

## Diversity of bryophilous fungi in desiccation-tolerance bryophyte plants

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### Abstract

Bryophilous fungi associated with two bryophyte species in the region of delta in Tamil Nadu that is, the mosses of *Polytrichum commune* and *Bryum capillare* (*Ptychostomum capillare*) mosses were studied during the winter season. These bryophytes were Desiccation-Tolerance Bryophytes plant (DTB), the ability to lose virtually all free intracellular water and then recover normal function upon rehydration is one of the most remarkable features of bryophytes. Our aims were to identify bryophilous fungi/ endophytic fungi using morphological taxonomy, to explore richness and estimate species frequency in two bryophytes, *Polytrichum* and *Bryum* in delta region of Tamil Nadu. We collected two bryophyte samples at old building wall, bricks localities on delta region. We thus identified 32 endophytic fungi species were isolated from these bryophyte plants tissues. In total, 32 endophytic fungal strains belonging to Ascomycetes (04), Coeleomycetes (02), Hyphomycetes (20), sterile forms (02), Yeast like strains (02) and Zygomycetes (02) taxa were obtained. Thus species RPO and diversity index suggest that our study still underestimate endophytes diversity because it does not provide complete samples. These results recommend the presence of a diverse range of fungal species even in a very limited area, and those bryophytes fungal diversity in this Desiccation-Tolerance environment. To our knowledge, this is the first report of endophytic fungi from DT Bryophytes in tropical region.

**Keywords:** Bryophyte; Bryophilous fungi; Desiccation-Tolerance; Ecology; Sere

### 1. Introduction

Fungal endophytes are a diverse group of fungal species that reside living plants at some time during their life cycle without causing apparent symptoms of infection (Wilson, 1995). Plants generally have multiple endophytes, and those may develop multiple endosymbiotic manners of the host. Endophytic fungi live within the healthy tissues of plants and can encourage host species tolerance to different environmental stresses. Bryophyte plants can be thrived in humid environments all over the world. Because they have no vascular tissue, they aren't able to take water from the soil and transport it to higher tissue. The Habitat of bryophyte occur in common on the forest floor, trees stem, rain forests, wetland ecosystems and at high altitudes and also Many bryophytes live in their urban life by establishing on areas such as bricks, driveways and cracks of surfaces. Phenotypic plasticity plants have appearing the ability of individual genotypes to produce different phenotypes when exposed to different environmental conditions (Pigliucci *et al.*, 2006). Some organisms have possibility to modify development and the ability of an individual organism to change its phenotypic state or activity in response to variations in environmental conditions (Garland and Kelly, 2006).

Most studies of fungal endophytes have focused on those species that live in vascular plants, but endophytes also live in nonvascular plants including bryophytes (i.e., mosses, liverworts, and hornworts). A great phylogenetic diversity of endophytes was found in the tissues of mosses and liverworts in boreal, temperate and tropical forests (Davis *et al.*, 2003; Davis and Shaw, 2008; Kausrud *et al.*, 2008). Associations between mosses and fungi were long thought to be

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uncommon and rare (Grasso and Scheirer, 1981). Although interactions between bryophilous ascomycetes and bryophytes have been investigated by Döbbeler (1997), endophyte and bryophyte interactions have remained largely unstudied (Davey and Currah, 2006).

Vascular plants species (Arnold *et al.*, 2000, Suryanarayanan *et al.*, 2011), aquatic plants and algae (Smith *et al.*, 1989, Venkatesan and Suryanarayanan, 2014, Venkatesan and Rameshkumar *et al.*, 2021, mosses and ferns (Petrini *et al.*, 1992, Raviraja *et al.*, 1996) examined to date are found to be hosts for endophytic bacteria and fungi (Nisa *et al.*, 2015). Endophytic microorganisms have been isolated from different parts of plant-like leaf and roots, stem, bark, petiole (Hata and Sone, 2001; Venkatesan, 2013; Venkatesan and Sharavanan, 2022), different forests (Suryanarayanan *et al.*, 2007, 2011) buds (Pirttilä *et al.*, 2008) and seeds (Panaccione *et al.*, 2014). In the past, mycological studies conducted in Antarctic regions have been generally restricted to soil (Boyd *et al.*, 1966; Bailey and Wynn-Williams, 1982) and only plants, mosses, and other organic substrates have been used as sources for the isolation of Antarctic microfungi (Pugh and Allsopp, 1982; Bradner *et al.*, 2000). The abundance and diversity of organisms decrease from the maritime to the continental Antarctic zone (Ruisi *et al.*, 2007). To our knowledge, no reports on the endophytic fungal diversity associated with bryophytes in the delta region of Tamil Nadu are available. The aim of this study was to investigate the diversity, distribution, and adaptation of the endophytes fungi associated with two Desiccation-Tolerance Bryophytes species (the moss of *Polytrichum commune* and the moss of *Bryum capillare*) in the Delta region of Tamil Nadu.

## 2. Material and methods

### 2.1. Study Area

These plants were studied in the Delta region (Mannargudi in city) of Tamil Nadu during the period from October 2021 to January 2021 (wet season) and over May 2022 to August 2022 (dry season). The district receives rainfall under the effect of both southwest and northeast monsoon. The hot season lasts for 4.3 months, from April to August, with an average daily high temperature above 94 °F. The hottest month of the year in Mannargudi in Thiruvavur District is June, with an average high of 97 °F and low of 83 °F. The cool season lasts for 3.0 months, from November to February, with an average daily high temperature below 86 °F. The coldest month of the year in Mannargudi is January, with an average low of 74 °F and high of 83 °F.

### 2.2. Collection of Plants

These DT - bryophyte species of *Bryum capillare* Hedw. (*Ptychostomum capillare* (Hedw.) D.T.Holyoak & N. Pedersen) *Ptychostomum capillare*, formerly designed as *Bryum capillare* is Capillary Threat - moss belonging to the family Bryaceae and *Polytrichum commune* Hedw., moss of family Polytrichaceae were collected from Mannargudi in Thiruvavur district (10.6651°-10° 39' 54N, 79.4525-79° 27' 9E), Tamil Nadu, India. These plants were collected to wet season at least 1.5 month old. Economically important species are those in the genus *Polytrichum* and *Ptychostomum* that form like peat mosses. These species can frequently be found in wet season will grow on hedge banks, old building wall, bricks, and driveways localities. Typically they are in areas that moist and humid in sites that are somewhat shaded.

### 2.3. Sterilization, inoculation and statistical methods

Thallus samples were collected from these bryophytes. Randomly select mosses and their one hundred and fifty tissue segments were cut from these mosses. However, sterilization techniques were followed in before cutting these segments. They were processed within a few hours of collection. The plant is washed thoroughly with running water and then the whole plant has sterilized as followed. Surfaces sterilization of leaves like thallus bits of 0.5 cm<sup>2</sup> each were cut and surfaces sterilized by the method of Suryanarayanan *et al.*, 1998. The samples were washed in running water, dipped in 70% ethanol for 60 seconds, immersed in 4% NaOCl (Sodium Hypochlorite) for 60 seconds, and then washed in sterile water for 120 seconds. The sterilized samples were placed on PDA medium amended with an antibiotic contained in Petri dishes. Incubation, isolation, and identification of endophytes ten segments were placed on PDA medium contained in a Petri dish. The Petri dish was sealed with Parafilm™ and incubated in a light chamber at 26+1 °C for 7 to 21 days (Bills and Polishook, 1992; Suryanarayanan, 1992). The light regimen given was 12 hours light followed by 12 hours darknesses. Fungi that grew from the segments were periodically observed and the endophytes were identified.



**Figure 1** DT - Bryophyte plants grown on building walls, A. *Polytrichum* commune, B. *Polytrichum* commune dense growth, C. *Ptychostomum* capillare

- Colonization frequency CF% of endophyte species was calculated by the method of Hata and Futai (1995). CF% = Number of colonies/Total number of segments X 100. Where, N Colonies and N Total are the number of segments colonized by each endophyte and the total number segments observed respectively.
- Relative Percentage of Occurrence (RPO) of each group (viz. Ascomycetes, Coelomycetes, Hyphomycetes, Sterile forms, Yeast like forms and Zygomycetes) of fungal species in each plant species was calculated as follows: RPO = Total colonization frequency of one group/ Total colonization frequency for all the groups of fungi X 100.
- Simpson's Index was calculated for fungal species richness.

$$D = S \sum_{i=1}^s (ni/N)^2$$

Where;

$ni$  is the number of individuals in species  $i$ ,

$N$  = total number of individuals of all species,

$ni/N = pi$  (proportion of individuals of species  $i$ ),

$S$  = species richness.

- Shannon -Weiner Index was calculated for fungal species evenness.

$$H' = - \sum_{i=1}^s pi \ln pi$$

### 3. Results

These fungi taxonomically were found out 32 species belonging to 19 genera of fungi were isolated from both bryophyte plants. In total 28 fungal species were isolated from *Polytrichum commune*. All fungal species has the small-scale isolation rate. Hyphomycetes (17) were the abundant group followed by Ascomycetes (03), Coleomycetes (02), and Sterile forms (02), Yeast like forms (02) and remain 02 species of Zygomycetes (Tables 1, 2 and 3). While a total of 26 fungal species were isolated from *Ptychostomum capillare*. In this Hyphomycetes (17) were rich diverse group by than Ascomycetes (03), Coleomycetes (01), Sterile forms (2), Yeast like forms (2) and continue one species of Zygomycetes (Figure 1 and 2). In this results a small number of (Fewer) fungal colonies are observed but some fungal species like *Collectotrichum* sp., *Cheatomium* sp., *Aspergillus flavus*, *Aspergillus niger*, *Fusarium oxysporum* and *Trichoderma* species are considered high frequency fungi due to slightly higher number (Table 1 and 2). Also, when we collected and examined the dried up moss/or soil like substrate form in places where the bryophytes grew, we found that the sere contained *Aspergillus* spp., *Cheatomium* sp., *Fusarium* sp, *Mucor* sp., *Trichoderma* sp., *Penicillium* sp., *Sordaria* sp., *Sporormiella* species etc. The dried - up moss study was carried out by Warcup and serial dilution method (Warcup, 1950). However, most studies have been of plants in humid habitats, and there are few reports of the advantages of such associations for plants of extreme environments.

*Chaetomium globosum* is a well-known saprophytic fungus that also reported to resides on plants endophytic, soil; dung (Li et al., 2014). *Fusarium oxysporum* is commonly documented as a wild causing phytopathogen in some plants or a

nonpathogenic plant growth promoting endophyte of other plants (Minerdi *et al.*, 2008). Several studies have reported the presence of *Aspergillus* sp., *Cladosporium* sp., *Mucor* sp., *Trichoderma* sp., *Penicillium* sp., in multiple environments around the world, such as air, land, water, plants and animals. In addition, it can be found in inhospitable locations such as deserts, highly salty and soils contaminated by radioactivity (Hasegawa *et al.*, 2008; Huyan *et al.*, 2012; Ribeiro *et al.*, 2008; Sterflinger *et al.*, 2012). *Sordaria* and *Sporormiella* species are common fungus that grows on dung, decaying matter in soil (Suryanarayanan *et al.*, 1998). *Trichoderma* sp., *Penicillium* sp., isolated from the Antarctic moss *Bryum argenteum* (Bradner *et al.*, 2000). When compared with earlier reports in bryophytes mosses, the endophytic fungi were isolated in to classes of Ascomycetes, Coelomycetes, Hyphomycetes, and Zygomycetes from Victoria Land, Arctowski and Jubany on King George Island by Möller and Dreyfuss, 1996, Tosi *et al.*, 2002. On the reported of Thormann *et al.* (2001) common saprobes are among Ascomycota: *Sporormiella* sp., *Sordaria* sp., Zygomycota: *Mortierella* sp., *Mucor* sp., Anamorphic fungi: *Cladosporium* sp., *Aspergillus* sp., *Botrytis* sp., etc.



**Figure 2** Petri plates showing on endophytic fungi growing on DT- Bryophyte plant (bits) tissues

**Table 1** Number of isolates, species and diversity index for endophytes fungi isolated from two bryophyte species

Sl.No	Details	<i>Polytrichum commune</i>	<i>Ptychostomum capillare</i>
1.	Total no. of species (Total species - 32)	28	26
2.	Total no. of colonizes	49	46
3.	Fisher's Alpha - diversity index	27.15	24.76
<b>Relative Percentage of Occurrence (RPO) of each group of fungal specie</b>			
4.	Ascomycetes	4 (13%)	3 (11%)
5.	Coeleomycetes	2 (6%)	1 (4%)
6.	Hyphomycetes	20 (63%)	17 (65%)
7.	Sterile forms	2 (6%)	2 (8%)
8.	Yeast forms	2 (6%)	2 (8%)
9.	Zygomycetes	2 (6%)	1 (4%)

#### 4. Discussion

Most studies of fungal endophytes have focused on those species that live in vascular plants, but endophytes also live in nonvascular plants including bryophytes (mosses, liverworts, and hornworts), which are a functionally important in tropical and ecology of desiccation tolerance in bryophytes where they produce much of the biomass. A great phylogenetic diversity of endophytes was found in the tissues of mosses and liverworts in tropical forests (U'Ren *et al.*,

2010). Both soil bacteria and fungi include heterotrophic microorganisms that are capable of rapid activation upon wet-up, although microbial communities have sometimes been shown to shift seasonally in water-limited systems (Clark *et al.*, 2009; Cruz-Martinez *et al.*, 2009).

**Table 2** An endophytes fungus was isolated from DT-Bryophytes plants and calculates Simpson's Index

No. of Species (i)	Name of fungus	<i>Polytrichum commune</i>			<i>Ptychostomum capillare</i>		
		Species count (ni)	ni/N	(ni/N) <sup>2</sup>	Speciescount (ni)	ni/N	(ni/N) <sup>2</sup>
<b>Ascomycetes</b>							
1	<i>Scopulariopsis sp. 1</i>				1	2.2	0.04
2	<i>Sordaria fimicola</i>	1	2	0.04			
3	<i>Sporormiella intermedia</i>	1	2	0.04	1	2.2	0.04
4	<i>Cheatomium sp. 1</i>	3	6.1	0.12	1	2.2	0.04
<b>Coeleomycetes</b>							
5	<i>Collectotrichum sp. 1</i>	5	10	0.2	3	6.5	0.13
6	<i>Phoma sp. 1</i>	1	2	0.04			
<b>Hyphomycetes</b>							
7	<i>Alternaria alternata</i>	1	2	0.04	1	2.2	0.04
8	<i>Alternaria sp. 1</i>	1	2	0.04			
9	<i>Aspergillus flavus</i>	4	8.2	0.16	5	11	0.22
10	<i>Aspergillus niger</i>	3	6.1	0.12	2	4.3	0.09
11	<i>Aspergillus sp. 1</i>	1	2	0.04	2	4.3	0.09
12	<i>Aspergillus sp. 2</i>	1	2	0.04			
13	<i>Aspergillus sp. 3</i>				1	2.2	0.04
14	<i>Aureobasidium sp. 1</i>	1	2	0.04			
15	<i>Botrytis sp. 1</i>	1	2	0.04	1	2.2	0.04
16	<i>Camarosporium sp. 1</i>				1	2.2	0.04
17	<i>Cladosporium herbarum</i>				3	6.5	0.13
18	<i>Curvularia lunata</i>	1	2	0.04	1	2.2	0.04
19	<i>Curvularia sp. 1</i>	1	2	0.04	2	4.3	0.09
20	<i>Drechslera sp. 1</i>	1	2	0.04	1	2.2	0.04
21	<i>Fusarium oxysporum</i>	5	10	0.2	4	8.7	0.17
22	<i>Fusarium sp. 1</i>	1	2	0.04	2	4.3	0.09
23	<i>Nigrospora orzae</i>	1	2	0.04	2	4.3	0.09
24	<i>Penicillium sp. 1</i>	2	4.1	0.08	1	2.2	0.04
25	<i>Stachybotrys sp. 1</i>	1	2	0.04	1	2.2	0.04
26	<i>Trichoderma aureoviride</i>	3	6.1	0.12	2	4.3	0.09
<b>Sterile forms</b>							
27	Unknown fungi 1	2	4.1	0.08	2	4.3	0.09

28	Unknown fungi 2	1	2	0.04	2	4.3	0.09
<b>Yeast forms</b>							
29	Yeast form 1	2	4.1	0.08	1	2.2	0.04
30	Yeast form 2	1	2	0.04	1	2.2	0.04
<b>Zygomycetes</b>							
31	<i>Mortierella</i> sp. 1	1	2	0.04			
32	<i>Mucor hiemalis</i>	2	4.1	0.08	2	4.3	0.09
Total no. of colonies		49	100	2	46	100	2
Total no. of species		28			26		

There are 32 species and 49 individuals on *Polytrichum commune* and 46 individuals on *Ptychostomum capillare*, but are they equally diverse.

**Note** that Simpson’s Index is often expressed (1-D), so the final answers are 2 and 2. This makes intuitive sense: a higher D is more diverse – which is *Polytrichum* because it is less dominated by one species.

**Table 3** An endophytes fungus was isolated from DT-Bryophytes plants and calculates Shannon –Weiner Index and the Evenness Index for endophytes fungi

No. of Species (i)	Name of fungus	<i>Polytrichum commune</i>				<i>Ptychostomum capillare</i>			
		count (ni)	Pi	In(Pi)	Pi*In(Pi)	count (ni)	Pi	In(Pi)	Pi*In(Pi)
<b>Ascomycetes</b>									
1	<i>Scopulariopsis</i> sp. 1					1	2.17	0.777	1.688106
2	<i>Sordaria fimicola</i>	1	2.04	0.713	1.455816				
3	<i>Sporormiella intermedia</i>	1	2.04	0.713	1.455816	1	2.17	0.777	1.688106
4	<i>Cheatomium</i> sp. 1	3	6.12	1.812	11.09365	1	2.17	0.777	1.688106
<b>Coleleomycetes</b>									
5	<i>Collectotrichum</i> sp. 1	5	10.2	2.323	23.70192	3	6.52	1.875	12.22918
6	<i>Phoma</i> sp. 1	1	2.04	0.713	1.455816				
<b>Hyphomycetes</b>									
7	<i>Alternaria alternata</i>	1	2.04	0.713	1.455816	1	2.17	0.777	1.688106
8	<i>Alternaria</i> sp. 1	1	2.04	0.713	1.455816				
9	<i>Aspergillus flavus</i>	4	8.16	2.1	17.13995	5	10.9	2.386	25.93442
10	<i>Aspergillus niger</i>	3	6.12	1.812	11.09365	2	4.35	1.47	6.389896
11	<i>Aspergillus</i> sp. 1	1	2.04	0.713	1.455816	2	4.35	1.47	6.389896
12	<i>Aspergillus</i> sp. 2	1	2.04	0.713	1.455816				
13	<i>Aspergillus</i> sp. 3					1	2.17	0.777	1.688106
14	<i>Aureobasidium</i> sp. 1	1	2.04	0.713	1.455816				
15	<i>Botrytis</i> sp. 1	1	2.04	0.713	1.455816	1	2.17	0.777	1.688106
16	<i>Camarosporium</i> sp. 1					1	2.17	0.777	1.688106

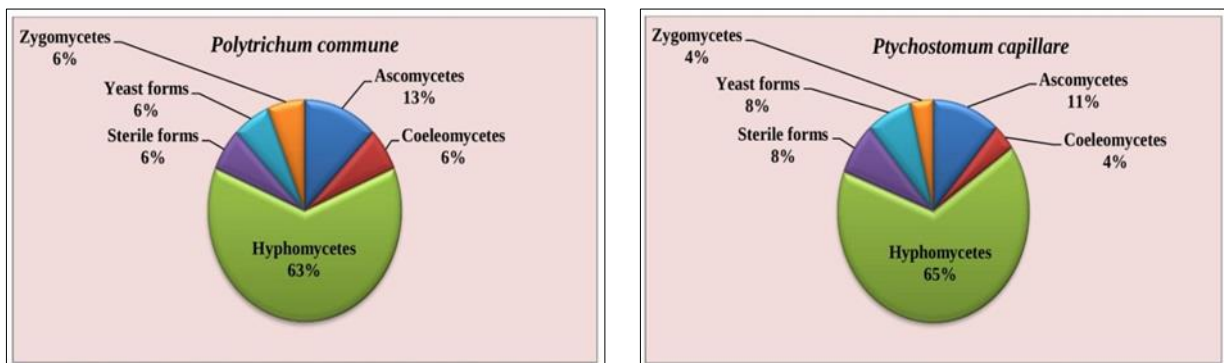
17	<i>Cladosporium herbarum</i>					3	6.52	1.875	12.22918
18	<i>Curvularia lunata</i>	1	2.04	0.713	1.455816	1	2.17	0.777	1.688106
19	<i>Curvularia</i> sp. 1	1	2.04	0.713	1.455816	2	4.35	1.47	6.389896
20	<i>Drechslera</i> sp. 1	1	2.04	0.713	1.455816	1	2.17	0.777	1.688106
21	<i>Fusarium oxysporum</i>	5	10.2	2.323	23.70192	4	8.7	2.163	18.80716
22	<i>Fusarium</i> sp. 1	1	2.04	0.713	1.455816	2	4.35	1.47	6.389896
23	<i>Nigrospora orzae</i>	1	2.04	0.713	1.455816	2	4.35	1.47	6.389896
24	<i>Penicillium</i> sp. 1	2	4.08	1.406	5.740804	1	2.17	0.777	1.688106
25	<i>Stachybotrys</i> sp. 1	1	2.04	0.713	1.455816	1	2.17	0.777	1.688106
26	<i>Trichoderma aureoviride</i>	3	6.12	1.812	11.09365	2	4.35	1.47	6.389896
<b>Sterile forms</b>									
27	Unknown fungi 1	2	4.08	1.406	5.740804	2	4.35	1.47	6.389896
28	Unknown fungi 2	1	2.04	0.713	1.455816	2	4.35	1.47	6.389896
<b>Yeast forms</b>									
29	Yeast form 1	2	4.08	1.406	5.740804	1	2.17	0.777	1.688106
30	Yeast form 2	1	2.04	0.713	1.455816	1	2.17	0.777	1.688106
<b>Zygomycetes</b>									
31	<i>Mortierella</i> sp. 1	1	2.04	0.713	1.455816				
32	<i>Mucor hiemalis</i>	2	4.08	1.406	5.740804	2	4.35	1.47	6.389896
Total no. of colonies		49	100	30.64	146.99	46	100	31.6	148.6
Total no. of species		28				26			

Again, according to the Shannon –Weiner Index, *Ptychostomum capillarie* is more diverse.

Evenness Index, From above we know that  $H_{max}$  is 148.6 (i.e. the highest of the Shannon –Weiner Index values).

The Evenness index for *Polytrichum commune* is  $J=H/H_{max} = 146.9/148.6 = 0.988$ .

The Evenness index for *Ptychostomum capillarie* is  $J=H/H_{max} = 148.6/148.6 = 1$ .



**Figure 3** Relative Percentage Occurrence (RPO) of endophytes belonging to different groups of fungi in two different hosts

In our study was based on isolated dried - up bryophytes plant fungal endophytes has been done location around open lands, wall during a few months of the winter season of the region. Thus, we found that the most widespread soil and

airborne fungi were also the almost all frequent namely, *Alternaria*, *Aspergillus*, *Cladosporium*, *Curvularia*, *Fusarium*, *Penicillium* were isolated (Table 2 and 3). This suggests that in cause of the above season of variation, these fungal species are ubiquitous in the Earth's atmosphere. *Aspergillus* and *Penicillium* spores have been shown to occur commonly in soil and air samples (Mitchell *et al.*, 2016; Umesha *et al.*, 2016). The Colonization Frequency (CF %) of the two bryophyte species by endophytic fungi were low frequency (below 50%) in the present study. Nevertheless, the endophytic fungi of *Colletotrichum* and *Phoma* species were very common isolate in this study, and they have been reported as the dominant fungal endophytes of various plant species from diverse environments (Suryanarayanan *et al.*, 2007). The results indicated that environmental condition is one of the most important factors affecting endophytes composition, especially the dominant endophytes. Hence, here isolated clusters can be seen as such. Some bryophytes species are amid the first to colonies open ground in very short period of life, which are lower in disturbed habitats because there is less shade.

Some authors have demonstrated that fungal endophytes community may be influenced by diverse biotic and abiotic factors, such as the type of plant tissues; a heterogeneous profile of microhabitats; and different substrates, climate and vegetation changes (Koide *et al.*, 2017; Shen *et al.*, 2007). Soil, airborne fungal spore concentrations and their diversity vary with the season of the year, geographical region, soil, air, meteorological parameters, presence of local resources, and vegetation.

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

The results show that the colonization frequency of fungal diversity analysis of two samples of bryophyte species, that there is no differences fungal diversity and that lower frequencies were observed.

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## 5. Conclusion

In this study, bryophyte plant species of host plant were found to be least colonized by endophytes. We assumed that they grow only in wet conditions and short periods of life; the distribution of fungi varied with that ecological condition. Even so the endophytic fungi may be accumulated in least amount of DT-Bryophyte plants. These hosts were found true fungi and much diversity of endophytes. Whatever, these endophyte fungi were isolated in the bryophyte plant tissues are usual present. Endophytic fungi may be rich sources of bioactive natural products that can be used to make someone value of pharmaceutical, medicinal, agriculture and industries.

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## Compliance with ethical standards

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### *Disclosure of conflict of interest*

No conflict of interest.

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