Research Article

Seasonal variation and diversity of endophytic fungi in *Chromolaena odorata* (L.) King and Robinson, an invasive alien weed of Tripura, Northeast, India

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Abstract

The endophytic fungal diversity of *Chromolaena odorata*, an invasive alien weed of Tripura, Northeastern India is scientifically documented in the present study. A total of, 177 endophytic fungal isolates were isolated from 480 tissue segments of *C. odorata* from eight different geographic locations. The isolates were identified into 14 genera and they belonged to the phylum Ascomycota, grouped into 3 classes, 9 orders, and 11 families. The most dominant orders were Glomerellales (22.60%), Hypocreales (22.03%), and Pleosporales (20.34%). *Colletotrichum, Fusarium, Corynespora, and Nigrospora* were the dominant genera in the present study. The colonization rate, isolation rate varied significantly among tissue type and sampling location. The colonization frequency (CF) and relative frequency (RF) of endophytic fungal strains showed variation considering tissue types, locations, and seasons. The total number of isolates recovered from leaf, stem, and root explants were 61, 67, and 49, respectively. There were considerable diversity in the number and population distribution of endophytic fungi in different seasons and plant parts of *C. odorata.* The highest biodiversity of endophytic fungi was observed in summer compared to winter. Among the different plant tissues, the *Shannon (H')*, *Simpson (1-D)* & Fisher alpha (α) diversity of the endophytic fungi was highest in the stem, followed by root and leaves. These findings suggest that the host plant tissue and sampling season are major factors of distribution of endophytic fungi.

Keywords: *Chromolaena odorata,* endophytic fungi, diversity, ascomycota, seasonal variation

Introduction

Endophytes are micro-organisms (fungi or bacteria) that spend the whole or part of their life cycle, invading the living tissues of host plants without developing symptoms of infection and even disease (Materatski et al., 2018; Wilson, 1995). The fungal endophytic community composition is highly influenced by both abiotic and biotic factors such as temperature, humidity, location, plant physiology, plant host genotype, tissue type, pathogen infections, and anthropogenic influences (Cevallos et al., 2017; Christian et al., 2016). Several studies mentioned that properties of soil, availability of nutrients, root exudation, and specific climatic conditions, e.g., temperature and precipitation, are also decisive drivers of endophytic microfungal communities in plant roots (Pecundo et al., 2021). Endophytic fungi are highly distributed among plants and it is estimated that each plant species harbors not less than one endophytic fungal species, although, this number may vary based on the host species subjected to isolation (Arnold et al., 2000; Correia et al., 2017). Fungal endophytes play a vital role in the successful population establishment of alien invasive species in newly invaded areas outcompeting native species (Clay et al., 2016; Seifert et al., 2009). The association of particular microbes with non-native invasive species poses severe threats to native plant competitors (Clay et al., 2016; Garnica et al., 2022). These microbes have the potential to alter the diversity and composition of entire plant

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communities (Rillig et al., 2016).

The alien-introduced species in a natural community constitutes a major threat to biological diversity throughout the world (Adair and Groves, 1997). These invasive plants affect the ecosystem structure and function in a habitat by changing soil nutrient status and altering geo-morphological processes (Cronk and Fuller, 1995; Macdonald et al., 1989). Endophytic fungi may influence plant growth and reproductive potential of invasive alien plant species (IAPS), or enhance resistance to both biotic and abiotic stress (Mayerhofer et al., 2013; Rho et al., 2018). Endophytic fungi improve the mineral uptake and nutrition of their host plants (Behie and Bidochka, 2014; García-Latorre et al., 2021; Tang et al., 2022). Association of endophytic fungi, *Curvularia geniculata* with *Parthenium hysterophorus* has been shown to promote plant growth through the production of phosphate solubilization and phytohormone (Priyadharsini et al., 2017), which might enable this IAPS to invade new areas. Endophytic fungi may gradually accumulate in the new geographical distributional ranges of invasive alien plants (Mei et al., 2014). Understanding the association between invasive species and their endophyte communities is important for the evaluation and management of the biological invasion of invasive alien plant species (Mei et al., 2014)

C. odorata formerly known as *Eupatorium odoratum* is a weedy perennial herb of the family Asteraceae. This plant species was indigenous to tropical and subtropical areas of Central and South America. *C. odorata* has been considered as one of the most invasive weeds in the world (Mandal & Joshi, 2014). It is widely distributed across five continents due to its remarkable tolerance capacity and adaptive nature to varied climatic conditions (Kriticos et al., 2005). It was introduced as an ornamental plant to the botanical garden in Calcutta, India in 1845 (CAB Reviews 2019 14, No. 009; Online ISSN 1749–8848) and became a harmful weed (Naidoo and Naidoo, 2018; Vaisakh and Pandey, 2012). In this study, an innovative lay out of the isolation and identification of the endophytic fungi inhabiting different plant parts like leaf, root, and stem explants of *C. odorata* collected from eight collection sites of Tripura, North-east, India were done and were compared with the biodiversity in different tissues and season of the host plant species.

Materials and methods

Plant sample collection

Five healthy and disease-free *Chromolaena odorata* plants were collected randomly from eight locations in Tripura (Table 1). The plants were transported to the laboratory in plastic zipper bags and processed within 24 hr of collection for isolation of endophytic fungal isolates. The plant sample was identified by the Biodiversity and Plant Taxonomy Laboratory, Department of Botany, Tripura University, and the herbarium was submitted to the Herbarium of Department of Botany, Tripura University.

Isolation of endophytic fungi

The roots, leaves, and stems were separated from the parent body and washed thoroughly in running tap water to remove dirt and debris attached to the surface. Plant parts were further cut into many small segments. The surface sterilization was carried out following standard isolation protocol (Schulz et al., 1993; Strobel and Daisy, 2003) with some minor modifications. These explants were surface disinfected by sequential washes in 70% (v/v) ethanol (1 min) and 3.5% (v/v) NaOCl (2 min), rinsing with sterile water and allowing the surface to dry under sterile conditions. Ten segments of each plant part were randomly selected from each collection site for the isolation of endophytic fungi. Segments were plated onto petriplates containing MEA (Malt Extract Agar) medium supplemented with streptomycin (100 µg/ml). All the plates were incubated at 28°C for a week and were observed daily for hyphal growth. All observed fungal growths were subcultured on MEA plates for purification. The sterilization protocol was validated using the leaf imprint method (Schultz et al., 1998) to confirm proper surface sterilization.

Morphological identification of endophytic fungi

The lacto phenol cotton blue staining technique was carried out for the microscopic identification of the fungal isolates. Based on macroscopic and microscopic characteristics, the endophytic fungi were identified using the standard manuals and literature (Domsch et al., 1980; Ellis, 1971; Watanabe, 2002).

Molecular identification of fungal strains

Isolates were identified at the sequencing facility of the National Centre for Microbial Resource (NCMR), National Centre for Cell Science, Pune. Genomic DNA was isolated by the standard phenol/chloroform extraction method (Sambrook et al., 1989), followed by PCR amplification of the SSU regions using universal primers NS1 [5'-GTAGTCATATGCTTGTCTC-3'] and NS8 [5'-TCCGCAGGTTCACCTACGGA-3']. The amplified SSU PCR product was purified by PEG-NaCl precipitation and directly sequenced on an ABI® 3730XLautomated DNA sequencer (Applied Biosystems, Inc., Foster City, CA) as per the manufacturer's instructions. Essentially, sequencing was carried out from both ends so that each position was read at least twice. Assembly was carried out using a Laser gene package followed by NCBI BLAST against sequences from type material for tentative identification (Boratyn et al., 2013).

Phylogenetic analysis

All four sequences were deposited in the NCBI-GenBank database and accession numbers were obtained. For phylogeny analysis of the 18S rRNA sequences, the BLAST program of the NCBI-GenBank database was used for searching the homologous sequence. For alignment, MEGA-XI software was used and the Neighbor-joining method was performed for phylogenetic analysis (Saitou et al., 1987).

Data analyses

The Colonization rate (**CR**) and Isolation rate (**IR**) were determined by following Rajini et al., 2019. The relative frequency (**RF**) (Photita et al., 2001, Huang et al., 2008), and colonization frequency (**CF**) (Hata and Futai, 1995) were determined using established formulas.

Diversity indices: The diversity of the endophytic fungal community was evaluated using various diversity indices, such as the Shannon–Weaver diversity index (*H'*), Simpson's dominance (*D*), the Dominance index (1- *D*), Equitability (*J*), Fisher's alpha diversity index (α), Berger-Parker dominance (*B*) and Brillouin index (*HB*) using R- programming software (R Core Team, 2022).

Results

Isolation and identification of endophytic fungi

The present investigation deals with the comparative evaluation of endophytic fungal diversity in leaf, stem, and root explants of *Chromolaena odorata* plant collected from eight different locations in Tripura, Northeast, India (Table 1). Based on morphological and molecular identification, only 177 isolates were identified. These 177 isolates were reported from leaves (61), stems (67), and roots (49). A total of fourteen endophytic fungal strains were isolated from the host plant (Table 2 & Table 3). The 14 fungal species grouped into 3 taxonomic classes (Sordariomycetes, Dothideomycetes and Eurotiomycetes), 9 orders (Eurotiales, Sordariales, Cladosporiales, Glomerellales, Pleosporales, Diaporthales, Hypocreales, Botryosphaeriales, and Xylariales), and 11 families (Aspergillaceae, Chaetomiaceae, Cladosporiaceae, Glomerellaceae, Corynesporascaceae, Pleosporaceae, Diaporthaceae, Nectriaceae, Botryosphaeriaceae, Apiosporaceae, Aspergillaceae, and Hypocreaceae) which were obtained from different *C. odorata* tissues across different seasons. All the isolates belonged to the phylum Ascomycota. The most dominant orders were Glomerellales (22.60%), Hypocreales (22.03%), and Pleosporales (20.34%) constituting about 64.97% of the total isolates, while Xylariales (10.73%), Botryosphaeriales (9.04%), Eurotiales (8.47%), Diaporthales (5.08%), Sordariales (1.13%) and Cladosporiales (0.56%) accounts for 35.02%.

Molecular identification and phylogeny analysis

The amplification of SSU regions of four isolates were successfully carried out with the universal primers NS1/NS8 respectively. The sequences were compared with GenBank database, and the results were represented in Table 2. Based on the molecular data endophytic fungal isolates were identified as *Apiospora aurea, Botryosphaeria rhodina Fusarium oxysporum* and *Hypocrea koningii (Trichoderma koningii)*. The 18S rRNA sequences of all these isolates were submitted to NCBI Genbank and accession numbers were obtained accordingly. Phylogenetic analysis using the Neighbor-joining method was performed. The Phylogenetic tree is shown in Figure 1.

Effect of locality on the endophytic fungi

The endophytic fungal assemblages in *C. odorata* were

Table 1. Geographic location of collection sites of *Chromolaena odorata* in Tripura, Northeast India

Collection sites	Latitude	Longitude	Elevation
Ambassa	23°55'26"N	91°51'21"E	195.2 feet.
Dhwajanagar	23°33'01"N	91°28'06"E	113.9 feet.
Duski	23°52'38"N	91°34'53"E	140.4 feet.
Jalefa	23°01'59"N	91°41'13"E	141.7 feet.
Kailashahar	24°18'57"N	91°59'43"E	170.8 feet.
Nandannagar	23°52'04"N	91°18'19"E	86.5 feet.
Padmanagar	23°35'08"N	91°20'50"E	92.3 feet.
Rajnagar	24°19'21"N	92°07'13"E	296.9 feet.

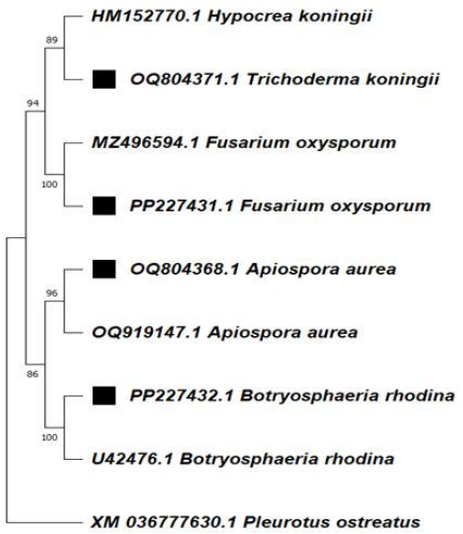

Figure 1: Phylogenetic tree showing the relationships of the isolates to closely related fungi. The numbers at branching points refer to bootstrap values, based on 1000 replicates

strongly shaped by sampling locations (Table 2). The data analysis indicated that almost 38.42% of fungal isolates (68 isolates, 34 in each site) were recovered at AMB & KAL sites, 16.95% at JAL (30 isolates), 11.30% at NAN (20 isolates),19.20% at DHA& DUS sites with 17 isolates each, 9.04% at PDM site with 16 isolates and least being 5.08% at RAN site with 9 isolates. Out of fourteen genera isolated from the host plant only *Fusarium oxysporum* was isolated from all the study sites and the genera like *Apiospora aurea, Chaetomium* sp., *Cladosporium* sp., & *Lasiodiplodia* sp. were isolated from a particular site (Table 2). For example, *Apiospora aurea* was isolated from the Kailashahar site, while the rest of the fungal genera were either isolated from two or more sites.

Effect of tissues on the endophytic Fungi

The fungal endophyte composition in *C. odorata* were strongly shaped by tissue type (Table 2). Among the 177 isolates generated, 61 were from leaves, 67 from stem, and 49 were from root tissues. Among the isolated endophytes, 10 fungal strains each were isolated from leaf and stem explants, and 11 fungal strains were obtained from root explants of the host plant collected from eight different locations in Tripura.

Some of the isolated fungal strains showed host tissue/ organ preference. Moreover, some of the strains were confined to one tissue only. The genera *Chaetomium* sp. was isolated only from leaf explants of Dhwajanagar; *Aspergillus* sp. was only isolated from leaf tissues of Ambassa and Jalefa sites, *Diaporthe* sp. from stem only from Ambassa, Dhwajanagar and Nandannagar sites; *Cladosporium* sp. was isolated from root tissues of Jalefa similarly, *Trichoderma* sp. was isolated from root tissues only from Ambassa and Rajnagar site; *Colletotrichum* sp. from leaf and stem; *Fusarium oxysporum* was from root and stem tissues.

Effect of season on endophytic fungi

The study showed that the seasons influenced the endophytic fungal composition in *C. odorata.* Seasonal effect was prominent with the maximum number of isolates occurring in winter (98 isolates), followed by summer (79

Table 2. Isolated endophytic fungal taxa, NCBI accession numbers, overall colonization frequency (CF%) and relative frequency (RF%) and other information of the endophytic fungi associated with *Chromolaena odorata* of Tripura, Northeast – India

Fungal Taxa	NCBI accession no.	Identity % age	Plant parts			Sampling sites							CF %	RF %
			L	S	R	AMB	DHA	DUS	JAF	KAL	NAN	PDM	RAN	
Apiospora aurea	OQ804368.1	98.15	2	1	0	0	0	0	0	3	0	0	0	1.88	1.64
Aspergillus sp.			2	0	0	1	0	0	1	0	0	0	0	1.25	1.12
Botryosphaeria rhodina	PP227432	100	2	9	1	4	0	5	0	0	2	1	0	7.50	6.77
Chaetomium sp.			1	0	1	0	2	0	0	0	0	0	0	1.25	1.12
Cladosporium sp.			0	0	1	0	0	0	1	0	0	0	0	0.63	0.56
Colletotrichum sp.			17	14	9	8	8	7	0	14	0	3	0	25.00	23
Corynespora sp.			9	9	2	4	0	0	6	7	3	0	0	12.50	11.3
Curvularia sp.			3	10	3	1	1	0	2	0	3	7	2	10.00	9.03
Diaporthe sp.			0	7	2	3	3	2	0	0	1	0	0	5.63	5.08
Fusarium oxysorum	PP227431	100	7	9	17	3	2	2	2	10	7	4	3	20.62	18.64
Lasiodiplodia sp.			0	4	0	0	0	0	4	0	0	0	0	2.50	2.25
Nigrospora sp.			11	2	3	3	1	0	9	0	2	1	0	10.00	9.03
Penicillium sp.			7	2	4	5	0	1	5	0	2	0	0	8.13	7.34
Hypocrea koningii	OQ804371.1	99.39	0	0	6	2	0	0	0	0	0	0	4	3.75	3.38
Total			61	67	49	34	17	17	30	34	20	16	9		

*AMB = Ambassa; DHA = Dhwajanagar; DUS = Duski; JAF = Jalefa; KAL = Kailashahar; NAN = Nandannagar; PDM = Padmanagar; RAN = Rajnagar

isolates) (Table 2 & Table 3). From the leaf, stem, and root tissues of the host plants collected from eight sites 10 taxa were isolated during the winter season and 11 taxa were isolated during the summer season. From leaf explants of host plants collected during the winter season, seven fungal strains were isolated; however, six from stem and five from root explants were isolated whereas during the summer season leaf tissues harbored five fungal strains, seven each from both stem and root tissues. Some of the endophytic fungal taxa were reported in only one or two seasons. For example, *Aspergillus* sp. & *Cladosporium* sp. were exclusively observed during the summer season, whereas, *Corynespora* sp., *Lasiodiplodia* sp. & *Nigrospora* sp. were recorded only during the winter season (Table 3). Some of the taxa of endophytic fungi like *Colletotrichum* sp., *Fusarium oxyspporum*, *Curvularia* sp., *Diaporthe* sp. *Penicillium* sp. were recorded in both sampling seasons. The colonization rate and isolation rate value varied in different tissue types collected from different sampling sites along with seasons (Table 3).

Colonization frequency (CF) and relative frequency (RF) of endophytic fungi

Considering tissue types, location, and seasonal attributes as factors the highest %CF was shown by *Colletotrichum* sp. (25.00), followed by *Fusarium oxysporum* (20.62) and *Corynespora* sp. (12.50) (Table 4 & Figure 2) similarly in the case of relative frequency *Colletotrichum* sp. (23.00) with highest values, followed by *Fusarium oxysporum* (18.64) and *Corynespora* sp. (11.30) (Table 3 & Figure 3). The occurrence and dominance of isolated endophytes were calculated by measuring colonization frequency (%CF) in all tissue types. In leaves, the highest %CF was shown by *Nigrospora* sp. (4.38) in the Jalefa site, followed by *Collectotrichum* sp. (3.75%), and *Penicillium* sp. of Jalefa site (2.5%) (Table 4). In stem tissues, the highest values of %CF were recorded in Jalefa site by *Botryosphaeria rhodina* (3.13%), followed by *Colletotrichum* sp. (2.5%) and *Curvularia* sp. (2.5%) of Duski & Padmanagar sites, respectively. In root tissues, *Fusarium oxysporum* (6.25%) with the highest %CF value was recorded from the Kailashahar site followed by *Colletotrichum* sp. (2.5%) of Kailashahar and *Fusarium oxysporum* (1.88%) of Ambassa sites, respectively.

Variations were also observed in the relative frequency of

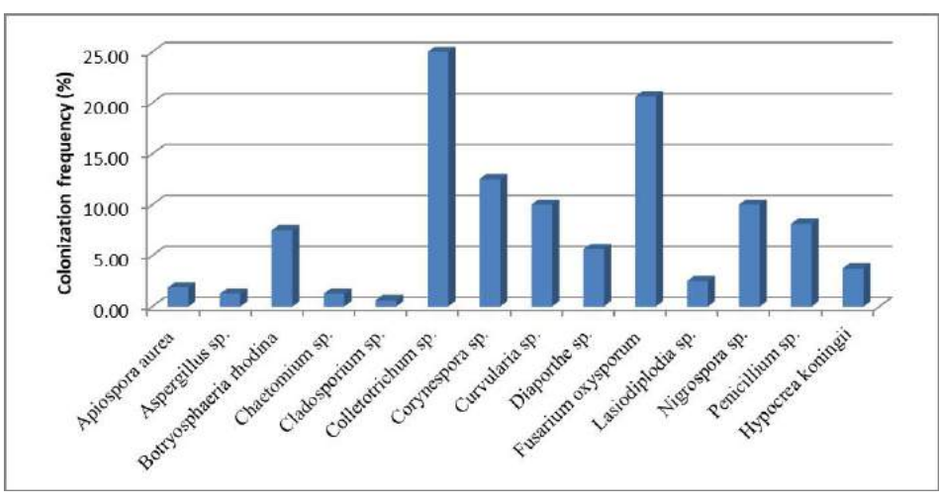

Figure 2. Colonization frequency (CF) of isolated endophytic fungi from *Chromolaena odorata*

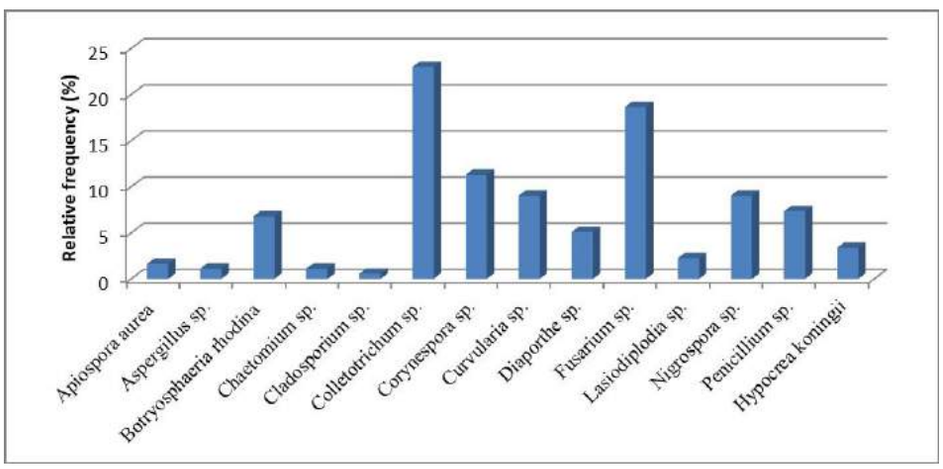

Figure 3. Relative frequency (RF) of isolated endophytic fungi from *Chromolaena odorata*

Table 3. Checklist of isolated endophytic fungal strains and their occurrence in explants of host plants in eight sampling locations and two growing seasons

Isolated endophytic fungal strains	AMB			DHA			DUS			JAF			KAL			NAN			PDM			RAN			Season		Family	Order	Class	Fungal phylum
	L	S	R	L	S	R	L	S	R	L	S	R	L	S	R	L	S	R	L	S	R	L	S	R	Summer	Winter				
Apiospora aurea	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	-	Apiosporaceae	Xylariales	Sordariomycetes	Ascomycota
Aspergillus sp.	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	Aspergillaceae	Eurotiales	Eurotiomycetes	Ascomycota
Botryosphaeria rhodina	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-		+	-	Botryosphaeriaceae	Botryosphaeriales	Dothideomycetes	Ascomycota
Chaetomium sp.	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	Chaetomiaceae	Sordariales	Sordariomycetes	Ascomycota
Cladosporium sp.	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	Cladosporiaceae	Cladosporiales	Dothideomycetes	Ascomycota
Colletotrichum sp.	+	+	+	+	+	-	+	+	-	-	-	-	+	+	+	-	-	-	-	-	+	-	-	-	+	+	Glomerellaceae	Glomerellales	Sordariomycetes	Ascomycota
Corynespora sp.	-	+	+	-	-	-	-	-	-	+	+	-	+	+	-	+	-	-	-	-	-	-	-	-	-	+	Corynesporascaceae	Pleosporales	Dothideomycetes	Ascomycota
Curvularia sp.	-	-	+	-	-	+	-	-	-	-	+	-	-	-	-	-	+	+	+	+	-	-	+	-	+	+	Pleosporaceae	Pleosporales	Dothideomycetes	Ascomycota
Diaporthe sp.	-	+	-	-	+	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	+	Diaporthaceae	Diaporthales	Sordariomycetes	Ascomycota
Fusarium oxysporum	-	+	+	-	+	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	+	-	+	+	Nectriaceae	Hypocreales	Sordariomycetes	Ascomycota
Lasiodiplodia sp.	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	Botryosphaeriaceae	Botryosphaeriales	Dothideomycetes	Ascomycota
Nigrospora sp.	+	-	-	+	-	-	-	-	-	+	-	+	-	-	-	-	+	+	-	+	-	-	-	-	-	+	Apiosporaceae	Xylariales	Sordariomycetes	Ascomycota
Penicillium sp.	+	+	+	+	-	-	-	-	-	+	-	+	-	-	-	-	-	+	-	-	-	-	-	-	+	+	Aspergillaceae	Eurotiales	Eurotiomycetes	Ascomycota
Hypocrea koningii	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	Hypocreaceae	Hypocreales	Sordariomycetes	Ascomycota

*AMB = Ambassa; DHA = Dhwajanagar; DUS = Duski; JAF = Jalefa; KAL = Kailashahar; NAN = Nandannagar; PDM = Padmanagar; RAN = Rajnagar, L= Leaf; S = Stem; R = Root

Table 4. Colonization frequency of isolated fungal endophytes from *Chromolena odorata* tissue wise and location wise data

Isolated endophytic fungal strains	CF (%) of Leaf								CF (%) of Stem								CF (%) of Root								
	AMB	DHA	DUS	JAF	KAL	NAN	PDM	RAN	AMB	DHA	DUS	JAF	KAL	NAN	PDM	RAN	AMB	DHA	DUS	JAF	KAL	NAN	PDM	RAN	Total CF(%
Apiospora aurea	0.00	0.00	0.00	0.00	1.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.63	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.88
Aspergillus sp.	0.63	0.00	0.00	0.63	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.25
Botryosphaeria rhodina	0.00	0.00	0.00	0.00	0.00	1.25	0.00	0.00	2.50	0.00	0.00	3.13	0.00	0.00	0.63	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.50
Chaetomium sp.	0.00	0.63	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.63	0.00	0.00	0.00	0.00	0.00	0.00	1.25
Cladosporium sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.63	0.00	0.00	0.00	0.00	0.63
Colletotrichum sp.	1.88	3.13	1.88	0.00	3.75	0.00	0.00	0.00	1.88	1.88	2.50	0.00	2.50	0.00	0.00	0.00	1.25	0.00	0.00	0.00	2.50	0.00	1.88	0.00	**25.00**
Corynespora sp.	0.00	0.00	0.00	1.88	1.88	1.88	0.00	0.00	1.25	0.00	0.00	1.88	2.50	0.00	0.00	0.00	1.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**12.50**
Curvularia sp.	0.00	0.00	0.00	0.00	0.00	0.00	1.88	0.00	0.00	0.00	0.00	1.25	0.00	1.25	2.50	1.25	0.63	0.63	0.00	0.00	0.00	0.63	0.00	0.00	10.00
Diaporthe sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.88	1.88	0.00	0.00	0.00	0.63	0.00	0.00	0.00	0.00	1.25	0.00	0.00	0.00	0.00	0.00	5.63
Fusarium oxysporum	0.00	0.00	0.00	0.00	0.00	1.88	2.50	0.00	0.00	1.25	0.00	0.63	0.00	2.50	0.00	1.25	1.88	0.00	1.25	0.63	6.25	0.00	0.00	0.63	**20.62**
Lasiodiplodia sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.50
Nigrospora sp.	1.88	0.63	0.00	4.38	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.63	0.63	0.00	0.00	0.00	0.00	1.25	0.00	0.63	0.00	0.00	10.00
Penicillium sp.	1.25	0.00	0.63	2.50	0.00	0.00	0.00	0.00	1.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.63	0.00	0.00	0.63	0.00	1.25	0.00	0.00	8.13
Hypocrea koningii	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.25	0.00	0.00	0.00	0.00	0.00	0.00	2.50	3.75

*AMB = Ambassa; DHA = Dhwajanagar; DUS = Duski; JAF = Jalefa; KAL = Kailashahar; NAN = Nandannagar; PDM = Padmanagar; RAN = Rajnagar

Table 5. Relative frequency of isolated fungal endophytes from *Chromolena odorata* tissue wise and location wise data

Isolated endophytic fungal strains	RF (%) of Leaf								RF (%) of Stem								RF (%) of Root								
	AMB	DHA	DUS	JAF	KAL	NAN	PDM	RAN	AMB	DHA	DUS	JAF	KAL	NAN	PDM	RAN	AMB	DHA	DUS	JAF	KAL	NAN	PDM	RAN	Total RF(%)
Apiospora aurea	0.00	0.00	0.00	0.00	**1.13**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.56	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.64
Aspergillus sp.	**0.56**	0.00	0.00	**0.56**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.12
Botryosphaeria rhodina	0.00	0.00	0.00	0.00	0.00	1.13	0.00	0.00	2.26	0.00	**2.82**	0.00	0.00	0.00	0.56	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.77
Chaetomium sp.	0.00	0.56	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.56**	0.00	0.00	0.00	0.00	0.00	0.00	1.12
Cladosporium sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.56**	0.00	0.00	0.00	0.00	0.56
Colletotrichum sp.	1.69	**2.82**	1.69	0.00	3.39	0.00	0.00	0.00	1.69	1.69	2.26	0.00	2.26	0.00	0.00	0.00	1.13	0.00	0.00	0.00	2.26	0.00	1.69	0.00	**23.00**
Corynespora sp.	0.00	0.00	0.00	1.69	1.69	1.69	0.00	0.00	1.13	0.00	0.00	1.69	**2.26**	0.00	0.00	0.00	1.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**11.30**
Curvularia sp.	0.00	0.00	0.00	0.00	0.00	0.00	1.69	0.00	0.00	0.00	0.00	1.13	0.00	1.13	**2.26**	1.13	0.56	0.56	0.00	0.00	0.00	0.56	0.00	0.00	9.03
Diaporthe sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**1.69**	**1.69**	0.00	0.00	0.00	0.56	0.00	0.00	0.00	0.00	1.13	0.00	0.00	0.00	0.00	0.00	5.08
Fusarium oxysporum	0.00	0.00	0.00	0.00	0.00	1.69	2.26	0.00	0.00	1.13	0.00	0.56	0.00	2.26	0.00	1.13	1.69	0.00	1.13	0.56	**5.65**	0.00	0.00	0.56	**18.64**
Lasiodiplodia sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**2.26**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.26
Nigrospora sp.	1.69	0.56	0.00	**3.95**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.56	0.56	0.00	0.00	0.00	0.00	1.13	0.00	0.56	0.00	0.00	9.03
Penicillium sp.	1.13	0.00	0.56	**2.26**	0.00	0.00	0.00	0.00	1.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.56	0.00	0.00	0.56	0.00	1.13	0.00	0.00	7.34
Hypocrea koningii	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.13	0.00	0.00	0.00	0.00	0.00	0.00	**2.26**	3.38

*AMB = Ambassa; DHA = Dhwajanagar; DUS = Duski; JAF = Jalefa; KAL = Kailashahar; NAN = Nandannagar; PDM = Padmanagar; RAN = Rajnagar

Table 6. Colonization rate in different tissue types collected from eight different sampling sites during summer and winter seasons

Tissue types	Sampling sites																	
	Colonization rate									Isolation rate								
Season: Summer																		
	AMB	**DHA**	**DUS**	**JAF**	**KAL**	**NAN**	**PDM**	**RAN**	**Overall**	**AMB**	**DHA**	**DUS**	**JAF**	**KAL**	**NAN**	**PDM**	**RAN**	**Overall**
Leaf	100	100	100	90	100	100	60	100	93.75	**0.6**	0.1	0.3	0.56	0	0	0.5	0	0.25
Stem	100	100	100	100	100	80	70	100	93.75	**0.6**	0.4	0.4	0.1	0.1	0.38	0	0.2	0.27
Root	100	100	100	90	100	80	90	100	95	0.6	0	0	0.22	**1**	0.38	0	0.4	0.32
Season: Winter																		
	AMB	**DHA**	**DUS**	**JAF**	**KAL**	**NAN**	**PDM**	**RAN**	**Overall**	**AMB**	**DHA**	**DUS**	**JAF**	**KAL**	**NAN**	**PDM**	**RAN**	**Overall**
Leaf	90	100	100	90	100	100	90	100	96.25	0.33	0.6	0.1	**1**	0.9	0.6	0.44	0	0.49
Stem	80	100	100	100	100	80	70	100	91.25	0.5	0.4	0	**0.9**	0.7	0.63	0.71	0.2	0.5
Root	100	100	80	80	100	30	70	30	73.75	0.5	0.2	**0.5**	0.38	0.4	0.33	0.43	0.33	0.38

*AMB = Ambassa; DHA = Dhwajanagar; DUS = Duski; JAF = Jalefa; KAL = Kailashahar; NAN = Nandannagar; PDM = Padmanagar; RAN = Rajnagar

Table 7. Analysis of the diversity of fungal isolates from *Chromolena odorata*

Diversity indices	Sampling sites								Tissue types			Season	
	AMB	DHA	DUS	JAF	KAL	NAN	PDM	RAN	Leaf	Root	Stem	Summer	Winter
Shannon (H')	2.143	1.498	1.395	1.838	1.265	1.996	1.719	1.369	1.061	1.921	2.095	2.101	1.85
Simpson (1 -D)	0.867	0.713	0.713	0.813	0.694	0.836	0.778	0.703	0.642	0.805	0.863	0.857	0.818
Pilou evenness (J)	0.376	0.324	0.324	0.37	0.278	0.359	0.434	0.437	0.309	0.732	0.442	0.526	0.745
Fisher alpha (α)	3.473	2.414	3.4	3.234	3.843	4.774	3.305	2.387	3.57	1.178	4.208	2.497	1.576
Brillouin (HB)	1.898	1.71	1.771	1.88	1.659	1.787	1.16	1.106	1.531	1.117	1.333	1.074	0.793
Berger -Parker (B)	0.228	0.276	0.279	0.206	0.354	0.235	0.471	0.412	0.3	0.412	0.389	0.438	0.444
Simpson Dominance_D	0.132	0.173	0.15	0.124	0.178	0.107	0.243	0.243	0.159	0.285	0.176	0.25	0.278

*AMB = Ambassa; DHA = Dhwajanagar; DUS = Duski; JAF = Jalefa; KAL = Kailashahar; NAN = Nandannagar; PDM = Padmanagar; RAN = Rajnagar

isolated endophytic fungal strains from different tissue types and locations (Table 5). The RF of endophytic fungal genera *Nigrospora* sp. (3.95) was highest in the leaf tissues collected from Jalefa site, followed by *Colletotrichum* sp. (2.82) from Dhwajanagar and *Penicillium* sp. (2.26) from Jalefa site and the lowest RF was shown by *Aspergillus* sp. from Ambassa and Jalefa sites, respectively. In stem tissues, *Botryosphaeria rhodina* was collected from Duski site showing the highest RF, immediately followed by *Corynespora* sp. (2.26) and *Curvularia* sp. (2.26) collected from Kailashahar and Padmanagar sites, whereas in root segments *Fusarium oxysporum* (5.65) shown highest RF collected from Jalefa site and was followed by *Hypocrea koningii* (2.26) from Rajnagar site (Table 5).

Colonization and isolation rate of endophytic fungi

In the present study, the colonization rate of endophytic fungi from different tissue types was determined. The highest colonization rate was recorded in leaves (95%) followed by stem (92.5%) and root tissue (84.37%), whereas the isolation rate was highest in stem tissues (0.77) followed by leaf tissues (0.37), and the least was observed in root tissues (0.35). Season-wise variations on colonization rates and isolation rates were observed (Table 6).

During the summer season, location-wise data on the colonization rate and isolation rate concerning different explants showed maximum CR in leaf tissues ranges from (100%) in most of the sites and the lowest was observed in the Padmanagar (60%) site, in the case of stem tissues except Nandanagar (80%) and Padmanagar (70%) sites, the rests showed 100% colonization whereas results in root tissues depicted 100% colonization in Ambassa, Dhwajanagar, Duski and Kailashahar sites while lowest was observed in Nandannagar (80%) site. The highest IR values in the leaf and stem tissues was recorded in Ambassa site whereas, the highest IR value in root explants was observed in Kailashahar. During the winter season also 70 - 100% colonization was recorded in all tissue types except in Padmanagar and Rajnagar (30%) site showing less colonization by the endophytic fungi in the case of root tissues. Isolation rate values from leaf tissues showed that the highest isolation was from Jalefa site (1.0) and the lowest (0.1) was observed in the Duski site. In stem tissues, the highest and lowest isolation of endophytic fungi was recorded at Jalefa (0.9) and Duski (0) sites, respectively. In contrast, in the case of root tissues highest (0.5) and lowest

(0.2) isolation were observed in the Dhwajanagar site.

Diversity analysis of endophytic fungi

The diversity of the endophytic fungal communities isolated from several tissue types and sampling locations were compared using diversity indices. The diversity indices of endophytic fungal species associated with *C. odorata* are summarized in Table 7. The highest biodiversity of endophytic fungi was observed in summer, with *Shannon (H')*, *Simpson (1-D),* Fisher alpha (α), and Brillouin (HB) indices reaching maximum values of 2.101, 0.857, 2.497, and 1.074 respectively. Among the different plant tissues, the highest biodiversity of endophytic fungi was in the stem, with *Shannon (H')*, *Simpson (1-D)* & Fisher alpha (α) diversity reaching maximum values of 2.095, 0.863 & 4.208, respectively. However, Berger-Parker (B) & *Simpson Dominance_D* was highest among the root tissues and winter season. Thus, there was considerable diversity in the number and population distribution of endophytic fungi in different seasons and plant parts of *C. odorata*. The highest Pielou's evenness index value of endophytic fungi was higher in the winter (0.745) compared to the summer (0.526) season. Among the different tissues, endophytic fungi in the root showed the highest Pielou's evenness index (0.732) followed by the stem and leaf tissue segments, whereas, Pielou's evenness index (0.437) value found to be highest in Rajnagar site in comparison to other sites Across the different locations, *Shannon (H')* and *Simpson (1-D)* was highest in the Ambassa site signifying the highest biodiversity of endophytic fungi whereas, Berger-Parker (B) & *Simpson Dominance_D* was highest in the Padmanagar site and Fisher alpha (α) values was recorded highest in Nandannagar site.

Discussion

Isolation and identification of fungal endophytes from invasive plants provide information about their composition in various atmospheric conditions, varied geomorpholocial regions, and tissue types. The current study on the biodiversity and seasonal variation of endophytic fungi from *Chromolaena odorata*, an invasive alien weed of Tripura, Northeastern India is one of the first attempts carried out from Tripura, a biodiversity hot spot region of India. In this study, a total of 177 fungal endophytes were isolated from 480 different plant parts of *C. odorata*. Some of these isolates were morphologically similar after macroscopic and microscopic observations. Only those fungal isolates that differed morphologically were identified and treated as different endophytic fungal isolates, whereas isolates that showed similar characteristics were regarded as repeats. A total of 14 endophytic fungi were isolated from the host plant, among which 11 fungal endophytes were identified using morphological- macroscopic characteristics till genus level whereas, 4 were identified till species using molecular techniques. In the current study, all the isolated endophytic fungal taxa belong to the phylum Ascomycota, which further grouped into 3 classes (Sordariomycetes, Dothideomycetes, and Eurotiomycetes) were similar to the findings of Gopane et al. 2021; Goveas et al., 2011; Kumar & Prasher., 2022, in their studies on *Coscinium fenestratum*, *Nigella sativa* and *Dillenia indica*. Studies suggested that most fungal strains that were reported as fungal endophytes isolated from different parts of plant species belongs to Ascomycota, Basidiomycota, and Zygomycota (Carvalho et al., 2012). Each host plant species is considered as store house of not less than one fungal endophyte taxa (Strobel and Daisy, 2003), which make up a huge biodiversity. Several studies by various researchers confirmed endophytic taxa from different host plants (Ferreira et al., 2015). The composition of the endophytic fungal assemblage within a plant was determined by both the fungi and the host plant species. Sadeghi et al., (2018) in their studies on diversity and spatiotemporal distribution of fungal endophytes associated with *Citrus reticulata* reported that the sampling season, collection site, and tissue type were the key determining factors of fungal endophyte composition. Fang et al., (2019) reported a significant level of tissue-specific endophytes from an invasive plant *Ageratina adenophora*. In the current study, both location-specific and tissue/organ-specific endophytic fungi were recorded. *Chaetomium* sp. and *Aspergillus* sp. were recorded as leaf endophyte, whereas, *Diaporthe* sp. as the stem endophyte while *Cladosporium* sp. and *Trichoderma* sp. as root endophyte. Okane et al. (1998), reported *Colletotrichum gloeosporioides* as a leaf endophyte. *Fusarium oxysporum* is the only genus which was present in all the plant tissues and location under study. The plant–endophyte relationship is very dynamic, in the sense that fungal endophyte composition varies temporally across months and seasons (Currie et al., 2014). In the present investigation, we recorded a significant level of seasonal variation with respect to the colonization of season-specific fungal strains. The genera *Aspergillus* sp. & *Cladosporium* sp. were isolated only during the summer season, whereas, *Corynespora* sp., *Lasiodiplodia* sp., and *Nigrospora* sp. were recorded only during the winter season while the taxa like *Colletotrichum* sp., *Fusarium oxyspporum*, *Curvularia* sp., *Diaporthe* sp. *Penicillium* sp. were recorded in both the sampling seasons. The highest biodiversity of endophytic fungi was observed in summer compared to winter. The differences in colonization frequency of different endophytic fungi in different tissues, season-wise, and location were recorded in our study, this may be due to the inhabiting potentiality of endophytic fungi showing varied degrees of affinities towards plant organs. Dhayanithy et al., (2019) reported similar findings in their studies on *Catharanthus roseus.*

Conclusions

This study analyzed the endophytic fungal diversity and seasonal variation of *Chromolaena odorata*, an invasive alien weedy perennial herb in Tripura, Northeastern India. This study identified a diverse fungal assemblages with dominant genera, including *Colletotrichum*, *Fusarium*, *Corynespora*, and *Nigrospora*. Seasonal and geographic variations were observed. Most of the isolated fungi belong to the class Sordariomycetes of the phylum Ascomycota. This research work shed light on the unique endophytic fungal diversity associated with *Chromolaena odorata.*

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