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RESEARCH ARTICLE

Seasonal diversity and spaciotemporal distribution of fungal endophytes associated with the medicinal plant *Coleus forskohlii* Briq.

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Abstract

Fungi that colonize the healthy tissues of the plants without showing any disease symptoms in the host plants are termed as fungal endophytes. The presence of fungal endophytes provides a positive effect on the host's growth and development and also triggers the production of some essential bioactive compounds in the host. This study was undertaken to isolate, identify and understand the spaciotemporal distribution and seasonal diversity of fungal endophytes associated with the leaf, stem and root of *Coleus forskohlii,* an important and endangered medicinal plant. Sampling was done for a period of 12 months between May 2020–April 2021. A total of 950 fungal endophytes were isolated from a total of 1680 tissues of the leaf, stem and root of *C. forskohlii.* The fungi were identified based on their morphological features and some of them were identified by molecular identification by 18S rRNA sequencing. The endophytic isolates belonged to 10 different orders belonging to 3 different classes-Sordariomycetes (Hypocreales, Xylariales, Microascales, Trichosphaeriales, Glomerellales and Sordariales), Dothiomycetes (Pleosporales, Capnodiales, Botryosphaeriales) and Eurotiomyctes (Eurotiales). About 81.26% of the isolates belonged to Ascomycota and 2.63% of the isolates belonged to Mucoromycota. *Chaetomium globosum, Collariella bostrychodes, C. robusta, Colletotrichum gloeosporioides, Fusarium chlamydosporum, Sterile hyaline mycelia, Aspergillus niger, Xylaria curta, X. grammica, Mucor circinelloides* and *Trichoderma harizianum* were the frequently isolated species of fungi. *C. globosum, C. bostrychodes*, *C. gloeosporioides,* sterile hyaline mycelia and *X. curta* were found distributed in all the tissues of the plant*. C. forskohlii* has thus revealed a rich diversity of fungal endophytes that could be isolated and cultured to yield some pharmacologically important bioactive compounds.

Keywords

Coleus forskohlii; fungal endophytes; seasonal diversity; spaciotemporal distribution; Ascomycota

Introduction

The Royal Botanic Gardens, Kew, estimates that nearly 390,900 plant species exist on earth, and studies have shown that almost all plants are associated with endophytes (1–4). Endophytic fungi are important group of micro-organisms that colonize the healthy tissues of the plants without instigating any disease symptoms into them. The term "Endophytae" was given by Heinrich Friedrich Link in 1809, who considered endophytes as fungi that are somewhat parasitic. It was Anton de Bary in 1866 who first gave the term "Endophytes" for those fungi residing inside the plant tissues. Fossil evidences of many plants indicate that plants have been associated with fungal endophytes for >400 million years (5–7). Plants have posed new niches

to endophytic fungi by establishing a beneficial symbiotic relation with them. Endophytic fungi colonize almost all parts of the plants including the leaves, stems, roots, flowers and seeds (8–10). The symbiotic association, organ preference and ecological functioning of fungal endophytes with their hosts could be vastly capricious. As much as endophytic fungi depend on the host for colonization and survival, the host plants also depend on the fungal endophytes for their growth and development, to solubilize minerals, to synthesis secondary metabolites, to synthesis phytohormones, to gain resistance against many pathogens and to withstand the environmental conditions (11, 12). Some endophytes are able to produce similar bioactive compounds as the host plant, while others are able to produce bioactive compounds that are not naturally synthesized by the host. These bioactive compounds could exhibit a wide range of applications in the field of agriculture and medicine (13).

Although fungal endophytes are reported from various plants distributed in different ecological habitats, their choice of host varies with the variation in climatic conditions and geographical regions. Seasonal changes have also known to affect the potential of the endophytic community harbouring the different tissues of the host plant. Variations in the climatic conditions such as temperature and atmospheric humidity is known to influence the endophytic community within the host tissues, because these factors affect the germination and growth of the fungal spore on the host. Only conducive environments can favour the above process. Alongside the external conditions, endophytic fungi also show tissue preference. They are sometimes explicitly found and isolated from specific tissues such as leaves, bark, root, etc., implying to their spaciotemporal distribution within the host (14).

Owing to the unexplored roles and bioactive potential of the endophytic fungal community associated with the tissues of *Coleus forskohlii,* the present study was conducted to decipher their diversity and tissue preference over the different seasons. This could pave way for isolating some useful bioactive compounds by fermenting these endophytic fungi.

C. forskohlii, a member of Lamiaceae is a well-known ancient perennial aromatic medicinal plant originating in the Indian sub-continent which occupies an important position in traditional medicine in countries like India, Kenya, Congo, Brazil, Gabon, Somalia, Rwanda and China (15). The wild variety of *C. forskohlii* is extensively cultivated for its fasciculate root tubers as they yield essential bioactive compounds of pharmacological repute (16). Nearly 68 pharmacologically important compounds are isolated from different parts of *C. forskohlii,* some of the most important ones are forskolin, rosmarinic acid, barbatusin, cyclobutatusin and essential oils (17). Harvesting the plant to obtain bioactive compounds is a plant devastating process and hence an alternative eco-friendly method needs to be employed to isolate the essential compounds. One such method is to isolate endophytes from the plant to obtain the necessary compounds.

Isolating bioactive compounds from the endophytic fungi associated with *C. forskohlii* is essential and hence as a first step. The present study was conducted mainly to seasonally isolate the fungal endophytes, understand their spaciotemporal distribution in different tissues of the plant and identify them.

Materials and Methods

Sampling site for collecting plant material

In order to isolate endophytic fungi, the selection of the host plant could be based on the following strategies: plants which grow in unusual environmental conditions, plants growing endemically in the areas hosting rich biodiversity or plants having a history of being used in folk medicine. *Coleus forskohlii* was chosen because of its medicinal value and ethnobotanical use. Based on the unreported studies that were conducted on the diversity of fungi from the soil of St. Joseph's University campus, this location was chosen hypothesizing that we would obtain a rich diversity of fungal endophytes from the host plant.

Leaves, stem and root of *C. forskohlii* were collected from the medicinal plant garden of St. Joseph's University, Bengaluru located in the altitude of 3020 ft/900 m above sea level, longitude: 77°35'49.56" E and latitude: 12° 57'45.72" N, Karnataka, India. The average maximum and minimum temperature and average rainfall during different seasons between May 2020 to April 2021 was 33.07°C and 20.32°C and 57.7 mm rainfall during summer (February to May), 28.4°C and 20.52°C and 173.07 mm rainfall during monsoon (June to October) and 27.6°C and 17.93°C and 33.27 mm rainfall during winter (November to January). Voucher specimen of *C. forskohlii* was submitted to Foundation for Revitalisation of Local Health Traditions for authentication and identification bearing the voucher number FRLH Coll. No. 124372. Healthy and mature plants of *C. forskohlii* were harvested every month, for a period of twelve months, between May 2020–April 2021 to obtain the leaves, stem and roots. The collected plant material was transferred to an aseptic plastic bag, taken to the laboratory, and immediately subjected to surface sterilization.

Isolation and identification of fungal endophytes

The plant parts were segregated into leaves, stem and roots and were washed thoroughly under running tap water for 10–15 minutes to get rid of the debris and rinsed with sterile distilled water to wash off the epiphytic microbes. Surface sterilization was done following the methodology of Suryanarayanan *et al.* and Srivastava and Anandrao (18, 19). The standardized surface sterilization protocol is mentioned in Table 1.

The surface sterilized plant materials were dry blotted using a sterile blotting paper. The leaf segments were cut to a size of approximately 5 × 5 mm, the stem was cut to small cylinders of size 6 × 6 mm (Fig. 1) followed by a longitudinal cut to obtain two halves and the roots were cut to small cylinders of size 4 × 4 mm using a flame sterilized scalpel.

Table 1. Standardized protocol for surface sterilization of *C. forskohlii.*

Plant part	Root	Stem	Leaf
70% ethanol	2 min.	30s	30s
Sterile distilled water	Rinse in sterile distilled water		
4% sodium hypo-chlorite	1 min.	10s	30s
Sterile distilled water	Rinse in sterile distilled water		
70% ethanol	30s	10s	10s
Sterile distilled water	Rinse in sterile distilled water (twice)		

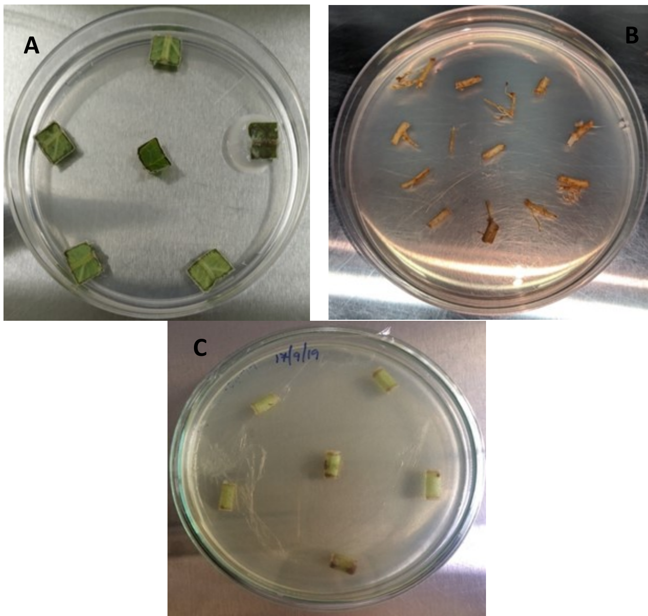

Fig. 1. Surface sterilized leaf (A), root (B) & stem bits (C) of *C. forskohlii.*

About 35 young leaf segments, 35 old leaf segments, 20 stem cylinders (40 halves) and 30 root cylinders were taken from the plant every month for isolation of endophytic fungi. In total, around 420 young leaf segments, 420 old leaf segments, 240 stem cylinders (480 halves) and around 360 root cylinders were placed on PDA (Potato Dextrose Agar) medium supplemented with chloramphenicol (200 mg/L) in order to prevent the growth of bacteria. The plates were incubated at natural conditions of light and darkness, and were regularly monitored for endophytic fungal growth, the emerging mycelia were sub-cultured on the PDA media for further studies.

The fungal endophytes were then identified based on their morphological features like colony characters, mycelia, structure and arrangement of conidia, and other reproductive structures if any, by using standard manuals and recent research articles (20–29). For microscopic studies, a drop of lactophenol cotton blue stain was added on a clean glass slide, the fungal hyphae with/without reproductive structures were taken on the glass slide containing the stain, a cover slip was placed on the mycelium and the microscopic features were carefully studied. The cultures which failed to sporulate were labelled as sterile mycelia. The fungal cultures were stained using lactophenol cotton blue and studied under a binocular microscope.

Some common fungi that were isolated among these, were chosen for molecular identification by 18S rRNA sequencing-the DNA was extracted by CTAB (cetyltrimethylammonium bromide) method. About 1 g of the fresh fungal mycelium was taken from the pure cultures grown on PDA media. The fungal mycelium was crushed in a chilled pestle and mortar using the CTAB extraction buffer, the extract was transferred to a tube and the volume was made up to 1 mL using the buffer. DNAse-free RNAse A (10 mg/mL) was then added and the tubes were incubated for 15 minutes at 37°C then for 30 minutes at 65°C and inverted regularly. 1 mL of phenol:chloroform:isoamyalcohol (25:24:1) was added to the tubes and centrifuged at 10,000 rpm for 10 minutes. The upper layer containing the DNA was transferred to a 2 mL Eppendorf tube containing 2 mL of chloroform:isoamyalcohol (24:1), and then centrifuged at 10,000 rpm for 10 minutes. The upper aqueous layer containing the DNA was then transferred to a fresh Eppendorf tube, equal volumes of 100% isopropyl alcohol and one-tenth 3 M sodium acetate was added to precipitate the DNA. The tubes were then incubated for 30 minutes at room temperature followed by centrifugation for 10 minutes at 10,000 rpm. The DNA pellet was washed by subjecting to centrifugation at 5,000 rpm for 5 minutes using 70% ethanol, air dried and suspended in Tris-EDTA buffer. The DNA was then amplified by Polymerase Chain Reaction (PCR), followed by subjecting the DNA of the PCR product to gel electrophoresis to check its purity (30).

The DNA was sequenced using Sanger's dideoxy method, and lastly the forward and reverse sequences were subjected to NCBI BLAST (National Centre for Biotechnology Information Basic Local Alignment Search Tool) for identifying the fungi (31). The identified sequences were submitted to GenBank in fast-all format and accession numbers were obtained for the same. All isolates were identified and preserved in storage vials in duplicates with 15% glycerol and sterile distilled water for future use.

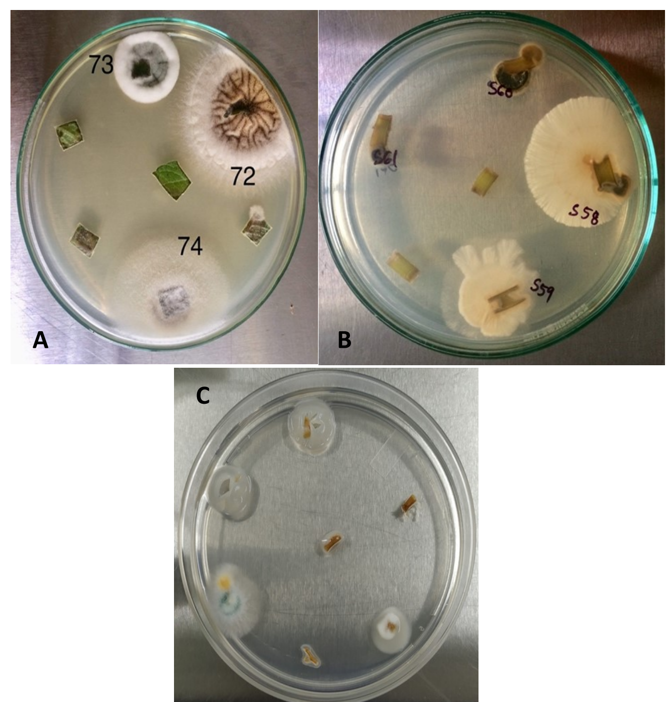

Fig. 2. Endophytic fungi emerging from leaf (A), stem (B) & root bits (C) of *C. forskohlii.*

Statistical analysis

To understand the seasonal diversity of endophytic fungi associated with *C. forskohlii*, the endophyte isolation rate, colonization frequency, relative percentage occurrence and relative species frequency were calculated as per the formulae mentioned below. Shannon-Wiener index, Simpson's Diversity index, Menhinick's index of Evenness, Simpson's Dominance index and Margalef's Richness index were calculated using the formulae below.

Colonization frequency (CF)

The CF% of the endophytic fungi was calculated using the formula given by Hata and Futai (32).

$$CF\ (\%) = \frac{\text{Number of segments colonized by each endophyte}}{\text{Total number of segments examined}} \times 100 \quad (\text{Eqn. 1})$$

Endophytic Isolation Rate (EIR)

The EIR was calculated using the below mentioned formula (33).

$$EIR = \frac{\text{Number of isolates obtained from plant segments}}{\text{Total number of segments incubated}} \quad (\text{Eqn. 2})$$

Relative Percentage Occurrence (RPO)

The RPO of the various groups of fungi was calculated using the below formula (34).

$$RPO\ (\%) = \frac{\text{Number of segments colonized by a group of fungi}}{\text{Total number of segments colonized by all the groups of fungi}} \times 100 \quad (\text{Eqn. 3})$$

Relative species frequency (RF)

Relative species frequency of one species in comparison with the other isolated species was calculated using the below mentioned formula (35).

$$RF = \frac{\text{Number of isolates of one species}}{\text{Total number of isolates}} \times 100 \quad (\text{Eqn. 4})$$

Fungal diversity index

Simpson's Diversity index: Simpson's diversity index was calculated by using the formula:

$D=\sum_i n_i(n_i-1)/N(N-1)$ (Eqn. 5)

where n_i = number of isolates of each species, while N=total number of isolates of all species.

Simpson's Dominance index: It was calculated by using the formula:

$1- (\sum_i n_i(n_i-1)/N(N-1))$ (Eqn. 6)

where n_i = number of individuals of each species, while N=total number of individuals of all species. Simpson's dominance index is 1-D.

Shannon-Wiener index: Shannon-Wiener index (H) was calculated by using the formula:

$\sum_i (n_i/N \,.\, \ln (n_i/N))$ (Eqn. 7)

where ln = natural logarithm.

Menhinick's index of Evenness: Species evenness was calculated using the formula:

$s/\sqrt{\sum i\, ni}$ (Eqn. 8)

where S = total number of endophytic species isolated and n_i = sum total of isolates of all the endophytic species.

Margalef's Richness index: Species richness was calculated using the formula:

$S-1/\ln N$ (Eqn. 9)

where S = number of species, ln is natural logarithm, while N = total number of individuals (35).

Results

Isolation and identification of fungal endophytes

About 950 endophytic fungi were isolated from 1,680 plant tissue segments of *Coleus forskohlii* during 2020–2021. Of these, 313, 296, 170 and 171 fungal endophytes were isolated from the old leaves, young leaves, stem (Fig. 2) and roots, respectively. A total of 21 genera of fungal endophytes were obtained. Morphological identification revealed about 55 species of endophytic fungi which are as follows: *Alternaria alternata, Amesia cymbiformis, Arcopilus flavigenus, Aspergillus flavus, A. fumigatus, A. nidulans, A. niger* (Fig. 4), *A. ochraceus, A. terreus, Chaetomium angustispirale* (Fig. 5), *C. coarctatum, C. cochliodes, C. globosum, C. globosum sensu stricto, C. indicum, C. madrasense, Chaetomium* sp., *C. spinosum, C. subspirale, C. tectifimeti, Cladosporium cladosporioides, Cladosporium* sp., *C. sphaerospermum, Collariella bostrychodes, C. robusta* (Fig. 6), *Colletotrichum endophytica, C. falcatum, C. fruticola, C. gloeosporioides, C. siamense, Colletotrichum* sp., *C. tropicale, C. truncatum, C. asianum, Epicoccum sp., Fusarium chlamydosporum, F. equiseti, F. solani, Fusarium* sp., *F. verticillioides, Lasiodiplodia theobromae, Mucor circinelloides, Nigrospora oryzae, Nigrospora* sp., *N. sphaerica, Paecilomyces carneus, Paecilomyces* sp., *Penicillium chrysogenum, P. paradoxum, Penicillium* sp., *Phoma herbarum, Phoma* sp., *Psuedotorula* sp., *Sarocladium strictum, Scopulariopsis brevicaulis, Scopulariopsis* sp., *Trichoderma harizianum, Trichoderma* sp., *Xylaria acuta, X. apoda, X. curta, X. feejeensis and X. grammica* and *Xylaria* sp., (20–29) and the mycelia which did not sporulate were called sterile mycelia. Some of the fungi isolated as a part of this study have been identified by 18S rRNA sequencing and reported in a previous study by Crasta and Raveesha (36) as denoted (*) in Table 4. A phylogenetic tree was also constructed in the above study to understand the phylogenetic affiliations between the fungal endophytes using MEGA X software (37).

Statistical analysis

The total colonization frequency (CF) of fungal endophytes associated with all plant tissues of *C. forskohlii* was found to be 56.54%. The old and young leaves of *C. forskohlii* showed maximum colonization rate of 74.52% and 70.47%, followed by roots with 50.27% and stem showed least CF of 35.41% as indicated in Fig. 7.

The seasons during these 12 months between May 2020–April 2021 were Summer, Monsoon and Winter. Seasons influenced the rate of colonization of endophytic fungi, and it was found that maximum colonization occurred during monsoon with CF of 71%, followed by winter 52.85% and least CF occurred during summer with CF of 40.17% as indicated in Fig. 8.

The RPO of Ascomycota was found to be the highest

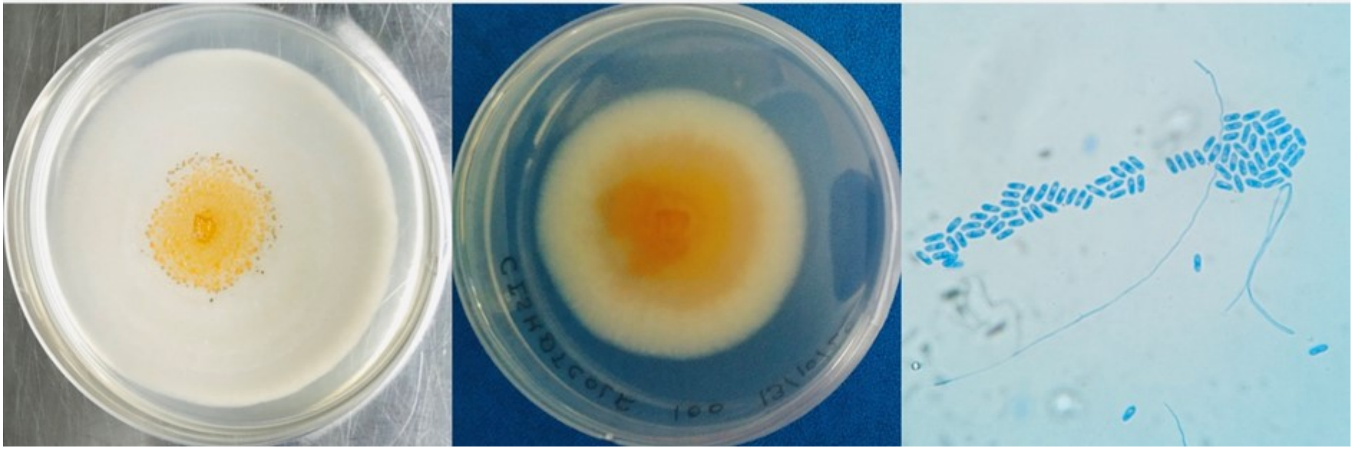

Fig. 3. Endophytic fungus isolated from old leaf-CISHDTCOLF98-*Colletotrichum gloeosporioides.*

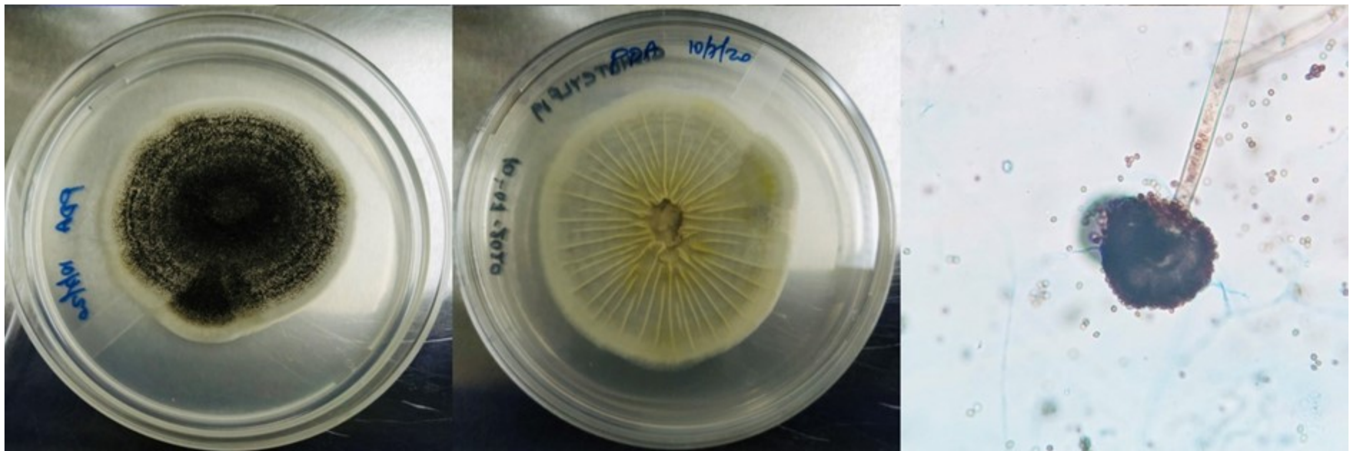

Fig. 4. Endophytic fungus isolated from young leaf-CISHDTCYLF19-*Aspergillus niger*.

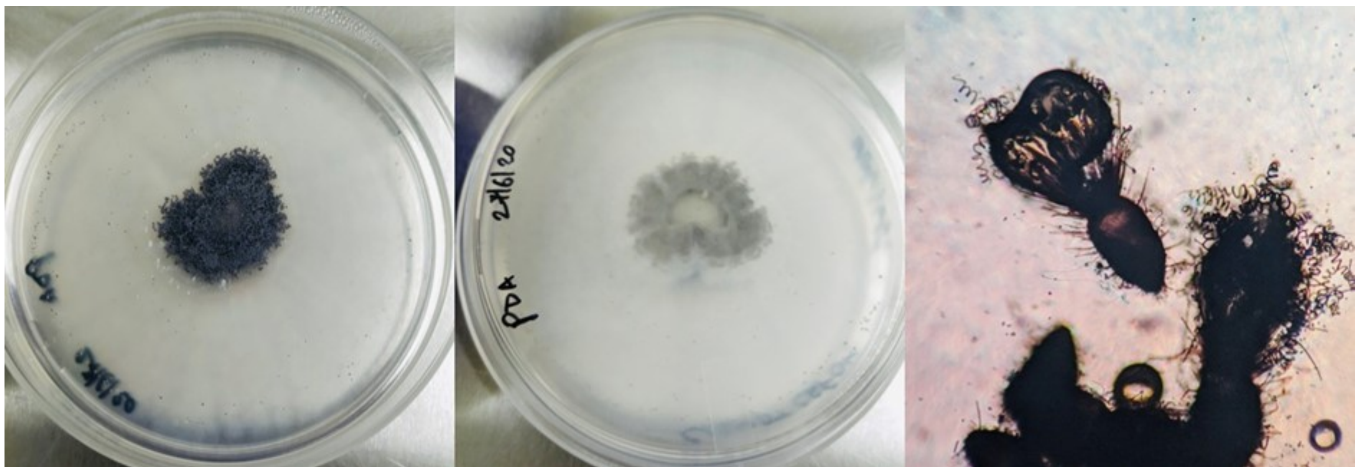

Fig. 5. Endophytic fungus isolated from stem-CISHDTCSF04-*Chaetomium angustispirale.*

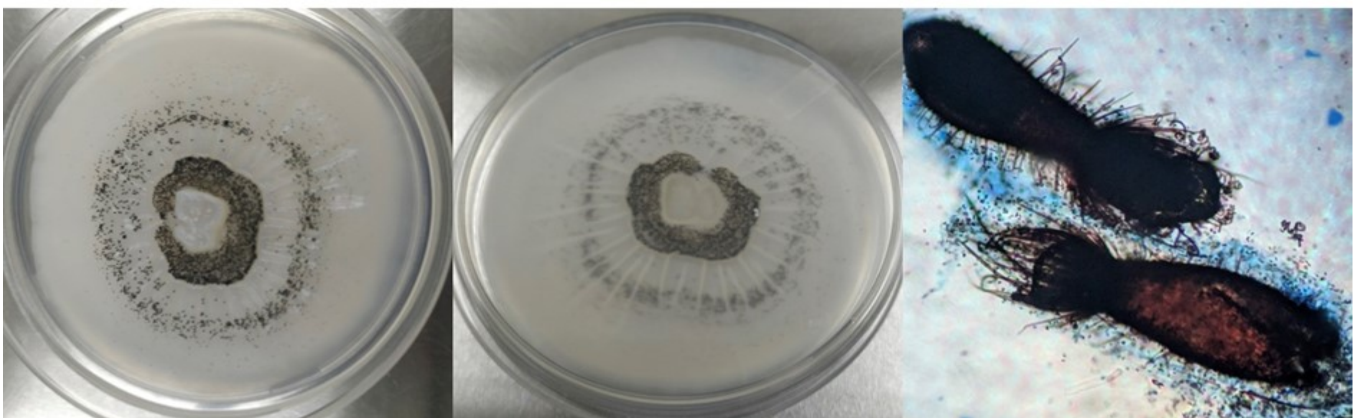

Fig. 6. Endophytic fungus isolated from root-CISHDTCRF22-*Collariella robusta.*

Table 4. Diversity, species richness and evenness of endophytic fungi associated with the different tissues of *C. forskohlii* in different seasons.

Index	By tissue type				By season		
	Old leaf	Young leaf	Stem	Root	Summer	Winter	Monsoon
Simpson's Diversity index	0.049	0.042	0.093	0.0416	0.03	0.036	0.05
Simpson's Dominance index	0.95	0.957	0.906	0.958	0.969	0.963	0.949
Shannon- Wiener index	3.227	3.215	2.651	3.238	3.581	3.494	3.534
Menhinick's index of Evenness	2.035	1.744	1.687	2.447	3.333	3.222	2.826
Margalef's Richness index	6.091	5.096	4.089	6.029	9.047	8.699	9.986
CF%	74.52	70.47	35.41	50.27	40.17	52.85	71

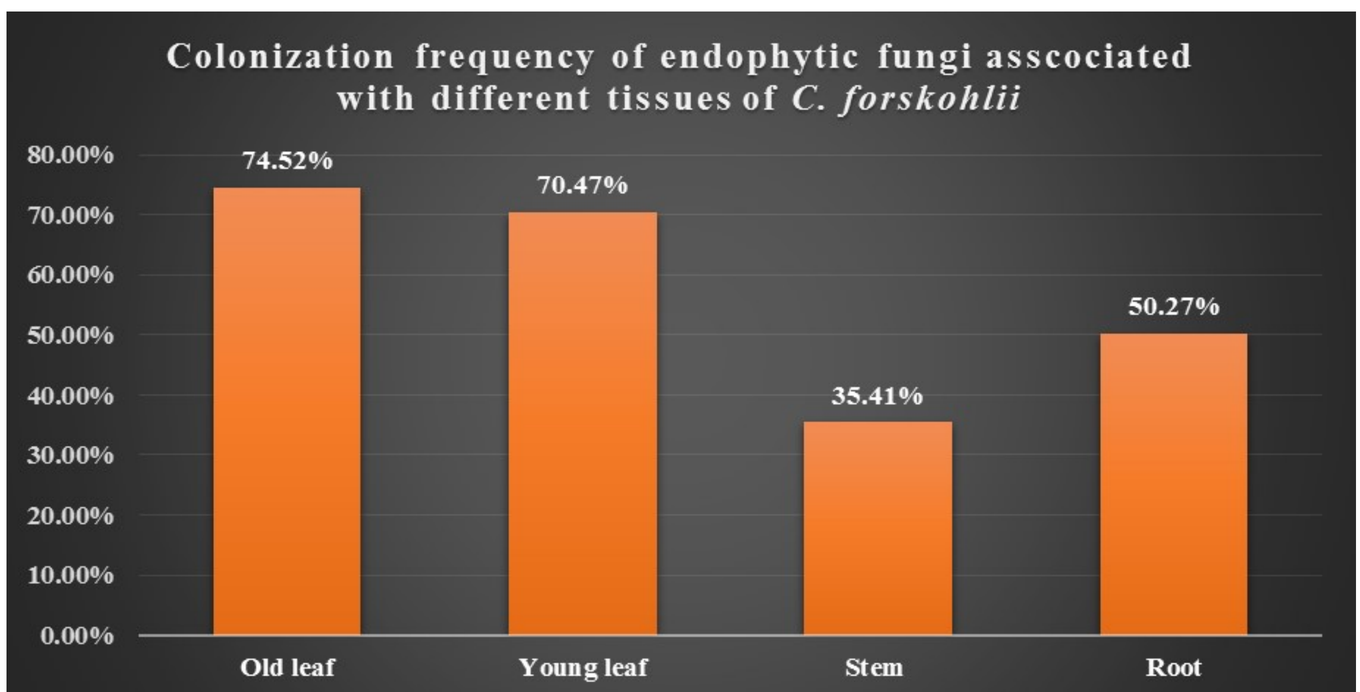

Fig. 7. Colonization frequency of endophytic fungi associated with *C. forskohlii.*

with 81.26%, Sterile mycelia with 16.10% RPO and the RPO of Mucoromycota was found to be 2.63% and is represented in the Fig. 9. The EIR is interpreted in the Table 2. The RF for each species of fungal endophytes is given in Table 3.

The fungal diversity index of the entire plant was as follows-Simpson's Diversity index was found to be 0.022 and Simpson's Dominance index was found to be 0.977, Shannon-Wiener index was found to be 4.203, Menhinick's

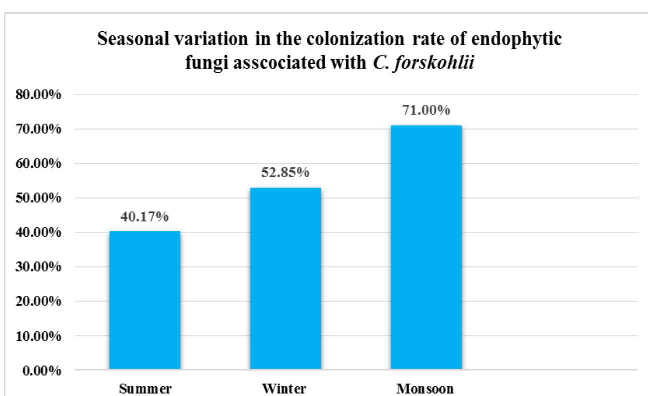

Fig. 8. Seasonal colonization rate of endophytic fungi associated with *C. forskohlii.*

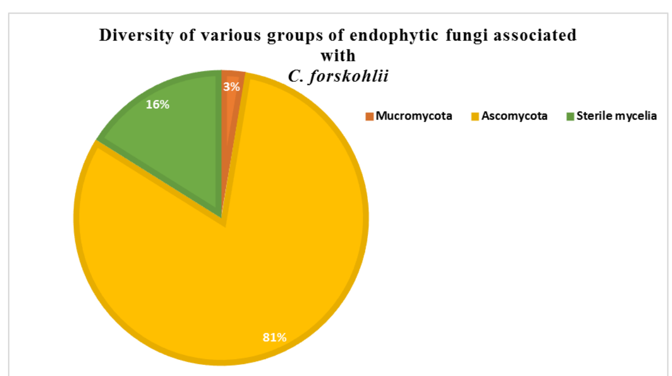

Fig. 9. Relative percentage occurrence of fungal endophytes associated with *C. forskohlii.*

Table 3. The relative species frequency (RF) of endophytic fungi associated with different plant tissues of *C. forskohlii.*

List of endophytic fungi	Old leaf fungi	Young leaf fungi	Root fungi	Stem fungi	Total	RF
Alternaria alternata *MT770930	7				7	0.73
Amesia cymbiformis	9				9	0.9
Arcopilus flavigenus			2		2	0.21
Aspergillus flavus * MT740749		1	8		9	0.9
Aspergillus fumigatus			2	4	6	0.63
Aspergillus nidulans		10			10	1.05
Aspergillus niger * MT750290	11	9	11		31	3.26
Aspergillus ochraceus * MT762907			6		6	0.63
Aspergillus terreus		11			11	1.15
Chaetomium angustispirale				4	4	0.42
Chaetomium cochliodes	14				14	1.47
Chaetomium coarctatum			7	4	11	1.15
Chaetomium globosum * MT770936	15	4	5	32	56	6.31
Chaetomium globosum sensu stricto	2				2	0.21
Chaetomium madrasense	1				1	0.1
Chaetomium sp.	3	7	1	7	18	1.89
Chaetomium spinosum		3	3	7	14	1.36
Chaetomium subspirale		7			7	0.73
Chaetomium tectifimeti			4		4	0.42
Cladosporium cladosporioides				5	5	0.52
Cladosporium sp. * MT770944	1			1	2	0.21
Cladosporium sphaerospermum	1				1	0.1
Collariella bostrychodes	16	10	15	25	66	6.94
Collariella robusta	14	17	5		36	3.78
Colletotrichum coccodes *MT750014				3	3	0.31
Colletotrichum endophytica		15			15	1.57
Colletotrichum falcatum	8				8	0.84
Colletotrichum fruticola	8	11	3		22	2.31
Colletotrichum gloeosporioides	15	10	8	4	37	3.89
Colletotrichum siamense	1				1	0.1
Colletotrichum sp. *MT762814	3	2			5	0.52
Colletotrichum tropicale			4		4	0.42
Colletotrichum truncatum *MT762340			5		5	0.52
Colletotrichum asianum	14				14	1.47
Epicoccum sp.				3	3	0.31
Fusarium chlamydosporum	14	20			34	3.57
Fusarium equiseti				10	10	1.05
Fusarium solani			4		4	0.42
Fusarium sp.	11		3		14	1.47
Fusarium verticillioides	10	12			22	2.31
Lasiodiplodia theobromae	3				3	0.31
Mucor circinelloides		23	2		25	2.63
Nigrospora oryzae *MT762908	10	10		2	22	2.31
Nigrospora sp.		4			4	0.42
Nigrospora sphaerica	13				13	1.36
Paecilomyces carneus		5	1	3	9	0.9
Paecilomyces sp.			3		3	0.31
Penicillium chrysogenum			7		7	0.73
Penicillium paradoxum			1		1	0.1
Penicillium sp. *MT770931	1	9			10	1.05
Phoma herbarum	1				1	0.1
Phoma sp.	4	1		1	6	0.63
Psuedotorula sp.			3		3	0.31
Sarocladium sp.			4		4	0.42
Sarocladium strictum		5	9		14	1.47
Scopulariopsis brevicaulis *MT770935			6		6	0.63
Scopulariopsis sp.			4	7	11	1.15
Sterile hyaline mycelia	49	25	16	20	114	12
Sterile septate mycelia	8	14	13	4	39	4.1
Trichoderma harizianum	6	15			21	2.21
Trichoderma sp.			3		3	0.31
Xylaria acuta	11				11	1.15
Xylaria apoda	3	14			17	1.78
Xylaria curta	11	9	3	6	29	3.05
Xylaria feejeensis	7			4	11	1.15
Xylaria grammica	8	8		11	27	2.84
Xylaria sp.				3	3	0.31
Total	**313**	**296**	**171**	**170**	**950**	

Table 2. Colonization frequency (CF) & endophyte isolation rate (EIR) of endophytic fungi associated with the different tissues of *C. forskohlii* in different seasons.

Plant part	Sampling season	No. of tissues studied	No. of isolates obtained	CF%	EIR
Old leaf	Summer	140	77	55	0.55
	Winter	105	75	71.42	0.71
	Monsoon	175	161	92	0.92
Young leaf	Summer	140	77	55	0.55
	Winter	105	71	67.61	0.67
	Monsoon	175	148	84.57	0.84
Stem	Summer	160	30	18.75	0.18
	Winter	120	39	32.5	0.32
	Monsoon	200	101	50	0.50
Root	Summer	120	43	35.83	0.35
	Winter	90	39	43.33	0.43
	Monsoon	150	89	59.33	0.59

index of Evenness was found to be 4.03 and Margalef's Richness index was found to be 18.26. The fungal diversity indexes of the different tissues during different seasons are mentioned in Table 4.

Discussion

A recent estimate of the number of species belonging to the kingdom fungi ranges from 2.2 to 3.8 million species, of which about 150,000 species have been described so far (38). Fungi have established associations with various life forms on Earth, including soil, dead and decaying matter, animals, and plants, demonstrating their ability to survive, absorb nutrients, and reproduce. One of the beneficial symbiotic relationships that fungi exhibit is their association with plants as endophytes. It is reported that fungi have been found in association with almost all groups of plants, and these symbiotic relationships profoundly impact the plant's fitness, ecology, and evolution. Fungi shape the internal communities of plants by producing secondary metabolites and also have significant effects on the diversity of other organisms such as insects, nematodes, and, bacteria associated with the host plant (39–41).

Coleus forskohlii, an important aromatic medicinal plant, has been extensively used in folk remedies for centuries across many tropical countries. Due to increased demand, the overharvesting of *C. forskohlii* has led to the endangerment of its population. In recent decades, researchers have turned their attention to plant protection and preservation using biological approaches. Fungal endophytes, known for their ability to protect plants against both abiotic and biotic stresses, play a crucial role in enhancing plant growth and development, safeguarding them from extreme environmental conditions. Understanding the diversity of fungal endophytes associated with different tissues of *C. forskohlii* is essential. This knowledge can aid in isolating some of the most beneficial fungal endophytes capable of producing bioactive molecules similar to those found in the host plant. By doing so, we can harness these bioactive compounds without further endangering the plant through overharvesting. As a first step, this study was conducted over a period of one year to comprehensively understand the diversity and spaciotemporal distribution of endophytic fungi associated with *C. forskohlii.* This research marks the inaugural report on the seasonal diversity of fungal endophytes associated with *C. forskohlii.*

A total of 950 endophytic fungi were isolated from 1680 segments of *C. forskohlii*, indicating a colonization frequency of 56.54%. These results align with the percentage of fungal communities isolated and reported in studies on *Pinus taeda* (42). When considering the seasonal diversity of fungal endophytes associated with *C. forskohlii,* it was observed that the maximum isolates were obtained during the monsoon season, with a CF of 71%. In the winter, the CF was 52.85%, while the summer season exhibited the lowest CF of 40.17%. This trend is consistent with earlier studies (43), suggesting that fungal endophytes spore tend to germinate and colonize host tissues more readily when the humidity is moderate to high, and temperature range between 20–30ºC. Moreover, the majority of isolates were found in the old/mature leaves, with a CF of 74.52%. This finding supports the results by Hilarino *et al*. and Arnold *et al*. (44, 45).

Fungal endophytes were identified using both morphology and 18S rRNA gene sequencing. In total, 21 genera and 55 species were identified, aligning with similar studies conducted on medicinal plants such as *Salvia multicaulis* (46), *Citrus reticulata* (35), *etc.* Notably, 81.26% of the isolates belonged to Ascomycota, a consistent trend found in many other studies (47), while 2.63% were classified under Mucoromycota. These endophytes fell into 10 different orders spanning three classes: Sordariomycetes (Hypocreales, Xylariales, Microascales, Trichosphaeriales, Glomerellales and Sordariales), Dothiomycetes (Pleosporales, Capnodiales, Botryosphaeriales) and Eurotiomyctes (Eurotiales).

Among these, Sordariomycetes were predominant, constituting 52% of the identified endophytes, followed by Eurotiomyctes at 9.6%, and Dothiomycetes (8.8%).

Simpson's Diversity index of 0.022 and Simpson's Dominance index of 0.977 highlights the high diversity of fungal endophytes in this location. Additionally, the Shannon-Wiener index of 4.203 and Margalef's Richness index of 18.26 underscores the extensive species richness and diversity among the fungal endophytes. Some of the predominant genera of fungal endophytes associated with *C. forskohlii* include *Chaetomium*, *Collariella*, *Colletotrichum*, *Aspergillus, Fusarium, Nigrospora* and *Xylaria*, aligns with finding from other researchers (48, 49). The predominant species include *Sterile mycelia* (narrow width)*, Chaetomium globosum, Collariella bostrychodes, C. robusta, Colletotrichum gloeosporioides, Fusarium chlamydosporum, Aspergillus niger, Xylaria curta, X. grammica,* and *Mucor circinelloides.* These finding suggests the potential for sourcing bioactive compounds of pharmaceutical value from these fungi. Among the isolated species, *Trichoderma harizianum. C. globosum, C. bostrychodes*, *C. gloeosporioides,* sterile hyaline mycelia and *X. curta* were found distributed in all parts of *C. forskohlii*, indicating their ecological and physiological adaptations within the tissues of *C. forskohlii* during different seasons. Moreover, species like *C. globosum, T. harizianum* and *X. curta,* identified in our study, have been recognized as plant growth promoting endophytes (50). These endophytes are also employed as potential biocontrol agents in agriculture, safeguarding plants against pathogenic microorganisms such as *Rhizoctonia solani, Trichoderma* spp. *Gliocladium* spp., *Chaetomium* spp., *Bacillus* spp., *Psuedomonas* spp. and *Aspergillus* spp. These findings underscore the potential benefits that the fungal endophytes could offer *C. forskohlii.* They not only contribute to its growth and development, but maintain a healthy internal environment, augmenting its medicinal value.

Conclusion

In conclusion, isolating endophytic fungi from the healthy tissues of the *Coleus forskohlii* could help minimize the overharvesting of this medicinally important plant. We found that fungi belonging to Mucoromycota and Ascomycota predominantly colonize the healthy tissues of *C. forskohlii.* Moreover, changes in environmental conditions during different seasons can affect the rate of isolation of the fungal endophytes. This study has also identified the optimal season for isolating endophytes from *C. forskohlii*, which could facilitate the examination and discovery of bioactive compounds with pharmaceutical potential. Notably, sterile hyaline mycelium, *Chaetomium globosum*, *Collariella bostrychodes* and *Fusarium chlamydosporum* were the most dominant species of endophytic fungi associated with *C. forskohlii.* This suggests that these isolates may contribute to the medicinal properties of *C. forskohlii* by producing essential secondary metabolites. Further studies on these isolated fungal endophytes could decipher their antagonistic potential, enhancing the antimicrobial properties of the secondary metabolites they produce.

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Authors' contributions

KAR designed the experiment, shared his expertise, provided guidance and support. GLC carried out the research, wrote the article, analysed and interpreted the results.

Compliance with ethical standards

Conflict of interest: The authors declare no conflict of interest.

Ethical issues: None.

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