium*, *Curvularia lunata*, *Fusarium oxysporum*, and *Trichoderma* species are considered high-frequency fungi with a slightly higher number recorded (Table 2).

Table 2: Fungal endophytes isolated from bryophytes: the leaves of *Bryum capillare*, *Polytrichum commune*, and *Riccia bifurca*.

		Host Code					
Sl. No	**Name of Fungus**	**BC**		**PC**		**RB**	
		Colonies	CF%	Colonies	CF%	Colonies	CF%
	Ascomycetes						
1	*Scopulariopsis* sp. 1	1	0.67	1	0.67	2	1.33
2	*Sordaria fimicola*	1	0.67	3	2	2	1.33
3	*Sporormiella intermedia*	1	0.67	1	0.67	3	2
4	*Cheatomium* sp. 1	3	2	1	0.67	1	0.67
	Coeleomycetes						
5	*Collectotrichum* sp. 1	4	2.67	6	4	2	1.33
	Hyphomycetes						
6	*Alternaria alternata*	1	0.67	2	1.33	2	1.33
7	*Alternaria* sp. 1	1	0.67	1	0.67		0
8	*Aspergillus flavus*	1	0.67	1	0.67	1	0.67
9	*Aspergillus niger*	3	2	1	0.67	1	0.67
10	*Aspergillus* sp. 1	1	0.67			1	0.67
11	*Aureobasidium* sp. 1	1	0.67			1	0.67
12	*Botrytis* sp. 1	1	0.67	2	1.33		
13	*Cladosporium herbarum*	2	1.33	3	2		
14	*Curvularia lunata*	2	1.33	4	2.67		
15	*Curvularia* sp. 1	1	0.67	1	0.67		
16	*Drechslera* sp . 1	1	0.67	2	1.33		
17	*Fusarium* oxysporium	2	1.33	4	2.67	2	1.33
18	*Fusarium* sp. 1	1	0.67	2	1.33	1	0.67
19	*Nigrospora orzae*			2	1.33		
20	*Stachybotrys* sp. 1			1	0.67		
21	*Trichoderma aureoviride*	1	0.67	2	1.33	1	0.67
	Sterile forms						
22	Sterile forms 1	3	2	2	1.33	1	0.67
23	Sterile forms 2	1	0.67	1	0.67		
	Yeast forms						
24	Yeast white form 1	2	1.33	1	0.67		
25	Yeast yellow form 2	1	0.67	1	0.67	1	0.67
	Zygomycetes						
26	*Mortierella* sp. 1	1	0.67				
27	*Mucor hiemalis*	2	1.33	1	0.67	1	0.67
	Total CF%		26.0		30.7		15.3
	Total no. of colonies	39		46		23	
	Total no. of species	25		24		16	

Table 3: Similarity coefficients between the number of species, total number of colonization frequency (CF%) of the endophyte in the host of Bryum capillare, Polytrichum commune and Riccia bifurca, relative percentage of occurrence (RPO), species richness (R1), species evenness (E5), and species diversity (Fisher's α) of the endophyte assemblages of the bryophytes plants

Sl. No.	**Statistics**	***Bryum capillare***	***Polytrichum commune***	***Riccia bifurca***
1.	Species	25	24	16
2.	Individuals	39	46	23
4.	CF%	26.0	30.7	15.3
5.	R1(Margalef's)	6.55	6.01	4.78
6.	H' (Shannon Index)	3.09	3.0	2.69

7.	E5 (Hill's Ratio)	1.72	1.30	2.23
8.	Fisher's Alpha	30.05	20.25	23.30
9. Relative Percentage of Occurrence (RPO) of each group of fungal specie				
9.1	Ascomycetes	4 sp. (15.4%)	4 sp. (12.9%)	4 sp. (35.5%)
9.2	Coeleomycetes	1sp. (7.7%)	1 sp. (12.9%)	1 sp. (8.7%)
9.3	Hyphomycetes	14 sp. (48.7%)	14 sp. (60.2%)	8 sp. (44.4%)
9.4	Sterile forms	2 sp. (10.4%)	2 sp. (6.5%)	1 sp. (4.7%)
9.5	Yeast forms	2 sp. (7.7%)	2 sp. (4.3%)	1 sp. (4.7%)
9.6	Zygomycetes	2 sp. (7.7%)	1 sp. (2.6%)	1 sp. (4.7%)

DISCUSSION

The majority of research on fungal endophytes has concentrated on species that inhabit vascular plants. No reports exist on the endophytic fungal diversity associated with bryophytes in the delta region. Still, endophytes also live in nonvascular plants, including bryophytes (mosses, liverworts, and hornworts), which are functionally important in tropical and temperate ecology and the ecology of tolerance to desiccation in bryophytes, which generate an enormous portion of biomass. A great phylogenetic diversity of endophytes was found in the tissues of mosses and liverworts in tropical forests (U'Ren *et al*., 2010). A total of 27 endophytic fungi (EF) were isolated from 108 colonies of 450 tissue segments from three bryophytes.

Our investigation was based on seasonal bryophytes, plant-fungal endophytes found in open lands during a few months of the region's rainy season. Thus, we found that the most widespread soil and airborne fungi were also the most frequent, namely *Alternaria*, *Aspergillus*, *Cladosporium*, *Curvularia*, *Fusarium*, and *Trichoderma aureoviride* were isolated (Hernandez and Martinez, 2018) (Table 2). Ubiquitous fungi genera contain common saprophytes in soil and air, clinical and plant pathogens. The common fungi species are frequently isolated from plants as both an endophyte and a pathogen. It has been demonstrated that soil and air samples frequently contain *Aspergillus* and *Penicillium* spores (Mitchell *et al*., 2016; Umesha *et al*., 2016).

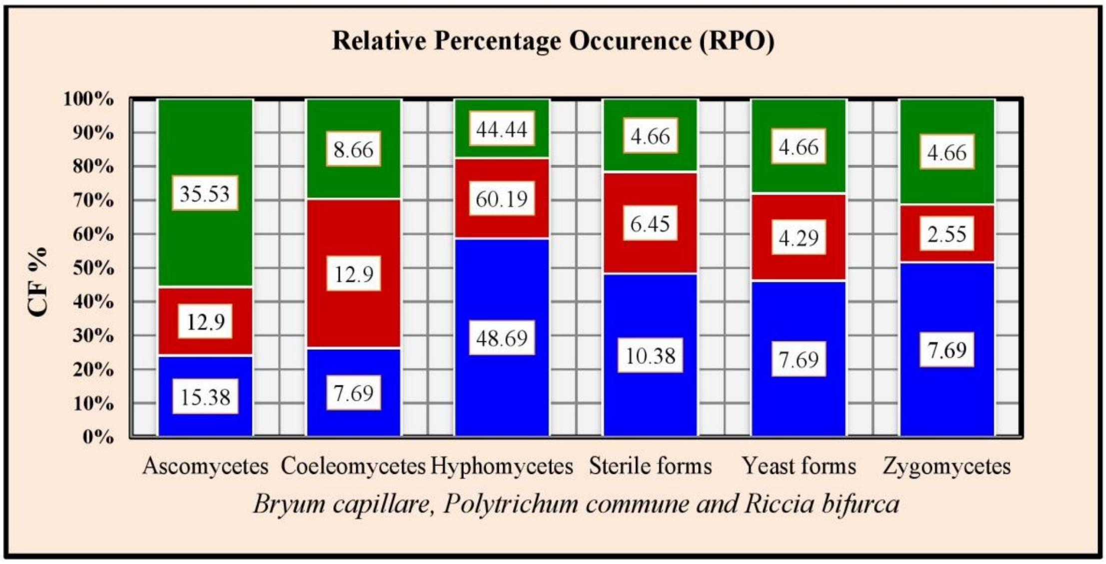

Figure 2: Relative Percentage Occurrence (RPO) of endophytes belonging to different groups of fungi.

Colletotrichum is a ubiquitous genus of fungi that are symbionts to plants as endophytes or phytopathogens. *Colletotrichum* species are common isolates of endophytes and pathogenic fungi; some species may have a mutuality relationship with hosts. In the past reported as the dominant fungal endophytes of various plant species from diverse environments (Suryanarayanan *et al*., 2011; Jayawardena *et al*., 2016; Talhinhas and Baroncelli, 2021). According to Morton and Smith (1963), *Scopulariopsis* is a common genus of anamorphic fungi that are saprobic and harmful to animals. These species are commonly found in soil, decaying wood, and other plant and animal *Sordaria fimicola* and *Sporormiella intermedia*, is an ascomycete fungus that is normally found and grows on decaying organic material and animal and cow dung and other products (Ivanová *et al*., 2018; Dustin *et al*., 2022). *Chaetomium globosum* is a saprophytic fungus that primarily resides on plants, soil, straw, and dung. This endophytic fungus assists in the cellulose decomposition of plant cells (Abou-Alhamed and Shebany, 2012; Elshahawy and Khattab, 2022). *Aureobasidium pullulans* is a common fungus that grows in diverse settings. It is universally acknowledged as an endophyte or epiphyte of different plant parts that occurs naturally. *Botrytis cinerea* is a necrotrophic fungus that affects many plants (Vikram *et al*., 2021). *Cladosporium* is a common fungus found worldwide in organic and inorganic matter and is the most frequently occurring fungal species. *Curvularia*, *Drechsleram*, *Fusarium*, and *Stachybotrys* are fungi that can cause disease in plants and humans, pathogens, and a diverse range of nonpathogens (Edel-Hermann and Lecomte, 2019; Hodgson *et al*., 1998). *Trichoderma aureoviride* is a genus of fungi present in all soils, where they are the most prevalent cultural fungi (Rosmana *et al*., 2016; Karlsson *et al*., 2017). Mucor and *Mortierella* are growing number of evidence indicating the importance of endophytic fungi in plant growth, fitness and adaptation to the environment (Doreswamy *et al*., 2022).

The results show the relative percentage of occurrence (RPO), species richness (R1), species evenness (E5), and species diversity (Fisher's α) of the endophyte assemblages of the bryophyte plants. In the samples of bryophyte species, there are differences in fungal diversity, and also lower frequencies were observed (Table 3, Figure 2). The diversity of fungi in the *Riccia* plant was very low compared to *Bryum* and *Polytrichum*. The results suggest that environmental conditions are the most important of the factors affecting endophyte composition, especially the dominant endophytes. Likewise, isolated bryophyte fungi have been seen as such. Some bryophyte species grow to the ground in a very short period of life; these habitats are less shady.

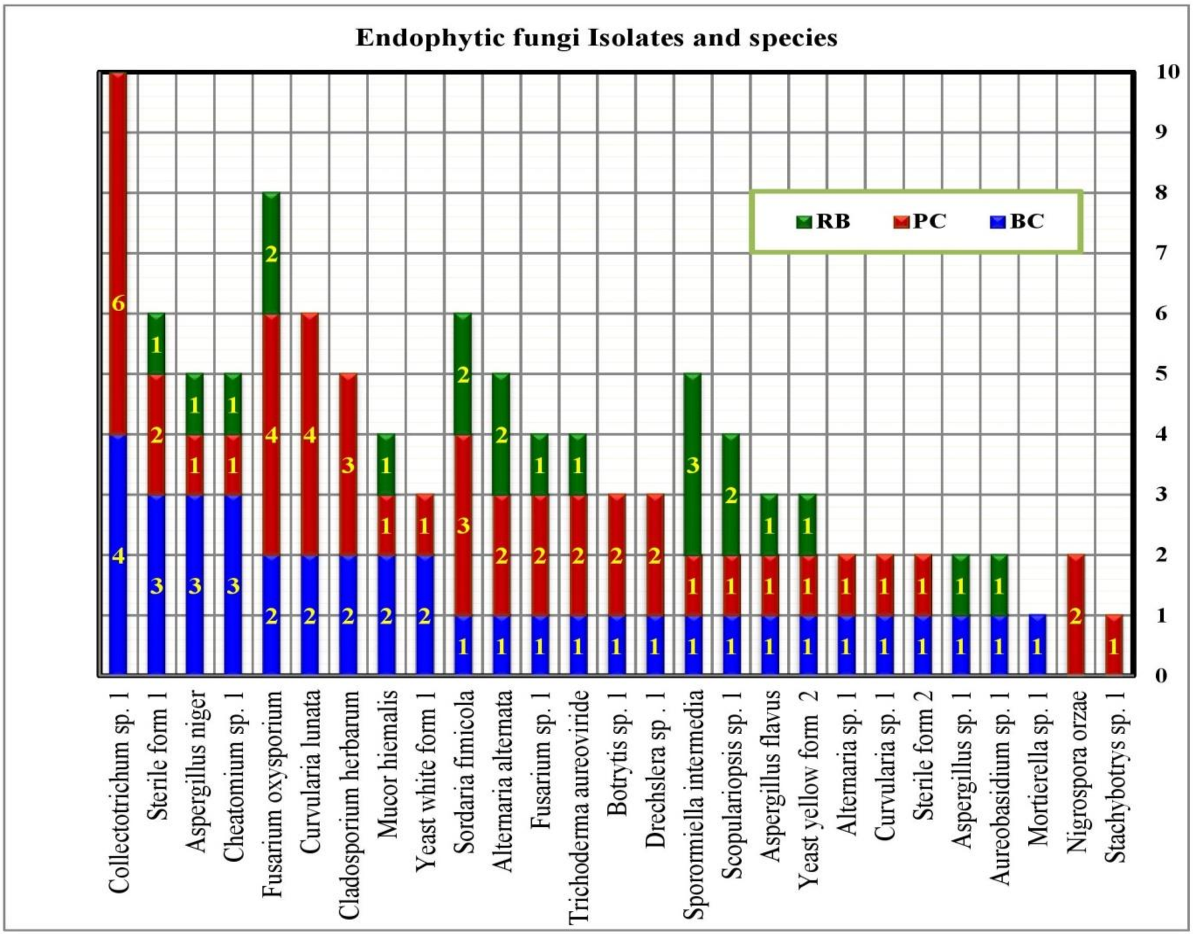

Figure 3: A histogram that shows the diversity of endophytic fungi isolates and species

Some authors have demonstrated that fungal endophyte communities may be influenced by diverse biotic and abiotic factors, such as the type of plant tissues; a heterogeneous profile of microhabitats; and variability throughout vegetation, climate, and substrates (Koide *et al.*, 2017). Soil, airborne fungal spore concentrations, and their diversity vary with the season of the year, geographical region, soil, air, meteorological parameters, the presence of local resources, and vegetation.

Our report states that although the above fungi are found in many environments, they are considered endophytic fungi in our study, and that endophytic fungi are part of the plant life cycle and are found on the leaf surface through either air or soil contact.

A few fungi that failed to sporulate were designated as "mycelia sterile" and can be identified later with different incubations, such as sporulation in UV, so for colony characteristics, the mycelia were transferred into PDA agar media.

CONCLUSION

According to this study, endophytes least commonly colonized host plant species, which were bryophyte plants. The distribution of fungi varied with that natural ecosystem, prompting us to assume that they only grow in wet conditions and have short life cycles. Even so, the endophytic fungi may contribute to the least amount of seasonal bryophyte plants. These hosts were found to be true fungi and had much diversity of endophytes. However, the bryophytes endophyte fungi were isolated very ubiquities fungi. Endophytic fungi may be rich sources of bioactive components and novel metabolites that can be used to be valuable in pharmaceuticals, medicine, agriculture, and industries.

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