J.Mycopathol.Res. 62(2) : 293-303, 2024, ISSN : 0971-3719 (Print), 2583-6315 (Online)
10.57023/JMycR.62.2.2024.293

Endophytic Fungal Diversity and Seasonal Variation in *Parthenium hysterophorus* L.: an invasive plant species of Tripura, Northeast India

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Received : 22.01.2024 Accepted : 20.04.2024 Published : 24.06.2024

Endophytic fungi are microbes that colonize or infect different living tissues of host plants without developing any disease symptoms. The diversity and seasonal variations of endophytic fungal colonization across different tissues of *Parthenium hysterophorus*, an invasive herbaceous weed of Asteraceae, were analyzed in this study. A total of 256 fungal isolates were recovered from different tissues of this weed collected from eight different geographical locations of Tripura. The total number of isolates recovered from leaf, stem, and root tissue segments were 108, 77, and 71, respectively. Based on the morphological and molecular identification,the isolated endophytic fungi were grouped into 13 genera representing 11 families of the phylum Ascomycota along with two non-sporulating forms. The colonization rate of endophytic fungi was highest in leaves (95%) followed by stem (88.12%) and root tissue (65%), respectively, while the isolation rate was highest in root tissues (0.75) followed by leaf tissues (0.72) and the least was observed in stem tissues (0.56). Tissue-specific and season-specific fungal strains were observed. The diversity of fungal endophyte composition varied significantly across sampling locations, tissue types, and seasons. This study is the first attempt to study endophytic fungal diversity and seasonal variation from the invasive herbaceous weed species *P. hysterophorus* of Tripura, Northeastern India.

Keywords: Ascomycota, Asteraceae, diversity, endophytic fungi

INTRODUCTION

Fungal endophytes are a hyperdiverse group of microorganisms that colonize inside healthy and disease-free tissues of almost all known plants **(**Arnold, 2007**).** These organisms provide a higher rate of resistance in the host plant against biotic and abiotic stresses by producing novel, biologically active diverse secondary metabolites that are produced by the endophytic fungi but not by the plant themselves (Moncrieff *et al.* 2015; Mishra *et al.* 2016; Pieterse *et al*. 2018).

Endophytic fungi promote the growth of host plants by producing growth-promoting substances. Endophytic fungal communities are highly influenced by several components, like host species studied, tissue types, geographic locations, and edaphic factors (Yadav *et al*. 2016). The endophytic fungal strains help in protecting the host from toxicity effects and in doing so, it contributes in developing resilience against inclement climatic conditions (Martinez-Arias *et al.* 2020). Seasonality is the driving factor in influencing endophytic fungal composition (Martins *et al.* 2016; Yadav *et al.* 2016). The analysis of the diversity of fungal endophytes in different host plants, tissue types, seasons, and geographic locations is important, as it paves the way to illuminate and understand their utility and

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frequency within a particular host plant (Yokoya *et al.* 2017). Endophytes degrade dead and decomposing tissues of plants, which are vital for nutrient cycling in an ecosystem (Saikkonen *et al.* 2015). Endophytes are reported from plants thriving in various environmental conditions ranging from tropical, temperate, xerophytic, and aquatic habitats (Rather *et al.* 2018). The abundance and distribution of fungal endophytes differ among plant species andare influenced by the selected host plant, the time of sampling, and the location (Unterseher *et al.* 2007).

Alien invasive plants are exotic plants introduced deliberately or unintentionally to a particular area or a region and tend to spread causing damage to the native biodiversity with subsequent disturbance to the ecosystem services and/or human health (Potgieter *et al.* 2018). Invasive plants, whenever introduced into unique areas, tend to develop a novel relationship with the micro-organisms and might bring about an impact on ecosystems. The endophytic fungi isolated from economically and medicinally important plants exhibited the potentiality to synthesize secondary metabolites or their derivatives that are useful in the pharmaceutical industry (Strobel, 2001). However, less priority was given to characterizing the endophytic fungal assemblages from invasive species. These invasive species invade new ranges and alter the compositions of local plant populations by producing novel allelochemicals that might positively or negatively impact the surrounding ecosystem (Shipunov *et al.* 2008; Motmainna *et al.* 2021). Studies on endophytic fungal assemblages in these plants may provide valuable insights into bringing out new sources of secondary metabolites for biotechnological applications.

Parthenium hysterophorus is an annual herbaceous invasive alien plant species of the family Asteraceae (Bashar *et al.* 2021). This plant is native to South and North America and has invaded nearly 46 countries to date. In India, this species is commonly called Congress grass and was reported around 1950 (Kaur *et al.* 2021). Nowadays this plant is known as one of the most destructive weed species in the world. It is harmful to agriculture and is responsible for multiple human diseases, although the species is used as folklore medicine to treat various diseases in several parts of the world (Bashar *et al.* 2021). It is a potential agent to be used as a source of herbicide, insecticide, and waste treatment management (Bashar *et al.* 2021). The knowledge of the composition and diversity of endophytic fungi in invasive plant species *P. hysterophorus* is meager. So, this study aimed to isolate and identify the fungal endophytes inhabiting different vegetative parts like leaf, root, and stem explants of *P. hysterophorus* collected from different sampling sites of Tripura, North-east, India and to compare the diversity composition in different tissues and seasons.

MATERIALS AND METHODS

Plant sample collection

Five healthy and disease-free *Parthenium hysterophorus* plants were collected randomly from eight locations in Tripura (Table 1; Figs. 1& 2). 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 was identified by the expert researchers of Plant Taxonomy and Biodiversity Laboratory, Department of Botany, Faculty of Science, Tripura University under the guidance of Prof. B. K. Datta. A voucher specimen was kept in the herbarium with proper tagging in the Department of Botany, Tripura University, India.

Isolation of endophytic fungi

To isolate endophytic fungi, first of all, the roots, leaves, and stems were separated from the parent body. Samples were 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; Hatamzadeh *et al.* 2020) 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 - 5 mins,depending on the type of tissue), rinsing with sterile distilled water, and allowed to surface dry under sterile conditions.In order to assure proper surface

sterilization, the surface sterilization protocol was authenticated using leaf imprint method (Schulz *et al.* 1998; Bhattacharya *et al.* 2019). Ten segments of each explant were randomly chosen from each sampling location for isolation. 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 1-2 weeks and were observed for hyphal growth. All observed fungal growths were sub-cultured on MEA plates for purification.

Morphological identification of endophytic fungi

Both macroscopic characteristics (color, consistency, etc.) and microscopic characteristics (morphology of vegetative and reproductive structures) were considered for the primary identification of endophytic fungi. The Lactophenol cotton blue staining technique was followed for the microscopic identification of isolates. The standard manuals and literature were used for the identification of isolated fungi. (Ellis, 1971; Domsch *et al*. 1980; 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

Sequences were deposited to 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; Tamura *et al.* 2021).

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.

CR = 100 x (total no. of segments yielding e" 1 isolate) / (total no. of segments incubated). This could be used for comparative purposes and could compare the degrees of infection by endophytic fungi between different plants and tissues.

IR = (Total no. of isolates yielded in a segment e" 1 isolate)/ (total no. of segments incubated). IR could measure fungal richness in a given sample of plant tissues and the incidence of multiple infections per plant segment in this study.

The Relative frequency (RF) was used to measure a specific endophytic taxon studied from the tissue or plant and was calculated as the number of isolates of one individual species divided by the total number of isolates of all species and expressed as a percentage. The Colonization frequency (%CF) of endophytic fungi represents the extent of endophytic colonization and was calculated using the formula CF = Ncol/ Nt x 100, where,Ncol is the number of segments colonized by each fungus and Nt is the total number of incubated segments studied.

Diversity indices

The diversity of the endophytic fungal community was evaluated using various diversity indices,

such as the Shannon–Weaver diversity index (*H*2), Simpson's dominance (*D*), the Dominance index (1- *D*), Equitability (*J*), Fisher's alpha diversity index (á), Berger-Parker dominance (*B*) and Brillouin index (*HB*) using R- programming software (R Core Team, 2022).

RESULTS AND DISCUSSION

Isolation and identification of endophytic fungi

A total of 256 fungal endophytes were isolated from 480 explants containing 160 explants of stem, root, and leaf tissues, respectively, collected from 8 different geographical locations in Tripura (Fig.1; Table 2). Among them, 108 isolates were recovered from leaves, 77 from stems, and 71 from root tissue segments. All the fungal isolates were assessed for macroscopic (colony color, consistency, etc.) and microscopic characteristics (morphology of vegetative and reproductive structures-spores).Based on macroscopic and microscopic observations, the fungal isolates were grouped into 13 genera belonging to 11 families along with two non-sporulating forms (non sporulating hyaline form and non sporulating dematiaceous form) (Table3). During this study it was observed that *Curvularia lunata* (RF = 13.67%) and *Fusarium* sp.(RF = 11.72%) werethe most dominant among the total isolates followed by *Penicillium oxalicum* (RF = 9.77%). The other genera, such as *Aspergillus*, *Alternaria*, *Chaetomium*, *Colletotrichum*, *Corynespora*, *Diaporthe*, *Nigrospora*, *Corynespora smithii*, *Botryosphaeria rhodina*, and *Trichoderma* were also recorded at low proportions.In the present study, all the isolated endophytic fungi belonged to the phylum Ascomycota. The dominant classes of endophytic fungi were-Sordariomycetes (26.56%), Dothideomycetes (25.8%) and Eurotiomycetes (16.01%). The genera *Penicillium oxalicum*, *Curvularia lunata*, *Fusarium* sp.,*Corynespora smithii*, *Colletotrichum* sp., *Corynespora* sp., *Diaporthe* sp., were commonly obtained from leaves, stem and root explants. *Botryosphaeria rhodina* and *Trichoderma* sp. were isolated only from root tissues whereas *Nigrospora* sp. was obtained from leaf tissue only. *Alternaria* sp. was isolated from above-ground parts only while *Aspergillus* sp. and *Chaetomium* sp., were isolated from leaf and root segments except that of stem. Non sporulating hyaline form and Non sporulating dematiaceous form were isolated from all tissue types and in both the season. Some of the endophytic species occurred preferentially in one or two of the seasons. *Nigrospora* sp. and *Trichoderma* sp. were not recovered during the sampling season summer; similarly, *Alternaria* sp. and *Botryosphaeria rhodina* were not isolated in the winter season.

Molecular identification and phylogeny analysis

Amplification of SSU regions 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 3. Based on the molecular data endophytic fungal isolates namely, PH6, PH8, PH9, PH11 and PH12 were identified as *Curvularia lunata*,*Corynespora smithii*, *Fusarium* sp., *Penicillium oxalicum* and *Botryosphaeria rhodina*. According to 18S rRNA gene similarity isolate PH6 showed 100% sequence homology with *Curvularia lunata*, PH8 showed 100% sequence homology with *Corynespora smithii*, PH9 showed 100% sequence homology with*Fusarium* sp., PH11 showed 95% sequence homology with *Penicillium oxalicum* and PH12 showed 100%sequence homology with *Botryosphaeria rhodina*, respectively. The18S rRNA sequences of all the isolates were submitted to NCBI Genbank and accession numbers were obtained accordingly. Phylogenetic analysis using Neighbor-joining method was performed. The Phylogenetic tree was shown in Fig. 3.

Colonization and isolation rate

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 (88.12%) and root tissue (65%) respectively, whereas the isolation rate was highest in root tissues (0.75) followed by leaf tissues (0.72) and the least was observed in stem tissues (0.56) (Table 2).

Table 1: Details of the sampling sites explored in this study

Collection sites	(code)	Latitude	Longitude	Elevation
Ambassa	(AMB)	23°55'26"N	91°51'21"E	81.3
Badharghat	(BAD)	23°48'15"N	91°16'21"E	21.8
Bishalgarh	(BIS)	23°41'30"N	91°16'20"E	38.0
Nabincherra	(NAB)	24°12'11"N	92°08'02"E	56.6
Pecharthal	(PER)	24°10'59"N	92°05'53"E	65.2
Santir Bazar	(SAN)	23°18'56"N	91°33'35"E	47.4
Teliamura	(TEL)	23°50'37"N	91°38'09"E	57.9
Udaipur	(UDP)	23°31'03"N	91°27'31"E	23.3

by Teliamura (5) and Bishalgarh site (0), respectively.

During the winter season, 100% colonization was recorded in leaves and stem explants collected from all the sites whereas CR value was highest (100%) in Ambassa, Pecharthal, and Udaipur sites and lowest (50%) was observed in Nabincherra site in case of root tissues. Variations concerning IR were also recorded. Isolation rate values from leaf tissues showed that the highest isolation was from Badharghat site (1.2) and the lowest (0.1) was observed in the Santirbazar site. In stem tissues, the highest and lowest isolation of endophytic fungi was recorded at Udaipur (0.7) and Ambassa (0) sites, respectively. In contrast, in the case of root

Table 2:The recovery and colonization of endophytic fungal isolates in different tissues of *Parthenium hysterophorus*.

Plant Part	Season of Sampling	No. of Studied tissues	Isolates recovered/generated	Colonization rate (CR) CR%
Leaf	Summer	80	72	90
	Winter	80	80	100
	Total	160	152	95
Stem	Summer	80	62	77.5
	Winter	80	79	98.75
	Total	160	141	88.12
Root	Summer	80	37	46.25
	Winter	80	67	83.75
	Total	160	104	65

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 least was observed in the Badharghat (30%) site, in the case of stem tissues highest CR was observed in Nabincherra and Pecharthal (100%) sites compared to lowest in Ambassa (50%) site whereas results in root tissues depicted highest in Pecharthal (100%) and lowest in Bishalgarh (10%) site. IR values in the leaf tissues showed highest in Santirbazar (1.5) and lowest in Ambassa (0.22). The highest IR value in stem explant was observed in Udaipur (1.75) and lowest in Teliamura (0.11) whereas the higher and lower rate of IR in roots was shown tissues highest (1.00) and lowest (0) isolation were observed in the Badharghat and Bishalgarh sites, respectively.

Colonization frequency (CF) and relative frequency (RF)

The occurrence and dominance of isolated endophytes were calculated by measuring colonization frequency (%CF) in all tissue types(Table4). In leaves, the highest %CF was shown by the Non sporulating hyaline form (17.5%), followed by the Nonsporulating dematiaceous form (11.25%),*Curvularia lunata* (8.75%), and the least was shown by *Nigrospora* sp. (1.25%) (Table3). In stem, the highest was

Table 3: Evaluated characteristics profile of endophytic fungi associated with *Parthenium hysterophorus*

Isolate Id	GenBank accession number	Similarity (%)	Fungal Taxa	Fungal family	Fungal order	Class *
PH1	-	-	*Alternaria* sp.	Pleosporaceae	Pleosporales	Dothideomycetes
PH2	-	-	*Aspergillus* sp.	Aspergillaceae	Eurotiales	Eurotiomycetes
PH3	-	-	*Chaetomium* sp.	Chaetomiaceae	Sordariales	Sordariomycetes
PH4	-	-	*Colletotrichum* sp.	Glomerellaceae	Glomerellales	Sordariomycetes
PH5	-	-	*Corynespora* sp.	Corynesporascaceae	Pleosporales	Dothideomycetes
PH6	PP177488	100	*Curvularia lunata*	Pleosporaceae	Pleosporales	Dothideomycetes
PH7	-	-	*Diaporthe* sp.	Diaporthaceae	Diaporthales	Sordariomycetes
PH8	PP177489	100	*Corynespora smithii*	Corynesporascaceae	Pleosporales	Dothideomycetes
PH9	PP177486	100	*Fusarium* sp.	Nectriaceae	Hypocreales	Sordariomycetes
PH10	-	-	*Nigrospora* sp.	Apiosporaceae	Xylariales	Sordariomycetes
PH11	PP177487	95	*Penicillium oxalicum*	Aspergillaceae	Eurotiales	Eurotiomycetes
PH12	PP177485	100	*Botryosphaeria rhodina*	Botryosphaeriaceae	Botryosphaeriales	Dothideomycetes
PH13	-	-	*Trichoderma* sp.	Hypocreaceae	Hypocreales	Sordariomycetes
PH14	-	-	Nonsporulating hyaline form	-	-	-
PH15	-	-	Nonsporulating dematiaceous form	-	-	-

* All classes belong to Acomycota

Table 4: Colonization frequency of endophytic fungi in different tissues of *Pathenium hysterophorus*

Endophytic fungi	Total Leaf seg.	LEAF		Total Stem Seg.	STEM		Total Root Seg.	ROOT		
		NOI	%CF		NOI	%CF		NOI	%CF	Total %CF
Alternaria sp.	160	8	5.000	160	2	1.25	160	0	0	2.08
Aspergillus sp.	160	9	5.625	160	0	0	160	7	4.375	3.33
Chaetomium sp.	160	3	1.875	160	0	0	160	3	1.875	1.25
Colletotrichum sp.	160	5	3.125	160	4	2.5	160	5	3.125	2.92
Corynespora sp.	160	3	1.875	160	2	1.25	160	2	1.250	1.46
Curvularia lunata	160	14	8.750	160	18	11.25	160	3	1.875	7.29
Diaporthe sp.	160	4	2.500	160	7	4.375	160	4	2.500	3.13
Corynespora smithii	160	6	3.750	160	1	0.625	160	2	1.250	1.88
Fusarium sp.	160	4	2.500	160	8	5	160	18	11.250	6.25
Nigrospora sp.	160	2	1.250	160	0	0	160	0	0.000	0.42
Penicillium oxalicum	160	4	2.500	160	16	10	160	5	3.125	5.21
Botryosphaeria rhodina	160	0	0	160	0	0	160	5	3.125	1.04
Trichoderma sp.	160	0	0	160	0	0	160	1	0.625	0.21
Nonsporulating hyaline form	160	28	17.500	160	13	8.125	160	12	7.500	11.04
Nonsporulating dematiaceous form	160	18	11.250	160	6	3.75	160	4	2.500	5.83

Table 5: The relative frequency of endophytic fungi in different tissues of *Parthenium hysterophorus* in different seasons and sampling sites

Fungal Taxa	Plant parts			Sampling sites								Season					
	L	S	R	AMB	BAD	BIS	NAB	PER	SAN	TEL	UDP	Summer	RF	Winter	RF	Total	RF
Alternaria sp.	8	2	0	0	2	4	4	0	0	0	0	10	6.71	0	0.00	10	3.91
Aspergillus sp.	9	0	7	0	0	0	2	12	0	1	1	13	8.72	3	2.80	16	6.25
Chaetomium sp.	3	0	3	0	0	0	3	0	0	2	1	5	3.36	1	0.93	6	2.34
Colletotrichum sp.	5	4	5	3	5	0	2	0	0	4	0	6	4.03	8	7.48	14	5.47
Corynespora sp.	3	2	2	3	0	0	2	0	0	2	0	2	1.34	5	4.67	7	2.73
Curvularia lunata	14	18	3	0	13	2	5	0	8	0	7	12	8.05	23	21.50	35	13.67
Diaporthe sp.	4	7	4	3	1	1	0	1	2	7	0	10	6.71	5	4.67	15	5.86
Corynespora smithii	6	1	2	2	0	1	4	0	2	0	0	2	1.34	7	6.54	9	3.52
Fusarium sp.	4	8	18	6	7	0	10	1	0	5	1	19	12.75	11	10.28	30	11.72
Nigrospora sp.	2	0	0	0	0	0	0	0	0	2	0	0	0.00	2	1.87	2	0.78
Penicillium oxalicum	4	16	5	1	2	0	3	12	2	3	2	20	13.42	5	4.67	25	9.77
Botryosphaeria rhodina	0	0	5	0	0	0	0	5	0	0	0	5	3.36	0	0.00	5	1.95
Trichoderma sp.	0	0	1	0	0	0	0	1	0	0	0	0	0.00	1	0.93	1	0.39
Nonsporulating hyaline form	28	13	12	0	5	6	11	0	8	9	14	29	19.46	24	22.43	53	20.70
Nonsporulating dematiaceous form	18	6	4	3	2	0	2	6	6	6	3	16	10.74	12	11.21	28	10.94
Total	108	77	71	21	37	14	48	38	28	41	29	149	-	107	-	256	-

* L= Leaf, S = Stem, R = Root; AMB = Ambassa, BAD = Badharghat, BIS = Bishalgarh, NAB = Nabincherra, PER = Pecharthal, SAN = Santir Bazar, TEL = Teliamura, UDP = Udaipur

Table 6: Diversity indices ofendophytic fungal communitiesfrom different tissues of *Parthenium hysterophorus*, obtained from different sampling sites and locations

Diversity indices	Tissue type			Sampling location								Sampling season	
	Leaf	Root	Stem	AMB	BAD	BIS	NAB	PER	SAN	TEL	UDP	Summer	Winter
Species richness (S)	13	13	10	7	8	5	11	7	6	10	7	13	13
Shannon (H')	2.240	2.100	1.730	1.667	1.475	1.213	2.044	1.351	1.154	1.925	1.234	2.200	2.040
Simpson (1-D)	0.877	0.838	0.787	0.790	0.720	0.656	0.847	0.691	0.612	0.834	0.611	0.874	0.831
Evenness (J)	0.450	0.520	0.380	0.340	0.300	0.290	0.350	0.390	0.560	0.600	0.290	0.490	0.520
Dominance_D	0.200	0.260	0.130	0.120	0.160	0.110	0.150	0.160	0.250	0.340	0.130	0.290	0.330
Fisher alpha (α)	2.520	2.260	3.920	3.110	3.640	3.890	4.130	3.150	3.180	1.870	3.950	2.180	3.220
Berger-Parker (B)	1.546	1.247	1.721	2.022	1.826	1.985	1.834	1.319	0.842	0.893	1.573	1.150	0.897
Brillouin (HB)	0.310	0.433	0.286	0.192	0.324	0.226	0.327	0.333	0.500	0.570	0.270	0.375	0.583

*AMB = Ambassa, BAD = Badharghat, BIS = Bishalgarh, NAB = Nabincherra, PER = Pecharthal, SAN = Santir Bazar, TEL = Teliamura, UDP = Udaipur

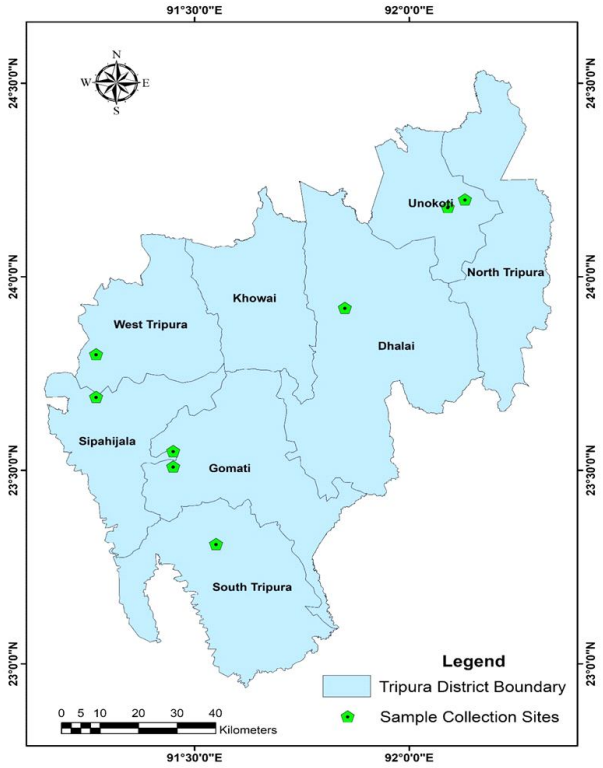

Fig.1: Map of study area showing the sample collection sites across different districts of Tripura

Fig.2: Photographs of *Parthenium hysterophorus* L. plant. A. Plants in their natural habitat; B. Whole plant body; C. Plant twig with flowering parts; D. Herbarium (TUH-2396)

recorded by *Curvularia lunata*(11.25%), followed by *Penicillium oxalicum* (10.00%), Nonsporulating dematiaceous form (8.12%), *Diaporthe* sp. (4.37%) and the lowest was in *Alternaria* sp. (1.25%). In root tissues, the highest %CF was observed in *Fusarium* sp.(11.25%) followed by Nonsporulating hyaline form (7.5%), *Aspergillus* sp.(4.37%) and the least was by *Trichoderma* sp. (0.62%). The relative frequency (Table5) of endophytic fungal genera is highest in

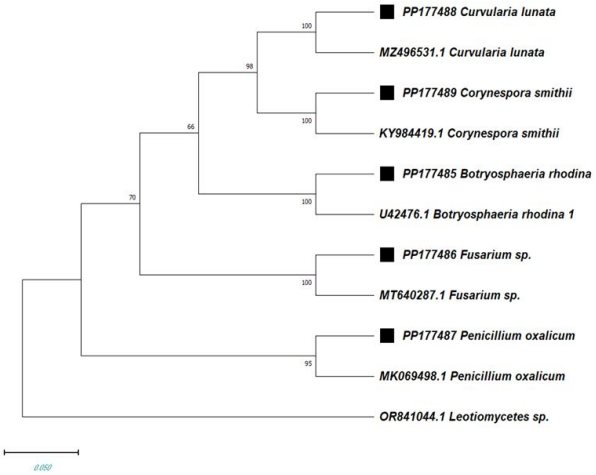

Fig. 3: Phylogenetic tree showing the relationships of the isolates to closely related fungi. The numbers at branching points refer to bootstrap values, based on 1000 replicates

Nonsporulating hyaline form (20.7%), followed by *Curvularia lunata* (13.67%), *Fusarium sp.* (11.72%), Nonsporulating dematiaceous form (10.94%), *Penicillium oxalicum* (9.77%) and the least was recorded in *Trichoderma* sp. (0.39%). Seasonal influence on RF was recorded. It was observed that the RF of *Curvularia lunata* was higher during winter (21.50%) compared to summer (8.05%), similar results were also observed for *Colletotrichum* sp., *Corynespora* sp., and *Corynespora smithii,* on the other hand, relative frequency of some of the isolates were higher in summer season in comparison to winter season. RF of *Penicillium oxalicum* was higher in summer (13.42%) and less during winter (4.67%) similar results were also recorded for fungal strains like *Aspergillus* sp., *Chaetomium* sp., and *Diaporthe* sp. (Table 4).

Diversity analysis

The fungal endophytes composition associated with *Parthenium hysterophorus* was determined, representing different sampling locations, tissue types, and seasons (Table 6). The higher Shannon diversity was observed at the Nabincherra site (2.044), followed by Teliamura (1.925), and the least Shannon diversity was found at Bishalgarh (1.213). The highest rate of fungal dominance was observed in Nabincherra (0.847), followed by Ambassa (0.790), and the lowest value was recorded in Udaipur (0.611). The Brillouin diversity was highest in the Ambassa site (0.570) followed by Santir Bazar (0.500), while the least was recorded in the Ambassa (0.192). The Evenness

value was highest at Teliamura (0.600), while the least evenness was found in Bishalgarh and Udaipur(0.290) sites, respectively (Table5). Similarly, the higher Fisher alpha value was observed at Nabincherra (4.130), followed by Udaipur (3.950), while the least was recorded at the Teliamura (1.870) site. Dominance_D values were highest in the Teliamura site (0.340) and the lowest was observed in Bishalgarh (0.110). Berger-Parker (B) indices showed the highest values in the Ambassa (2.022) site and the least was noted in the Santir Bazar (0.842) site.

The diversity of fungal endophyte composition varied significantly across tissue types (Table6). The Shannon diversity was highest in the leaf (2.240), followed by root tissues (2.100), and the least was observed in the stem (1.730). Simpson's (1-D) results also showed a similar pattern. The evenness value was highest in root tissues (0.520), followed by leaf (0.450) and stem (0.380). Similarly, the higher Fisher alpha index value was observed in the stem (3.920), followed by the leaf (2.520) and root (2.260), respectively. The highest Brillouin diversity was observed in root tissue (0.433), followed by leaf (0.310) and the least diversity was found in stem (0.286) tissues. Higher values of Dominance-D indices were recorded from root tissues followed by leaf and least values were observed in stem tissues. Similarly, Berger-Parker (B) indices were performed and results showed that the stem tissue possesses the highest value, followed by leaf and root tissues.Sampling site-wise variation in endophytic fungal composition was observed in our study. It was found that Nabincherra site with the highest number of endophytes followed by Teliamura site and the Bishalgarh site with the lowest number of endophytes. The influence of season on fungal endophyte diversity was noted. It was observed that diversity indices such as Shannon (H'), Simpson's (1-D), and Berger-Parker values were higher for summer endophytes than that of winter ones. Evenness (J), Dominance_D, Fisher alpha (á), and Brillouin (HB) indices were shown to have the highest values for winter endophytes compared to summer endophytes.

P. hysterophorus plants were studied for the composition of endophytic fungi (EF) from leaf, stem, and root tissues collected from eight geographical locations of Tripura, Northeast India. The endophytic fungal communities inhabiting the leaf, stem, and root tissues of *P. hysterophorus* were highly diverse. In this study, a total number of 256 EF were isolated and were assigned to 13 genera (Table 2). The highest number of isolates were generated by leaf tissues followed by stem and root tissues (Table 2). The diversity of EF is highly determined by the environmental conditions and geographical location of the host plant collected (Arnold *et al.* 2007; Chauhan *et al.* 2019).

The frequency of colonization was highest in the leaves and lowest in the roots, which may be because leaves provides highest surface area for inoculants (Chareprasert*et al.* 2006; Mishra *et al.*2012), and most importantly the leaf exposure time may also playa determining factor for the increased density of EF due to horizontal transmission(Chauhan *et al.* 2019). In addition, airborne spores from faraway sources can colonize leaves whereas roots get mainly colonized by the population of microorganisms present in the nearby soil (Mishra *et al.* 2012).In the present study, the fungal community was dominated by Ascomycetes and the endophytic fungal community belonged to the class Sordariomycetes, Dothideomycetes, and Eurotiomycetes which was in congruence with the previous studies (Mei *et al.* 2014; Rampadarath *et al.* 2018).Our study revealed that tissue specific endophytic fungi, for example, *Botryosphaeria rhodina* and *Trichoderma* sp. were isolated only from root tissues whereas *Nigrospora* sp. was obtained from leaf tissue only. Mishra *et al*. (2012) reported that the genera *Guignardia* and *Acremonium* were leaf tissue specific endophytic fungi, other than the rest of the isolates in their study from *Tinospora cordifolia*.

In the present study, it was noticed that the Evenness (J) index of EF was higher in root tissues compared to leaf and stem tissues which might be due to the fact that the environmental conditions in the soil were more stable and uniform than that of leaf tissues of the host plant, whereas diversity indices such as Shannon (H'), Simpson (1-D), Fisher alpha and Berger-

Parker, were higher in the leaves (Mishra *et al.* 2012).

The sampling seasons play an important role in the expression and diversity of endophytic fungal communities. Although in regards to the species richness, there was not much difference but diversity indices such as Shannon (H'), Simpson's (1-D), and Berger- Parker values were highest for summer endophytes in contrast to that of winter ones while Evenness (J), Dominance-D, Fisher alpha (α), and Brillouin (HB) indices were shown to have the highest values for winter endophytes compared to summer endophytes. The seasonal irregularities showed to contribute to the appearance of endophytic fungal assemblages in different plants and plant parts (Singh *et al.* 2016). In our study it was observed that the seasonal fluctuation contributed to the expression of fungal communities for example *Nigrospora* sp. and *Trichoderma* sp. could not be isolated during the summer season whereas *Alternaria* sp. and *Botryosphaeria rhodina* during the winter season. So, some of the endophytic species occurred preferentially in one or two of the seasons. Earlier studiesreported that (Kim *et al.* 2013; Mishra *et al.* 2016), the summer seasons favours the optimal growth ofhigher number of endophytic fungi than winter season which may be due to nutrient stress to the EF thus suppressing the expression of certain fungal species (Higgins *et al.* 2011).

CONCLUSION

This study on the invasive plant *P. hysterophorus* showed that the host species was highly enriched with culturable endophytic fungi. Our study further revealed that the culturable endophytic fungi of *P. hysterophorus* showed variation in colonization and isolation rates across different sampling sites as well as different tissues. The study demonstrated the occurrence of tissue and season-specific endophytic fungi in the host plant. Significant variations were observed in the colonization and relative frequency of isolated fungal strains. Variations were also observed in endophytic fungal composition in several sampling locations. The culturable endophytic fungi in the leaves showed more variation than those in the roots and stems. So, the study suggests that the alteration of environmental conditions is the driving force behind the fluctuations in the composition of endophytic fungi.

DECLARATIONS

Conflict of Interest: Authors declare no conflict of interest.

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