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Endophytic fungal communities in the leaves of pteridophyte plants: Diversity and distribution patterns

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Abstract

Two species of pteridophytes, viz., *Adiantum capillus* and *Nephrolepis cordifolia* pteridophyte plant species of the tropical herb, studied by endophytic fungi were collected from Coonoor, a city in (Ooty) Nilgiris district in the south India state of Tamil Nadu. They were screened for the presence of fungal endophytes from the plant parts. A total of 17 species of fungal endophytes were recorded. *Colletotrichum gloeosporioides*, *Fusarium oxysporium, Curvularia lunata* and *Phomopsis* sp. 1 occurred in all the pteridophyte species screened. The maximum number of endophytic fungal species was observed in *Adiantum capillus*, while *Nephrolepis cordifolia* ranked first in the number of isolates. The species composition, endophyte assemblage and pattern of colonisation among these vascular cryptogams were similar to those of the most studied phanerogams.

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Introduction

Endophytes have been known for a long time. The term endophyte was actually coined in 1884 by Heinrich Anton de Bary, who recognised that fungi and bacteria could dwell within plant tissues without causing any apparent harm. 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 *et al.,* 2022). These are ubiquitous microorganisms, reported in almost all the vascular plants and bryophytes studied to date (Hardoim *et al.,* 2015; Venkatesan and Mahalakshmi, 2022). Endophytes, organisms that are present in the internal parts of living plant tissues without causing any disease symptoms, are assumed to be present in all the plant species surveyed so far (Stone *et al.*, 2000). Fungal endophytes have been reported from mosses (Petrini, 1986), gymnosperms (Carroll and Carroll, 1978; Petrini and Carroll, 1981), and angiosperms (Arnold *et al.*, 2001; Suryanarayanan *et al.*, 2011). Studies in the tropics with regard to the taxonomy and ecology of fungal endophytes have gained interest in the recent past (Mishra *et al.*, 2012). This is due to the fact that the internal tissues of plants are relatively unexplored for fungi, and this niche of microbes could be sourced for novel fungi and potential compounds as well (Jacob and Bhat, 2000).

The fungal endophytes are being reported from two pteridophyte species, viz., *Adiantum capillus* and *Nephrolepis cordifolia*. Pteridophytes and their associates are primitive vascular plants that did not ever bloom and are referred to as reptiles. They are frequently discovered across wet and cold environments and the tropics are where they are most widely varied (Giri *et al.*, 2021). Pteridophytes are regarded as significant due to their evolutionary relevance, they are a notable ancient group of species with a significant number of relics and endemic ones (Patil *et al.*, 2016). The pteridophytic flora of Nepal is extremely diverse due to the country's significant altitudinal changes, which vary from humid tropical to mountain ranges, as well as the existence of a wide range of climatic conditions and soil types (Gurung, 1992). In contrast to seeded plants, they can be found in ecosystems from the tropics to the poles and reproduce by means of tiny spores (Moran, 2004). After Linnaeus published "Species Plantarum" in 1753, with 140 genera and 182 species of pteridophytes, the history of the study of pteridophytes began.

Research on the relationships between species and elevation has frequently focused on pteridophytes, with the highest diversity seen in tropical and subtropical highlands (Bhattrai *et al.*, 2004). Several studies of pteridophytes have already been conducted in various parts of Nepal, but comparative research on the diversity of pteridophytes in various aspects of Nepal is still insufficient. Few works done in Palpa district in the past include floristic research done by (Mahato, 2014) and (Shrestha *et al.*, 2018). Pteridophytes are higher cryptogams with a well-developed vascular system and there are around 13,271 live species listed in a global checklist of ferns and lycophytes (Hassler, 2018).

A wide range of plant species have been reported to be host to endophytic fungi (Rajamanikyam *et al.*, 2017; Souza and Santos, 2017; Toghueo and Boyom, 2019). These bacteria can be taken out of the inside of plants or separated from plant tissues that have been damaged on the surface (Hallmann *et al.*, 1997). Endophytic fungi are abundant in bioactive substances with functions essential to host plant health and resilience to stressful conditions as well as the survival and upkeep of endophytes in plants. These substances include hormones (such as auxin, gibberellins), cellulases, proteases and chitinases), antimicrobial agents (Eid *et al.*, 2019).

Materials and methods

Study area

Adiantum capillus and Nephrolepis cordifolia pteridophyte plant species of the tropical herb, studied by endophytic fungi are collected from Coonoor, a city in (Ooty Nilgiris district in the South India state of Tamil Nadu (Fig. 1). Situated in the Nilgiri District, which lies

in the southwest corner of Tamil Nadu in India, the little town of Coonoor can be described as the gateway to the much-famous tourist destination, Ooty. This city, Coonoor, is located at 11.345°N, 76.795°E. It has an average elevation of 1,650 metres (5413 feet) above sea level. It features a subtropical highland climate due to its high altitude. Hence, located at an elevation of 1861.07 metres above sea level, Coonoor has a tropical wet and dry, or savanna, climate. The district's yearly temperature is 25.74°C (78.33°F), which is -0.23% lower than India's averages. Coonoor typically receives about 225.71 millimetres (8.89 inches) of precipitation and has 229.64 rainy days (62.92% of the time) annually. *Adiantum capillus and Nephrolepis cordifolia* in plants were collected from individuals of each host species, and their fungal endophytes were isolated during 2022–2024 about once every three months throughout the year. For these studies, medicinally important plant species in *Adiantum capillus and Nephrolepis cordifolia* are members of the family (Pteridaceae and Nephrolepidaceae) widely distributed around hill stations in the world. Each plant sample was brought to the laboratory in sterile polythene bags and processed within 24 hours of collection.

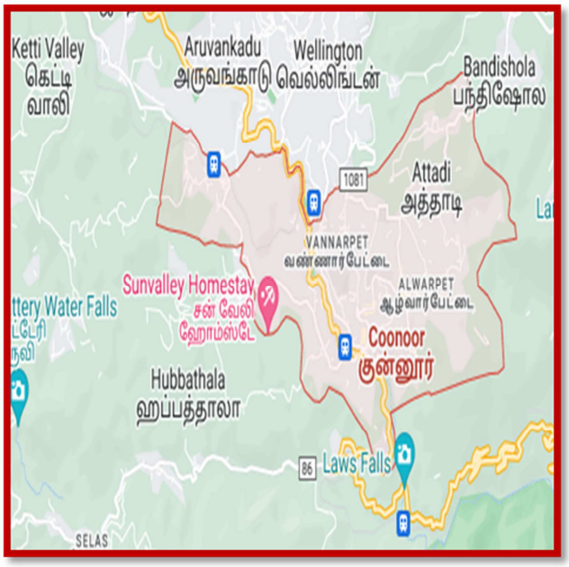

Fig. 1. Google map of Coonoor

Sterilisation and culture protocols

The host plant studied was collected from Coonoor (Ooty), Tamil Nadu, and South India. These stages of plant leaves were collected for the investigation.

In our studies, we have used culture-dependent approaches based on media culture. Leaf samples were collected from healthy plants. In these plants, leaves were randomly collected: two hundred leaves were collected from a few plants, and their one hundred and fifty tissue segments were cut from two hundred leaves. However, sterilisation techniques were followed before cutting these segments. The plant leaves are washed thoroughly with running water, and then the leaves are sterilised as follows: After the surface sterilisation, the leaves were cut approximately into 0.5 cm (segments) of each leaf. The samples were washed in running water, dipped in 70% ethanol for 60 seconds, immersed in 2.5% NaoC1 for 90 seconds, and then washed in sterile water for 10 seconds (Suryanarayanan *et al.*, 1998) or three times. The sterilised samples were placed on the PDA medium amended with antibiotics contained in Petri dishes. The Petri dish was sealed with Parafilm™ and incubated in a light chamber at 26˚C for 7 to 21 days (Bills and Polishook, 1992; Suryanarayanan *et al.*, 1998). The light regimen given was 12 hours of light followed by 12 hours of darkness. Fungi that grew from the segments were periodically observed, and the endophytes were identified.

Morphological identification of isolated endophytic fungi

Preliminary identification was done by studying the fungi's cultural characteristics, such as colony growth, colour, shape, etc. The morphological characters were examined by growing cultures on PDA plates for 21 days. Microscopic observations are Conidiophores, conidia, and mycelia characters were carried out by preparing slides stained with cotton blue and Congo red and observed under the compound microscope (Ellis, 1971; Subramanian, 1971; Barnett and Hunter, 1972; Sutton *et al.*, 1981).

Statistical analysis

Colonisation frequency

The number of segment colonies and the number of totals are the number of segments colonised by each endophyte and the total number of segments observed respectively, according to (Hata and Futai, 1995).

Colonisation frequency (CF %) = {(Number of colonies)/(Number of totals)}×100

Relative percentage 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 colonisation frequency of one group)/(Total colonisation frequency for all the groups of fungi)}×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

Pteridaceae family member Adiantum capillus-veneris Linn

The tufted fern known as maidenhair fern, *Adiantum capillus*-veneris Linn., is a member of the pteridaceae family. According to Gruenwald *et al.* (2008), the herb is commonly grown in warm tropical areas with high moisture content. Plants range in height from 15 to 30 cm, forming large colonies with fronds that emerge in clusters from creeping rhizomes; sori are present in both small and large fronds, sori are present at the top of the lobe of the pinnae, sori are white to brown. Fronds are glossy black, leaves tripinnate and pinnae compound with small rounded segments narrowing symmetrically to connate bases (Fig. 2).

Ecology: found in tropical zones, mixed forests, adhered to stones, damp areas on land and around streams.

Fig. 2. *Adiantum capillus* plant

Fig. 3. *Nephrolepis cordifolia* plant

Nephrolepis cordifolia (L.) C. Presl. (Nephrolepidaceae family)

The fern *Nephrolepis cordifolia* is indigenous to the tropics worldwide, which includes northeastern Australia and Asia. It can be identified by a variety of common names, such as herringbone fern, fishbone fern, upright sword fern, narrow sword fern, and tuber ladder fern. *Nephrolepis exaltata*, a related fern, is comparable to it. Rhizomes are upright plants that produce long, creeping runners that eventually develop into tubers and adventitious buds. The rhizome is covered in dense, pale brown scales, and the stripe is typically short, measuring up to 15 cm in

length. The central pinnae are oblong to lanceolate-oblong, straight to slightly falcate, and the base is auriculate-cordate. The AChls have an acroscopic overlap, with margins that range from entire to serrulate to smoothly crenate, and apex that is bluntly rounded. The indusial reniform to run rate or deltaterounded; Sori along the vines in two rows (Fig. 3).

Ecology: reported from moist paces, terrestrial or epiphytic plants grow.

Results and discussion

Adiantum capillus-veneris and *Nephrolepis cordifolia* plant belonging to pteridophyte in the (Pteridaceae *and* Nephrolepidaceae*)* (Fig. 2&3) families was studied for their foliar endophyte assemblages during 2022–2024 in different seasons. This line of inquiry was aimed at getting comprehensive knowledge of the endophyte's status in tropical herb plants. Isolation of fungal endophytes from the leaves of the *Adiantum capillus-veneris* and *Nephrolepis cordifolia* plants during the seasons, One hundred and fifty leaf segments of *Adiantum capillus-veneris* and *Nephrolepis cordifolia* were screened for the presence of fungal endophytes. The segments were cut from the entire portion of the basal leaves. Surface sterilised using ethanol and sodium hypochlorite (Sterilisation and Culture Protocols) and screed as mentioned under Materials and Methods. A total of 16 fungal species and 94 isolates were obtained from *Adiantum capillus,* and 7 fungal species and 102 isolates were obtained from *Nephrolepis cordifolia* (Fig. 4). *Colletotrichum gloeosporioides*, *Fusarium oxysporium, Curvularia* lunata and *Phomopsis* sp. 1 showed the highest colonisation frequency from *Adiantum capillus*. A total of 23 fungal species and 266 isolates were obtained during the dry season, and 7 fungal species and 102 isolates were obtained from *Nephrolepis cordifolia*. *Colletotrichum gloeosporioides* and *Phomopsis* sp. 1 showed the highest colonisation frequency during *Nephrolepis cordifolia*. Several species of endophytic fungi were investigated including *Aspergillus niger* and *Penicillium*, where the isolated endophytic flora of the *Aspergillus* genus was the most abundant (Subhashini, 2018).

Table 1. Fungal endophytes isolated from pteridophytes in the leaves of *Adiantum capillus* and *Nephrolepis cordifolia*

SL	Fungi	Hosts			
		Adiantum capillus - veneris		*Nephrolepis cordifolia*	
	Ascomycetes	Colonies	CF%	Colonies	CF%
1	*Lasiobolus papillatus*	1	0.76		
	Coelomycetes				
2	*Colletotrichum gleosporiodis*	26	20	47	36.1
3	*Phoma* sp. 1	2	1.5		
4	*Phomopsis* sp. 1	13	10	36	27.6
5	*Phyllosticta* sp. 1	1	0.76		
	Hyphomycetes				
6	*Alternaria alternata*	6	4.61	1	0.76
7	*Aspergillus flavipes*	1	0.76		
8	*A. niger*	1	0.76	3	2.3
9	*A. ochraceus*		0.76		
10	*A. flavus*	2	1.5		
11	*Curvularia lunata*	14	10.7		
12	*Fusarium culmorum*	1	0.76		
13	*Fusarium oxysporum*	21	16.1	9	6.9
14	*Penicillium* sp. 1	1	1.5		
	Sterile form				
15	Sterile form 1	1	0.76	5	3.8
	Unidentified				
16	Unknown sp. 1	2	1.5	1	0.76
17	Unknown sp. 2	1	0.76		0.76
	Total no. of species	94		102	
	Total no. of Colonies Frequency		73.49		78.98

Table 2. Similarity coefficients between the Number of species, total number of colonization frequency (CF %) of the most dominant endophyte in the host of *Adiantum capillus* and *Nephrolepis cordifolia*, Relative percentage of occurrence (RPO), species richness (R1), species evenness (E5) and species diversity (Fisher's α) of the endophyte assemblages of the pteridophyte plants

SL	Statistical analysis	*Adiantum capillus*	*Nephrolepis cordifolia*
1.	Species	16	7
2.	Individuals	94	102
3.	Total no. of segments	150	150
4.	Total colonization frequency (CF %)	73.49	78.98
5.	R1(Margalef's)	3.30	1.30
6.	E5 (Hill's Ratio)	0.74	0.74
7.	Fisher's alpha	5.54	1.70
8.	Relative percentage of occurrence (RPO) of each group of fungal species		
8.1	Ascomycetes (1 species/1 genera)	1.06	0.0
8.2	Coelomycetes (4 species/4 genera)	4.25	1.96
8.3	Hyphomycetes (9species/4 genera)	8.51	2.94
8.4	Sterile forms (1 species/- genera)	1.06	0.98
8.5	Unknown fungi (1 species/- genera)	2.12	1.96

A total of 17 endophytic isolates were collected from 300 segments both plants. Totally 17 endophytic isolates were categorized into 13 genera, comprising 1 Ascomycetes genera *Lasiobolus papillatus,* 4 Coelomycetes genera *Colletotrichum gloeosporioides*, *Phoma* sp, *Phomopsis* sp.1 and *Phyllosticta capitalensis*, 5 Hyphomycetes genera *Alternaria alternata*., *Aspergillus* spp., *Curvularia lunata*., *Fusarium* spp., *Penicillium* spp 1 sterile form and 2 Unidentified. All the leaf parts of plant tissues were found to harbour various endophytic fungal species with different colonization frequencies (CF %) and statistics analysis (PCO, CF%, E5, H1) (Table 1&2).

The present work is entirely new, and many descriptions and a few illustrations appear for the first time. The arrangement and numbering of genera are identical in numerous earlier works. For the dry season, we recorded 93 endophyte isolates from *Adiantum capillus* and 102 endophyte isolates from *Nephrolepis cordifolia* from 150 leaf segments (each host). In most cases, each tissue segment was infected by more than one fungal species (multiple infections), substantiating the view that tropical plants have high rates of endophyte colonisation (Suryanarayanan *et al.,* 2002). We found that there was no significant difference in endophyte colonisation between individuals. This was irrespective of the hosts. The dominant fungi were the same for both plants. *Colletotrichum gloeosporioides* and *Phomopsis* sp.1 have shown that more endophyte isolates occur in leaves screened in the *Adiantum capillus* and *Nephrolepis cordifolia plants*. In addition, in both plants, dominant endophyte species had a higher CF% in *Nephrolepis cordifolia*, thus bringing down the evenness index of the endophyte assemblages. In a comparison of the endophyte assemblages of the host, the endophyte diversity from *Nephrolepis cordifolia* was higher for *Adiantum capillus* due to higher mean annual rainfall. However, the diversity index was almost identical for both plants.

However, other studies indicate that the dry tropical forests do not support very high endophyte diversity (Suryanarayanan *et al.*, 2002; 2003), and the period is relatively dry. Earlier studies involving a few individual plant species revealed that precipitation and endophyte colonisation of leaf tissues are positively correlated (Suryanarayanan *et al.*, 1998). Earlier studies reveal that ascomycetes, colomycetes, hyphomycetes and sterile forms invariably constitute the endophyte assemblages of trees; basidiomycetes and oomycetes are rarely encountered (Petrini, 1986). In dry tropical forests, rainfall is seasonal and the rest is a relative percentage of occurrences (RPO) (Fig. 2). This also showed that the foliar endophytes of the *Nephrolepis cordifolia* were different from those of the *Adiantum capillus*. The present work stated the presence of major foliar endophytic fungi mainly belonging to the genera *Fusarium* spp.

Colletotrichum gloeosporioides, *Phomopsis* sp. 1 and *Phyllosticta capitalensis* were the second-most dominant endophytes in this plant. Several previous studies have reported the presence of *Colletotrichum gloeosporioides*, *Phomopsis* sp. 1 and *Phyllosticta capitalensis*. Fungal genera are a dominant group of endophytes residing in association with different medicinal plants (PrabhaToppo *et al.*, 2024). While the number of isolated species described in each plant fungus is compared to those recorded in the species richness (Table 1&2). Endophytic fungal genera such as *Colletotrichum gloeosporioides*, *Phoma* sp. 1, *Phomopsis* sp. 1 and *Phyllosticta capitalensis* had higher abundance rates and frequencies in both plants. Hyphomycetes have more fungal diversity in both plants.

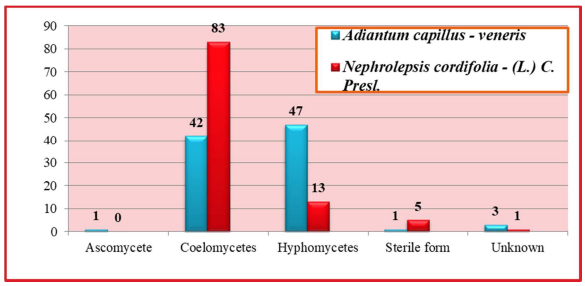

Fig. 4. Number of endophyte isolates recovered from *Adiantum capillus* and *Nephrolepis cordifoli* leaves

Endophytes can alleviate abiotic and biotic stressors such as drought, salinity, heavy metals, and other toxic compounds introduced by the environment, floods, extreme temperatures, predators, and pathogens (Clay *et al.*, 1999; Su *et al.*, 2021). Soil and 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.

Conclusion

The practical method to estimate the global diversity of species-rich genera such as leaf fungi is investigation from *Adiantum capillus* and *Nephrolepis cordifolia* sampling. The most promising several are endophytic fungi which are becoming an important source of bioactive chemicals for many applications in industry, agriculture and medicine.

In the current work, fungal endophytes were isolated, identified, and characterised using morphological, leaf, and stem explants of the pteridophytes plants *Adiantum capillus* and *Nephrolepis cordifolia*. In the past few decades, many researchers have mainly focused on the investigation of fungal endophytes for diversity and their relationships with their host plants. Because of this, we investigated the fungus in the plant tissues of a few significant and widely utilised plants from *Adiantum capillus, Nephrolepis cordifolia*, pteridophyte plant species of the tropical herb studied by endophytic fungi. This herbal plant was collected from Coonoor, a city in the Ooty Nilgiris district in the South India state of Tamil Nadu, and found a huge diversity of fungal species.

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