PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol x(x): xx–xx
https://doi.org/10.14719/pst.3045

HORIZON
e-Publishing Group
HePG

RESEARCH ARTICLE

Endophytic fungal diversity in *Terminalia arjuna* (Roxb.) Wight & Arn. of Tripura, Northeast India at different sampling sites and plant organs

Samrat Tripura[1], Prasenjit Debbarma[1,2], Suman Paul[3], Rahul Saha[1], Ajay Krishna Saha[1*]

[1]Mycology and Plant Pathology Laboratory, Department of Botany, Tripura University, Suryamaninagar, Tripura – 799 022, India
[2]Department of Botany, Netaji Subhash Mahavidyalaya, Dhwajanagar-799 120, Udaipur, Tripura, India
[3]Plant Taxonomy and Biodiversity Laboratory, Department of Botany, Tripura University, Suryamaninagar, Tripura – 799 022, India

*Email: aksaha.58@gmail.com

OPEN ACCESS

ARTICLE HISTORY

Received: 28 October 2023
Accepted: 12 February 2024

Available online
Version 1.0 : 13 June 2024

Check for updates

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc
See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

CITE THIS ARTICLE

Tripura S, Debbarma P, Paul S, Saha R, Saha A K. Endophytic fungal diversity in *Terminalia arjuna* (Roxb.) Wight & Arn. of Tripura, Northeast India at different sampling sites and plant organs. Plant Science Today (Early Access). https:/doi.org/10.14719/pst.3045

Abstract

Endophytic fungi are ubiquitous in plant kingdom and play a vital role in balancing the microenvironments within the host plants. Fungal endophytes isolated from ethno-botanically important plants were the source of several secondary metabolites with potential biological activities. The present study has documented the variability of culturable endophytic fungi from *Terminalia arjuna* (Roxb.) Wight & Arn. (Combretaceae), a widely used medicinal plant of Tripura, North-east, India. A total of 613 fungal strains were isolated from 720 tissue segments viz. leaves, bark and root of *T. arjuna*. The highest numbers of endophytic fungal isolates were found to be colonizing in leaves (257) followed by barks (182) and the least number of isolates were obtained from roots (174). The fungal isolates were classified into 27 individual fungal strains based on morphological and microscopic features. A total of 9 endophytic fungal strains were identified using the nuclear ribosomal DNA internal transcribe sequence and were subjected to phylogeny analysis. Most of the identified morphotypes belonged to phylum Ascomycota. Among all the isolates, *Diaporthe* sp., *Fusarium* sp., *Colletotrichum* sp., *Penicillium* sp. were the most abundant fungal isolates from *T. arjuna*. The fungal orders namely Sordariomycetes was the most prevalent followed by Eurotiomycetes and Dothideomycetes. Colonization rate, isolation rate, colonization frequency and relative frequency results suggest that leaf segments harbors maximum endophytic fungi assemblage compared to bark and root tissues. The analysis of Shannon (*H'*), Simpson (1 -*D*), Fisher alpha (*α*) and Brillouin (*HB*) indices had significant difference with locations and tissue type. The host plant harbor unique endophytic community composition in studied sampling locations and their colonization varied between the inoculated vegetative parts of the plant. As no such study has been undertaken in the North- eastern part of India, this pioneer study may enable to unearth novel fungal endophytes that might have beneficial role in plant growth promotion, stress tolerance etc.

Keywords

endophyte; ethnobotany; *Terminalia arjuna*; fungal diversity

Introduction

Endophytic fungi are micro-organisms that reside within the healthy plant tissues without causing any disease in the host plants (1). They coexist as symbionts with all terrestrial plants in diverse plant tissues in natural environments (2). According to fossil evidence, fungus and plants have coexisted as mutualists for 400 million years (3). These organisms are

common in phylogenetically and functionally diverse in natural environments (4). Endophytic fungi assist their host plants against several disease causing organisms such as pathogens, pests and herbivores (5). These organisms thrive in a variety of environmental circumstances, including aquatic, temperate, tropical and xerophytic habitats (6). Some endophytic fungi can manufacture metabolites that are identical to the ones made by their hosts, protecting their hosts by making natural substances that the host' plants lack. These substances have been utilized in industry, agriculture and medicines in recent time (7). Some of the biological activities of secondary metabolites produced by endophytic fungi are antioxidant, antibacterial, antimicrobial, antifungal, antiviral, anticancer and immunosuppressive capabilities (8).

The focus of current research on fungal endophytes has been on evaluating their function in plants in relation to stress tolerance (9), secondary metabolites biosynthesis (10), chemical profiling structure of host plants (11), functional trait expression in plants (12), growth promotion in plants (13), resistance of the disease (14) and resistance to abiotic and biotic stresses (15), produce different by-products with several biological aspects (16).

The compositions of the endophytic mycoflora are affected by the environmental conditions (17). Endophytic assemblages residing in particular host plant have exhibited tissue specificity (18) which attributed to anatomical, biochemical, morphological and physiological features of the host tissue (19). The diversity of endophytes in host plants is also greatly influenced by abiotic factors such soil type, moisture content, temperature, climatic and environmental factors (20). Host species, tissue types and abiotic factors have been demonstrated to have an impact on the colonization rate, diversity and community makeup of endophytic fungi (21). Several studies have been reported that geography influence the patterns of distribution of fungal endophytes further than the season (22). Geographical location has significant effect on the diversity and species composition of endophytic fungi (23). The location, period, geography, host physiology, tissues and organs of the host plants are just a few of the many variables that have a significant impact on the endophytic fungal composition and diversity (24). Earlier reports have indicated that the environmental factors and the study sites have significant influence on the frequency and the diversity of endophytic fungi in host plants (25).

The genus *Terminalia* (Combretaceae) consists of approximately 200-250 species are widely used in traditional medicine throughout the world (26). These species have been used extensively for curing the various diseases such as cardiovascular effects, wound healing, bacterial infections, conjunctivitis, dysentery, fever, gastric ulcers, hypertension, jaundice, leprosy, pneumonia and skin diseases (27). A great variation of endophytic fungi composition in twig tissues was noticed where *T. arjuna* grows (28). Majority of the fungal species were explored from *Terminalia* species belonging to the Ascomycota were recovered from various plant parts and described as dominant traits in tropical trees (29). These endophytic fungi exhibited various biological effects like antioxidant, antimicrobial, anticancer, antimalarial, anti-hypercholesterolemic, anti-inflammatory and biocontrol activities possibly by releasing the secondary metabolites (30). The diversity and community structure of fungal endophytes associated with the various plant tissues of *T. arjuna* are only sparsely reported. Therefore, the present study was designed (a) to understand the diversity and community composition of endophytic fungi among the 24 sampling locations and (b) to analyze the relationship between the diversity or community composition of endophytic fungi and plant tissues.

Materials and Methods

Study area and collection of plant materials

Tripura is a third smallest state that covers an area of 10491.69 km^2 in the North-East region of India. It represents a large biodiversity area and several medicinal plants still remain unexplored in terms of isolation of endophytic fungi. Healthy and asymptomatic plant samples including leaves, barks and roots of *T. arjuna* were collected from 24 different locations (Fig. 1 and 2). The plants were at least 5 km apart in the study areas. The selected study sites have distinctive variations in the location-specific characteristics (Table 1). During the field investigations, the collection ID, GPS coordinates, altitude, soil pH, soil moisture and soil temperature of the locations were recorded. Freshly healthy leaves and the bark samples were collected from different regions. However, the root samples were undertaken from the depth of 15-20 cm below the ground level by using the digging iron bar. Within 24 h of collection, the fresh samples were transported to the lab and processed for the enumeration of endophytic fungi in separate polythene bags.

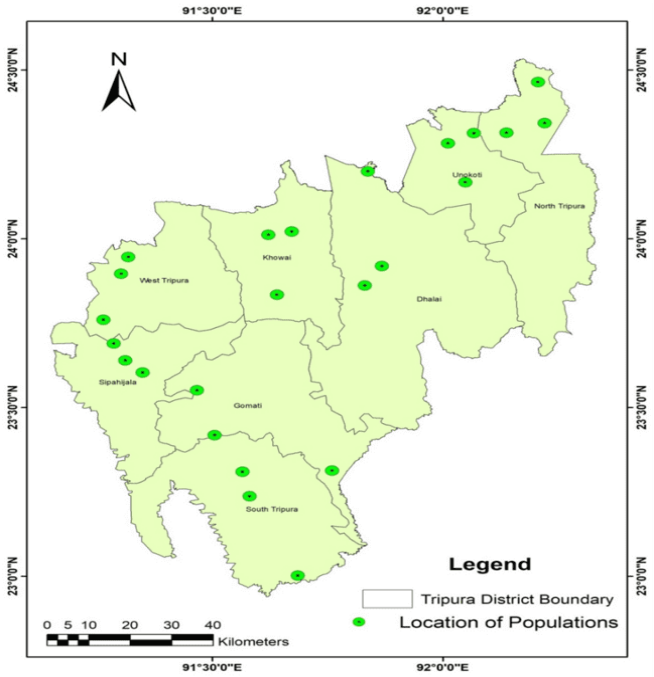

Fig. 1. Map showing the 24 sampling locations of Tripura, India where the *Terminalia arjuna* was collected.

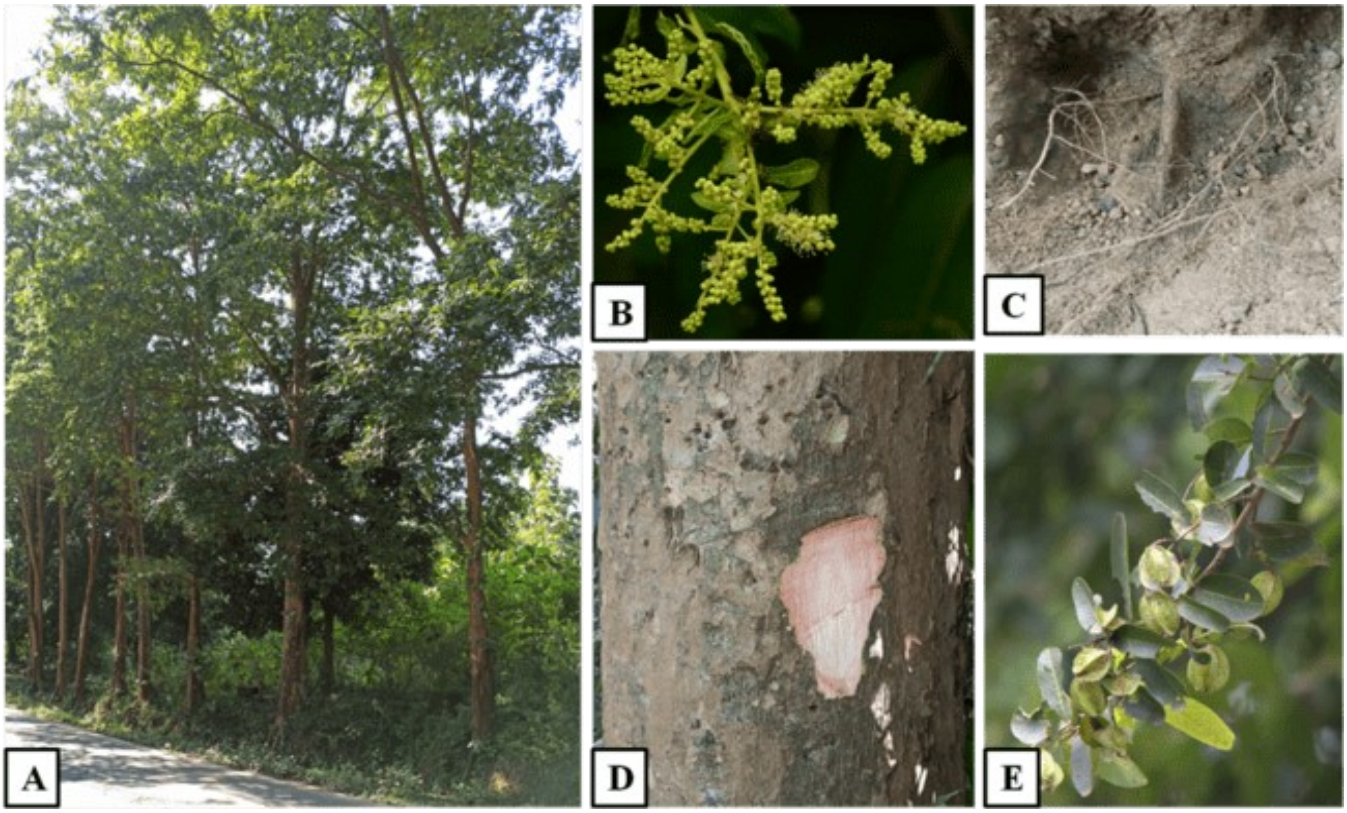

Fig. 2. *Terminalia arjuna* plant. A. Natural habitat; B. Flowers; C. Root sections; D. Bark and E. Twig with a bunch of fruits.

Table 1. Location, GPS co-ordinates, altitude and characteristics of the sampling locations of *Terminalia arjuna*

Locations (Code)	Geographic coordinates		Altitude (m)	Topography	Soil pH	Soil Temperature	Soil Moisture
	Latitude	Longitude					
Salbagan (SAL)	23°53′45″ N	91°18′08″ E	51	Plain	7	24°C	0.08
Fatikchhara (FAT)	23°56′44″ N	91°19′13″ E	37	Plain	7	25°C	0.05
Suryamaninagar (SUR)	23°45′34″ N	91°15′49″ E	41	Plain	7	27°C	0.06
Bishramganj (BRJ)	23°36′12″ N	91°20′56″ E	66	Plain	6	28°C	0.07
Bishalgarh (BSL)	23°41′22″ N	91°17′09″ E	49	Plain	7	28°C	0.08
Charilam (CHL)	23°38′21″ N	91°18′40″ E	45	Plain	7	28°C	0.08
Garji (GJI)	23°25′03″ N	91°30′17″ E	76	Plain	6.5	28°C	0.06
Jalaya (JAL)	23°18′45″ N	91°45′34″ E	86	Plain	7	25°C	0.07
Udaipur (UDA)	23°33′02″ N	91°28′00″ E	71	Plain	6.5	28°C	0.05
Paschim Jalefa (PAJ)	23°00′04″ N	91°41′07″ E	50	Plain	6.5	25°C	0.06
Muhuripur (MUH)	23°14′11″ N	91°34′50″ E	56	Gentle slopy	7	27°C	0.07
Santir bazar (STB)	23°18′32″ N	91°33′55″ E	53	Plain	7	28°C	0.05
Laxmipur (LAX)	23°50′01″ N	91°38′24″ E	89	Gentle slopy	7.5	17°C	0.09
Belphang (BEL)	24°00′42″ N	91°37′16″ E	70	Plain	7.5	27°C	0.1
Chebri (CHE)	24°01′16″ N	91°40′20″ E	76	Plain	7.5	28°C	0.08
Kulai (KUL)	23°51′40″ N	91°49′48″ E	134	Gentle slopy	7	19°C	0.08
Ambassa (AMB)	23°55′08″ N	91°52′03″ E	118	Plain	7	20°C	0.08
Kamalpur (KAL)	24°11′57″ N	91°50′12″ E	59	Plain	6.5	22°C	0.07
Uttar Unakoti (UTU)	24°18′46″ N	92°04′00″ E	138	Rough slopy	7.5	17°C	0.06
Debasthal (DEB)	24°16′58″ N	92°00′37″ E	49	Rough slopy	7.5	20°C	0.07
Kumarghat (KUM)	24°10′03″ N	92°02′53″ E	69	Plain	7	22°C	0.07
Kadamtala (KAD)	24°27′50.5″ N	92°12′20.4″ E	53	Plain	6	19°C	0.05
Rajnagar (RAJ)	24°18′50″ N	92°08′14″ E	73	Plain	6	20°C	0.05
Ganganagar (GGA)	24°20′34.2″N	92°13′12.4″ E	76	Plain	6.5	22°C	0.06

Isolation of fungal endophytes

All the fresh plant parts were thoroughly washed three times under running tap water to disinfect and remove the debris. It is followed by excising of each plant parts into 0.5 -1.0 cm pieces with the help of a sterile puncture and surgical blades or sterile scissors. The plant samples were then surface sterilized by immersing them in 70% ethanol for 1 min, 2% sodium hypochlorite for 3 min and 75% ethanol for 1 min. The plant pieces were then washed 3 times for 5 min each in sterile distilled water. After sterilization, the cut segments were placed on autoclaved filter papers and dried under aseptic conditions.

About 5 tissue segments of each plant sample were placed into each petridish include Malt Extract Agar (MEA) medium with antibiotic streptomycin (50 µg/mL). The petridish were then closed with parafilm and incubated at 27 ± 2 °C for 5-7 days. The plates were observed daily to check the growth of fungal mycelia. Using a sterile inoculating loop, the pure fungal hyphal tips are instantly placed one at a time onto the fresh MEA plates and stored at 4 °C with some modifications (31).

Morphological identifications

The endophytic fungi were observed morphologically using the lactophenol cotton blue reagent and examined under the Leica DM 750 microscope at various magnifications. The fungal isolates were identified based on their macroscopic and microscopic features, including colony colour, hyphal structure, fruiting structures, spore morphology and reproductive structures, in accordance with the standard taxonomic manuals (32).

Molecular identification of endophytic fungal strains

The molecular identification of fungal isolates was carried out at the sequencing facilities of the National Centre for Microbial Resource (NCMR), National Centre for Cell Science, Pune. After genomic deoxyribonucleic acid (DNA) was isolated using the standard phenol/chloroform extraction method, the ITS sections were amplified by PCR using universal primers ITS1 [5'-TCC GTA GGT GAA CCT GCG G -3'] and ITS4 [5'-TCC TCC GCT TAT TGA TAT GC -3'] (33). The amplified ITS PCR product was purified by PEG-NaCl precipitation in accordance with the manufacturer's instructions, and it was then immediately sequenced using an ABI® 3730XL automated deoxyribonucleic acid (DNA) sequencer (Applied Biosystems, Inc., Foster City, CA). Basically, sequencing was carried out from both ends, requiring at least 2 reads for each place. After the assembly was complete, the Lasergene package was used to perform a tentative identification using NCBI BLAST against sequences from type material (34).

Phylogenetic Analysis

The obtained sequences were compared with public databases at the NCBI website using the Basic Local Alignment Search Tool (BLAST) algorithm to determine sequence similarity (SS) (35). GenBank accession numbers were received after sequences were submitted. The MUSCLE program was used to align the internal transcribed spacer (ITS) and incomplete 16S rRNA sequences with the best BLAST matches in order to confirm the endophytes evolutionary history. MEGA version 11 was used for the phylogenetic and molecular evolutionary studies (36).

Data Analysis

The colonization rate (CR) of fungal endophytes was calculated as the number of segments infected by endophytes divided by the total number of segments incubated by 100 (37). The isolation rate (IR) was calculated as the number of isolates obtained from tissue segments divided by the total number of segments by 100 (38). Additionally, the colonization frequency (CF) percentages were calculated by the number of segments colonized by each endophytes divided by the total number of segments infected by 100 (39). The number of isolates of a species divided by the total number of isolates was used to calculate relative frequency (RF) (40). The diversity indices such as Shannon index (H'), Simpson's dominance (*D*), Simpson's diversity index (1-*D*), Fisher's alpha diversity index (*a*), Berger-Parker dominance (*B*), Brillouin index (*HB*) and Pilou evenness (*J*) were used to further assess the distinction of the endophytic fungal communities. All the analyses were performed using R statistical software version 4.2.0 (41).

Results

Isolation and morphological identification of fungal endophytes

A total number of 613 endophytic fungi were produced from 720 tissue sections from various plant parts of *Terminalia arjuna* across 24 different sampling sites. The leaf had the most fungal isolates (257), followed by the bark (182) and the roots (174) (Table 2). The isolation rate and colonization rate of fungal isolates in leaf was higher than the bark and the root tissues. On the other hand, the relative frequency of fungal strains was observed comparatively higher in the leaves (41.92%), then the barks (29.69%) and roots (28.38%) (Table 2). These results indicated that the CR, IR and RF value of endophytic fungi has exhibited maximum in the leaf tissue other than bark and roots.

Table 2. Isolation rate (IR %), Colonization rate (CR %) and Relative frequency (RF %) of fungal isolates from various tissues of *Terminalia arjuna*

Plant parts	No. of tissue segments inoculated	No. of tissue segments infected	Fungal endophytes isolates	IR (%)	CR (%)	RF (%)
Leaves	240	240	257	107.08	100.00	41.92
Bark	240	206	182	75.83	85.83	29.69
Root	240	183	174	72.50	76.25	28.38
Total	720	629	613	255.42	262.08	100.00

Some of the fungal isolates were morphologically identical after microscopic and macroscopic observations were considered as repeated. However, only those fungal strains that are structurally dissimilar were recognized as distinct fungal strains. Therefore, 27 different fungal strains were recovered from various tissues of *T. arjuna*. The most isolated fungal genera includes *Aspergillus* sp., *Alternaria* sp., *Aureobasidium* sp., *Chaetomium* sp., *Colletotrichum* sp., *Corynespora* sp., *Diaporthe* sp., *Fusarium* sp., *Mucor* sp., *Paecilomyces* sp., *Penicillium* sp., *Pestalotiopsis* sp., *Trichoderma* sp., white sterile, black sterile and yellow sterile were isolated from this plant (Table 5).

Molecular identification and phylogeny analyses of fungal endophytes

Based on the morphological characteristics of fungal endophytes from *T. arjuna*, the fungal isolates were divided into 27 distinct taxa. Nine of the fungal morphotypes including sterile strain were assigned to molecular identification and phylogenetic analysis. The molecular identification of fungal isolates were *Fusarium pernambucanum* TAL1A, *Corynespora torulosa* TAL1B, *Talaromyces australis* TAL3A, *Lasiodiplodia theobromae* TAB3A, *Penicillium oxalicum* TAR1A, *Fusarium solani* TAR1D, *Fusarium oxysporum* TAR2B, *Lasiodiplodia theobromae* TAR2D and *Fusarium crassum* TAR3A (Fig. 3). The accession numbers of 9 sequenced fungal isolates were generated have been shown in Table 3.

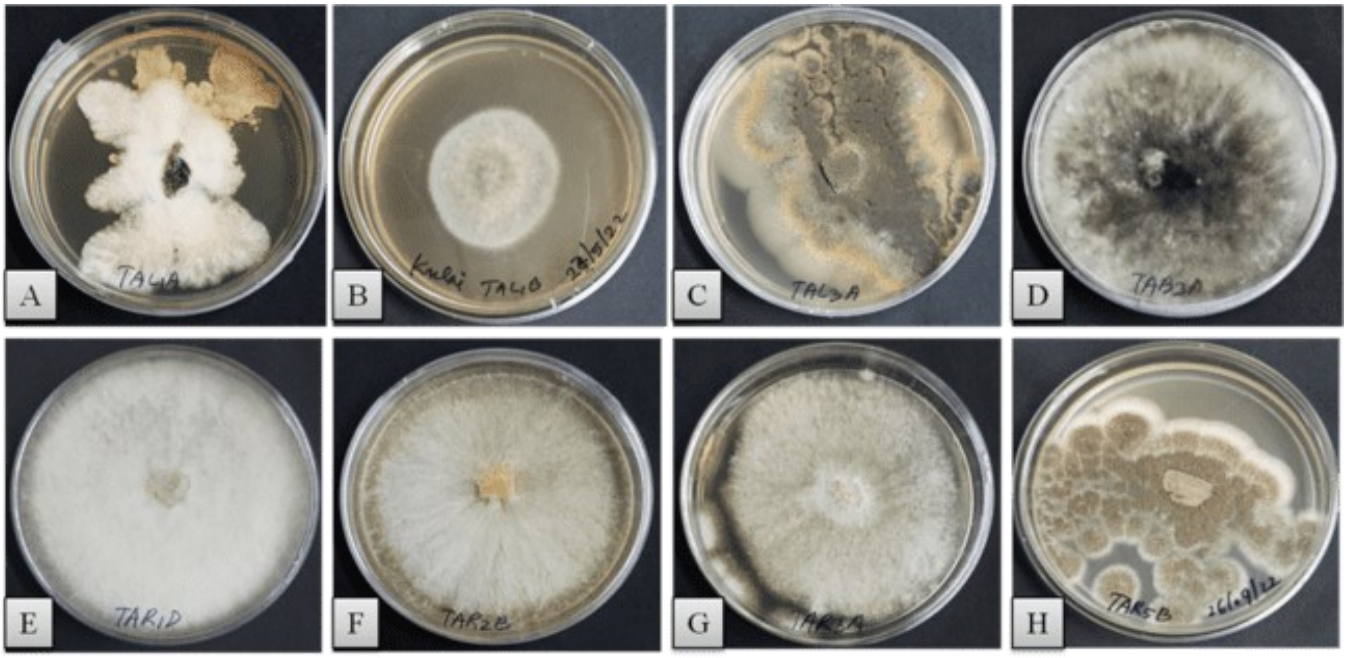

Fig. 3. Pure culture plates of the endophytic fungi isolated from the plant parts of *Terminalia arjuna*. A. *Fusarium pernambucanum*; B. *Corynespora torulosa*; C. *Talaromyces australis* D. *Lasiodiplodia theobromae*; E. *Fusarium solani*; F. *Fusarium oxysporum*; G. *Fusarium crassum*; H. *Aspergillus niger*.

Table 3. The ITS sequence identification of the endophytic fungal strains from *T*erminalia *arjuna*

Sl. No.	Fungal strain code	Most closely related strain	GenBank accession number	Sequence Identity %	Fungal phylum
1.	TAL1A	*Fusarium pernambucanum*	OR544493	100%	Ascomycota
2.	TAL1B	*Corynespora torulosa*	OR544494	97.15%	Ascomycota
3.	TAL3A	*Talaromyces australis*	OR544495	99.27%	Ascomycota
4.	TAB3A	*Lasiodiplodia theobromae*	OR544496	100%	Ascomycota
5.	TAR1A	*Penicillium oxalicum*	OR544497	99.47%	Ascomycota
6.	TAR1D	*Fusarium solani*	OR544498	99.64%	Ascomycota
7.	TAR2B	*Fusarium oxysporum*	OR544499	99.81%	Ascomycota
8.	TAR2D	*Lasiodiplodia theobromae*	OR544500	100%	Ascomycota
9.	TAR3A	*Fusarium crassum*	OR544501	100%	Ascomycota

In the present study, the endophytic taxa were divided into 2 phyla includes Ascomycota (Dothideomycetes, Sordariomycetes and Eurotiomycetes) and Mucoromycota (Phycomycetes) while the rest of the isolates are sterile. Ascomycota (73.89%) become the most common phyla followed by the sterile (21.69%) and Mucoromycota (4.40%). Sordariomycetes (46.81%) was the most prevalent class followed by Eurotiomycetes (18.43%), Dothideomycetes (8.64%) and Class Phycomycetes (4.40%) was the lowest level of richness (Supplementary Table S2). Nine morphotypes of internal transcribed spacer (ITS) rDNA sequences were generated and submitted to NCBI for BLASTn analysis. Table 3 lists the fungal endophyte strains that have been identified include their phylum along with their GenBank accession number and sequence identity. The ITS rDNA sequences of endophytic fungi had a maximum sequence identity of 97 to 100% when compared to those found in GenBank (Table 3). Endophytic fungi are thought to have been classified as species when their ITS gene sequences from reference isolates in GenBank have 100% base similarity. Others are deemed to belong to genera if their ITS gene sequences have a base similarity to those of the reference isolates between 98 and 99.9%. The phylogenetic tree was constructed of 9 fungal taxa based on their similarity sequence of internal transcribed spacer (ITS) rDNA region (Fig. 4). As a result, 9 fungal morphotypes were recognized at the species level out of 27 representative morphotypes and 18 of them were identified at the genus level (Table 3; Supplementary Table S2).

Effect of locations on endophytic fungal communities

Among 24 different sampling locations, the most abundant number of fungal endophyte isolates was recovered from the sampling site of Uttar Unakoti (38 isolates), followed by Ambassa (36 isolates) and Kadamtala (35 isolates) respectively. The lowest number of fungal isolates were distributed at Ganganagar (14 isolates) and Bishalgarh (12 isolates) (Supplementary Table S1). It was also noted that the 9 sampling locations such as, Suryamaninagar, Jalaya, Paschim Jalefa, Ambassa, Kamalpur, Uttar Unakoti, Debasthal, Kadamtala, Rajnagar have recorded the same colonization frequency with 100% showed the highest and secondly at Udaipur and Laxmipur with 96.66% and the lowest colonization frequency was obtained from Chebri (56.66%) respectively (Table 4). The highest relative frequency was observed in *Colletotrichum* sp. (17.62%) followed by the *Diaporthe* sp. (12.95%) and the lowest frequency was noticed in *Paecilomyces* sp. (0.30%) (Supplementary Table S1). Therefore, the endophytic fungi communities are significantly differences in the present study area.

In case of fungal endophytes occurrence, *Colletotrichum* sp. and *Diaporthe* sp. were the most prevalent isolates. The highest fungal isolates were recovered from the locations of Uttar Unakoti, Ambassa and Kadamtala. The fungal genera such as *Colletotrichum* sp., *Corynespora* sp., *Diaporthe* sp. and *Fusarium* sp. were found almost in every location of the study sites except Rajnagar. In addition, *Alternaria* sp. and *Paecilomyces* sp. were isolated from 2 locations. *Pestalotiopsis* sp. and *Aureobasidium* sp. are the 2 fungal species recovered from 1 location only (Supplementary Table S1).

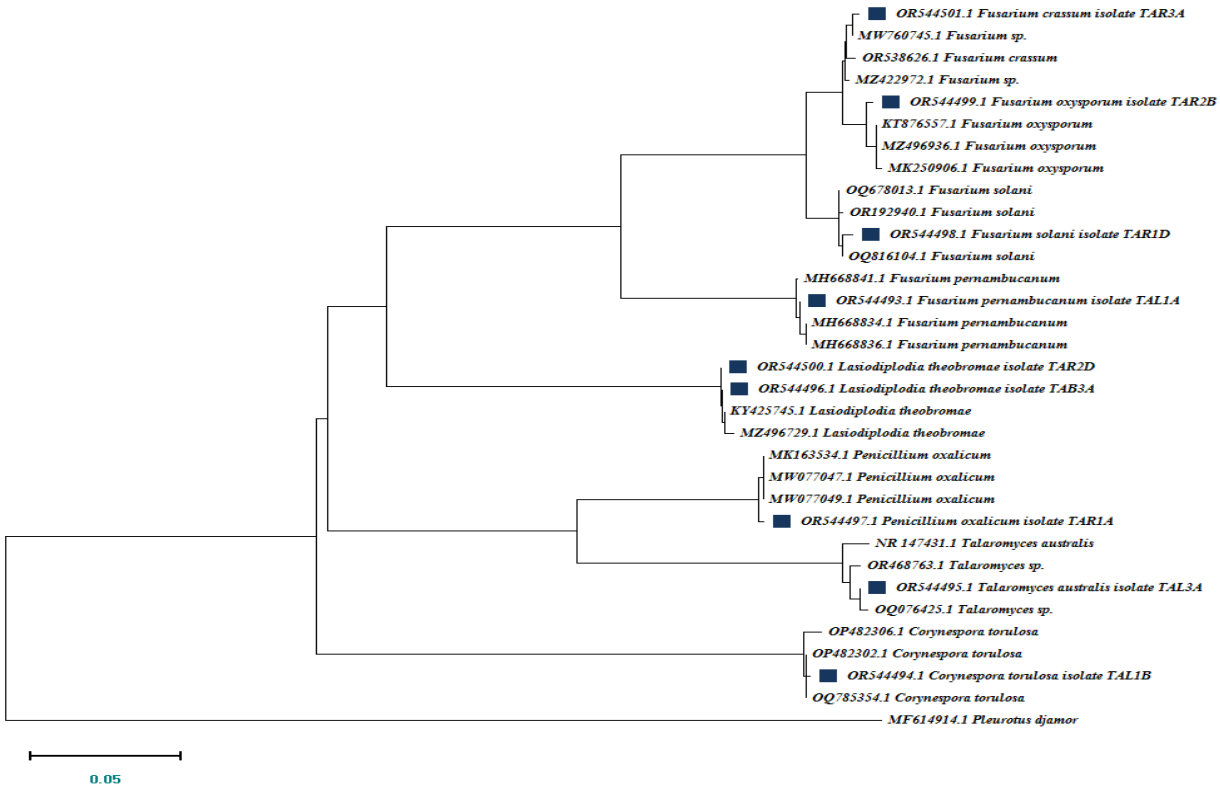

Fig. 4. Phylogenetic tree of fungal endophytes was constructed based on ribosomal ITS rDNA gene sequences. The neighbor-joining tree was drawn by Mega 11software.

Table 4. Colonization frequency of colonized segments of *Terminalia arjuna* according to the sampling locations of Tripura, India

Location	No. of segments inoculated	No. of segments obtained	Fungal endophytes isolates	CF%
Salbagan	30	18	22	60
Fatikchhara	30	20	19	66.66
Suryamaninagar	30	30	29	100
Bishramganj	30	26	25	86.66
Bishalgarh	30	28	12	93.33
Charilam	30	25	24	83.33
Garji	30	27	24	90
Jalaya	30	30	26	100
Udaipur	30	29	19	96.66
Paschim Jalefa	30	30	28	100
Muhuripur	30	28	25	93.33
Santir bazar	30	28	33	93.33
Laxmipur	30	29	30	96.66
Belphang	30	20	18	66.66
Chebri	30	17	30	56.66
Kulai	30	24	33	80
Ambassa	30	30	36	100
Kamalpur	30	30	31	100
Uttar Unakoti	30	30	38	100
Debasthal	30	30	23	100
Kumarghat	30	21	18	70
Kadamtala	30	30	35	100
Rajnagar	30	30	21	100
Ganganagar	30	19	14	63.33
Total	720	629	613	-

Endophyte communities in plant tissues

For endophytic fungal communities, different plant tissues of *T. arjuna* such as leaves, barks and roots were investigated. The highest numbers of endophytic isolates were found to be colonized in leaves (257) followed by barks (182) and the least number of endophytic isolates were obtained from roots (174) (Table 2). The colonization frequency of isolated endophytic fungi, *Colletotrichum* sp. was mostly abundant in leaves (29.17%) followed by barks (16.23%). However, in case of roots, *Fusarium* sp. 1 (9.58%) showed the highest colonization among the isolated fungal endophytes (Table 5; Fig. 5). The sterile fungal isolates are mostly found in the leaves followed by the roots and bark tissues.

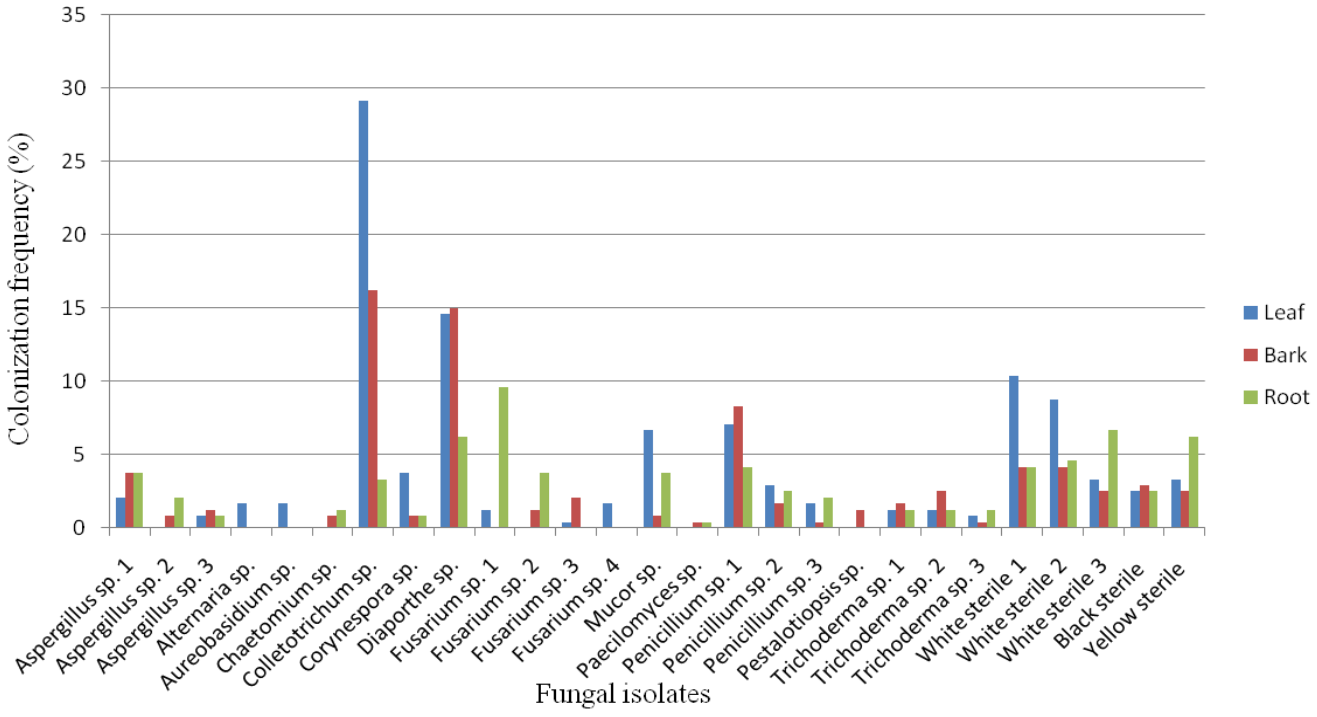

Fig. 5. Colonization frequency (%) of endophytic fungi isolated from different tissues of *Terminalia arjuna*.

Table 5. Colonization frequency of endophytic fungi isolated from different tissues of *Terminalia arjuna*

Endophytic fungi	% CF of endophytic fungi			
	Leaf	Bark	Root	Total
Aspergillus sp. 1	2.08	3.75	3.75	9.58
Aspergillus sp. 2	0.00	0.83	2.08	2.92
Aspergillus sp. 3	0.83	1.25	0.83	2.92
Alternaria sp.	1.67	0.00	0.00	1.67
Aureobasidium sp.	1.67	0.00	0.00	1.67
Chaetomium sp.	0.00	0.83	1.25	2.08
Colletotrichum sp.	29.17	16.25	3.33	48.75
Corynespora sp.	3.75	0.83	0.83	5.42
Diaporthe sp.	14.58	15.00	6.25	35.83
Fusarium sp. 1	1.25	0.00	9.58	10.83
Fusarium sp. 2	0.00	1.25	3.75	5.00
Fusarium sp. 3	0.42	2.08	0.00	2.50
Fusarium sp. 4	1.67	0.00	0.00	1.67
Mucor sp.	6.67	0.83	3.75	11.25
Paecilomyces sp.	0.00	0.42	0.42	0.83
Penicillium sp. 1	7.08	8.33	4.17	19.58
Penicillium sp. 2	2.92	1.67	2.50	7.08
Penicillium sp. 3	1.67	0.42	2.08	4.17
Pestalotiopsis sp.	0.00	1.25	0.00	1.25
Trichoderma sp. 1	1.25	1.67	1.25	4.17
Trichoderma sp. 2	1.25	2.50	1.25	5.00
Trichoderma sp. 3	0.83	0.42	1.25	2.50
White sterile 1	10.42	4.17	4.17	18.75
White sterile 2	8.75	4.17	4.58	17.50
White sterile 3	3.33	2.50	6.67	12.50
Black sterile	2.50	2.92	2.50	7.92
Yellow sterile	3.33	2.50	6.25	12.08
Total	107.08	75.83	72.50	255.42

Diversity analysis of fungal endophytes

The results generated on diversity indices of endophytic fungal strains were based on their locations and tissues are displayed in Table 6. The highest species richness (S), as determined by a comparison of 3 different plant tissues was found in the leaves (257), followed by the barks (182) and the roots (174). The fungal dominance on tissue specificity was more prevalent in the leaves (0.12) and barks (0.11) as compared to roots (0.06). The Simpson (0.93), Shannon (2.86), Berger-Parker (0.27) and Brillouin (2.65) indices indicated that the diversity of fungal species was mostly occurred in the roots, while bark exhibited maximum diversity of Fisher's alpha (6.97) and Brillouin (2.51) indices. However, Pilou evenness (0.35) occurred equally in the leaves and roots more than barks respectively (Table 6).

By comparing 24 sampling locations, we have found that the species richness of fungal species showed a high consistency in Uttar Unakoti (UTU) (14) and Ambassa (AMB) with 12 species. On the other hand, Ganganagar (GGA) (6) and Bishalgarh (BSL) (4) locations exhibited a least species richness among the sampling site respectively. The highest value of the diversity indices of Shannon was observed highest at Uttar Unakoti (UTU) (2.47) followed by Debasthal (DEB) (2.38) and Bishalgarh (BSL) (1.14) was the lowest. Simpson index was mostly found maximum at Uttar Unakoti (UTU) with 0.91 followed by Ambassa (AMB) and Paschim Jalefa (PAJ) locations with 0.90 values. However, Pilou evenness was recorded highest at Debasthal (DEB) (0.64) and Ambassa (AMB) (0.29). The Dominance index of the endophytic fungal diversity was found comparatively higher at Bishalgarh (BSL) (0.30) than the rest of the locations. However, Fisher alpha and Berger-Parker indices was occurred maximum at Debasthal (DEB) (12.38) and Charilam (CHL) (0.50) sampling sites. In addition, Brillouin index of fungal species was observed highest in Uttar Unakoti (UTU) (2.04) (Table 6).

Table 6. Diversity indices of endophytic fungi from different plant tissues and locations of *Terminalia arjuna*

	Diversity index							
	Species richness (S)	**Shannon (*H'*)**	**Simpson (1 -*D*)**	**Pilou evenness (*J*)**	**Dominance (*D*)**	**Fisher alpha (*a*)**	**Berger-Parker (*B*)**	**Brillouin (*HB*)**
By tissue type								
Leaf	22	2.52	0.88	0.35	0.12	5.76	0.27	2.37
Bark	23	2.58	0.89	0.28	0.11	6.97	0.21	2.38
Root	22	2.86	0.93	0.35	0.06	6.67	0.13	2.65
By sampling location								
SAL	8	1.86	0.82	0.31	0.14	4.52	0.27	1.48
FAT	7	1.77	0.80	0.41	0.15	4.00	0.32	1.39
SUR	9	1.80	0.77	0.32	0.20	4.47	0.41	1.47
BRJ	8	1.91	0.83	0.43	0.13	4.07	0.28	1.55
BSL	4	1.14	0.64	0.33	0.30	2.10	0.42	0.87
CHL	8	1.54	0.69	0.32	0.28	4.20	0.50	1.22
GJI	6	1.69	0.81	0.39	0.16	2.57	0.25	1.40
JAL	8	1.86	0.82	0.41	0.15	3.95	0.31	1.52
UDA	8	1.76	0.78	0.42	0.18	5.21	0.37	1.36
PAJ	14	2.41	0.90	0.37	0.07	9.43	0.18	1.91
MUH	11	2.21	0.87	0.37	0.09	7.50	0.20	1.75
STB	10	2.00	0.82	0.41	0.15	4.88	0.33	1.66
LAX	11	2.26	0.88	0.42	0.09	6.26	0.20	1.84
BEL	8	1.94	0.84	0.28	0.11	5.52	0.22	1.49
CHE	12	2.31	0.89	0.40	0.08	7.41	0.17	1.87
KUL	10	2.14	0.87	0.39	0.11	4.88	0.21	1.77
AMB	12	2.35	0.90	0.29	0.08	6.30	0.14	1.95
KAM	7	1.51	0.70	0.30	0.28	2.82	0.48	1.26
UTU	14	2.47	0.91	0.51	0.07	8.01	0.16	2.04
DEB	13	2.38	0.89	0.64	0.07	12.38	0.22	1.82
KUM	7	1.88	0.84	0.36	0.11	4.21	0.22	1.47
KAD	11	2.04	0.83	0.32	0.15	5.52	0.31	1.69
RAJ	7	1.72	0.78	0.38	0.18	3.68	0.38	1.37
GGA	7	1.81	0.82	0.49	0.12	5.57	0.29	1.35

Note: Salbagan (SAL); Fatikchhara (FAT); Suryamaninagar(SUR); Bishramganj (BRJ); Bishalgarh(BSL); Charilam (CHL); Garji (GJI); Jalaya (JAL); Udaipur (UDA); Paschim Jalefa (PAJ); Muhuripur (MUH); Santir bazar (STB); Laxmipur (LAX); Belphang (BEL); Chebri (CHE); Kulai (KUL); Ambassa (AMB); Kamalpur (KAM; Uttar Unakoti (UTU); Debasthal (DEB); Kumarghat (KUM); Kadamtala (KAD); Rajnagar (RAJ); Ganganagar (GGA).

Discussion

The present investigations explored the community structure and diversity of endophytic fungi across the 24 sites and various plant tissues of *Terminalia arjuna*. A total of 613 endophytic fungal isolates were isolated from 720 inoculated tissue segments of this plant. The colonization rate and the isolation rate has recorded highest value in leaves while the barks and roots shown notably less. It was clearly indicated that endophytic fungi favour colonizing the leaf tissues because of their higher surface area, rich nutrients and thin walls (42). In contrast to the rhizome and fibrous roots, recent investigations have shown that the leaves are more significant in holding the majority of endophytic fungus (43). In previous studies, the recovery of endophytic fungi from leaf tissue was higher than that of the stem and petiole tissue demonstrating that the characteristics of leaf tissues and the size of the surface area are widely open up to the environment, favour spore deposition and dispersion that may result in endophytes in due time and conditions (44). The stomata, roots, wounds as well as their direct secretion of hydrolytic enzymes, could have allowed the endophytic fungi to penetrate into the host tissues (45). Additionally, endophytic fungi spread throughout various plant tissues by horizontal (9) or vertical (46) transmission via spores.

The fungal isolates were divided into 9 fungal species and 5 genera belonging to the phylum Ascomycota based on their molecular characteristics. In fact, the newly introduced endophytic fungi were from 4 different fungal classes with 10 different fungal orders, including Ascomycota-Eurotiomycetes (Eurotiales), Dothideomycetes (Dothideales, Pleosporales), Sordariomycetes (Amphisphaeriales, Diaporthales, Eurotiales, Glomerellales, Sordariales, Hypocreales) and Phycomycetes (Mucorales) comes from Mucoromycota. Ascomycota (73.89%) become the dominant morphotypes followed by the sterile phyla (21.69%) and the lowest occurrence was found in Mucoromycota (4.40%) (Supplementary Table S2). Similar findings have also reported that Ascomycota was a common characteristic of the endophytic microbiome and remain consistent dominant phyla in many medicinal plants (47). According to recent studies, ascomycetous fungi are the common representative fungal endophytes in *Coptis chinensis* Franch. (43).

In the present examination, the most frequent isolates of fungal endophytes community of *T. arjuna* comprised the fungal genera includes *Colletotrichum* sp., *Corynespora* sp., *Diaporthe* sp., *Fusarium* sp., *Penicillium* sp. and white sterile while the less frequently isolated genera were *Alternaria* sp., *Aureobasidium* sp., *Chaetomium* sp., *Paecilomyces* sp. and *Pestalotiopsis* sp. The fungal endophytes such as *Colletotrichum* sp., *Corynespora* sp., *Diaporthe* sp., *Mucor* sp., *Penicillium* sp. and white sterile preferred the leaves inhabitant in contrast to bark and roots. These results show a strong affinity of tissue specificity for endophytic fungi colonization in leaves. Similar studies have also reported that *Colletotrichum* sp. and *Diaporthe* sp. colonized in the leaf tissues as endophytes in *T. arjuna* (48). The frequent genera such as *Aspergillus* sp., *Colletotrichum* sp., *Diaporthe* sp., *Fusarium* sp., *Penicillium* sp. and *Trichoderma* sp. were considered as endophytes. Similarly, several authors have reported that the fungal genera such as *Aspergillus* sp., *Diaporthe* sp., *Penicillium* sp., *Pestalotiopsis* sp. and *Trichoderma* sp., were mostly isolated endophytic fungi investigated in *T. arjuna* (49). Additionally, *Pestalotiopsis* sp. become the only isolated fungi found in the barks of *T. arjuna* which was also supported with previous reports (28). However, the most isolated endophytic fungi in the roots of *T. arjuna* was *Fusarium* sp. which corresponds with earlier reports on endophytic colonization in the roots of *Citrus reticulata* (2). These results indicated that *T. arjuna* harbor rich fungal species that are yet to be explored of the fungal community.

The endophytic fungal diversity were significantly different and varies across sampling locations. The diversity and species richness in this plant were strongly affected by the tissues and locations. The diversity of fungal endophytes was higher in leaf than that of barks and roots. This result was similar to the endophytic fungi diversity in *Croton chinensis* (43). Similar research was also observed in *Dillenia indica* where the frequency of endophytic fungi was showing higher in the leaf tissue (8). Furthermore, tissue specificity of fungal endophytes from *T. arjuna* was exhibited after the analysis of diversity indices. The community structure of endophytic fungi of the leaf was entirely distinct from the roots. It could be due to the variations of micro-environments in various plant tissues provide for hosting different microbiome (43).

There was a similar colonization frequency of fungal endophytes in nine sites out of 24 sampling locations suggests that the host may have the same climatic conditions and micro-environmental characteristics, which have less impact on the diversity and species richness of fungal endophytes (50). On the other hand, abiotic factors such as soil type, moisture content, temperature, climatic and other environmental conditions, have a significant impact on the diversity of host plant endophytes (20). Indeed, Uttar Unakoti (UTU) was the highest number of fungal isolates among the sampling site in the present study. Ambassa (AMB) and Kadamtala (KAD) were placed in second and third position of isolation. In contradict, Bishalgarh (BSL) showed the lowest isolation of endophytic fungi (Table 6). The possible differences could be due to the ecological conditions of sampling location including altitude, topography, soil temperature, soil pH and soil moisture (Table 1) affect the endophytic communities in the study sites. Due to the different environmental circumstances at each site, there were high dominance and low fungal richness in the community structure of endophytic fungi in *T. arjuna*. We have also noticed that certain endophytic fungi at least at genus level displayed location-specific distributions of *Aureobasidium* sp. exclusively isolated from Chebri (CHE) whereas *Pestalotiopsis* sp. was restricted to Paschim Jalefa (PAJ), *Alternaria* sp. in Fatikchhara (FAT) and Chebri (CHE),

Fusarium sp. 4 in Rajnagar (RAJ). While *Paecilomyces* sp. was recovered from Paschim Jalefa (PAJ) and Uttar Unakoti (UTU). These endophytes restricted distribution patterns revealed the spatial community structures of endophytic fungus. Previous studies have shown that the impact of distance on the spread of endophytic fungus may be connected to variations in geographic and ecological factors, like the plant association as well as the local climate just before sampling at each location (23). Similarly, it was also reported that differences in environmental, ecological characteristics as well as the climatic conditions of each location could differed in endophytic fungal diversity and fungal richness in *Salvia multicaulis* (51). Locality and tissue have a significant impact on the patterns of spread of endophytic fungus. Endophytic fungi living on *Taxus chinensis* var. *mairei* showed differences depending on their location and tissue (50).

Conclusion

The present study has reported the diverse groups of endophytic fungi from *Terminalia arjuna* belonging to Ascomycota. The endophytic fungi showed significant variations in their composition in different sampling sites and tissue types. The dominant genera such as *Diaporthe* sp., *Corynespora* sp., *Colletotrichum* sp., *Penicillium* sp., *Fusarium* sp. and white sterile were recorded from this plant. It was also noticed that the tissue types, climatic conditions and ecological factors of sampling locations may influence the diversity of the endophytic fungi community in *T. arjuna*. The isolation and colonization rate of fungal endophytes was showed highest in leaves as compared to that of bark and roots. We have also observed that the analysis of species richness index of endophytic fungi showed maximum at Uttar Unakoti (UTU) and the lowest at Bishalgarh (BSL) location. Similarly, Shannon index was also found highest at Uttar Unakoti (UTU) and the lowest was Bishalgarh (BSL) location. Several studies have reported that endophytic fungi have the capability of producing secondary metabolites, resistance to biotic and abiotic stress, potential in plant growth and antimicrobial agents etc. Therefore, the isolated endophytic fungi from this study will open a new platform for the discovery of endophyte-derived chemical compounds for their bio-applications in restoration of the host plants in extreme ecosystems and will also be crucial for the sustainability and productivity of agricultural crops.

Acknowledgements

The authors are gratefully thankful to the Head, Department of Botany, Tripura University, Tripura, India for availing the laboratory facilities, instrumentation and infrastructure during our research work. The authors would like to acknowledge for the Senior Research Fellowship (Sanctioned Order No.: BT/PR25260/NER/95/1102/2017) by the Department of Biotechnology, Government of India, for providing all the necessary facilities and financial support to carry out the research work. The authors would like to thank Mr. Biplab Banik, Department of Botany, Tripura University, Tripura, India for construction of Tripura map for the study sites. The authors also deeply thankful to Prof. Badal Kumar Datta, Department of Botany, Tripura University, Tripura, India for proper identification of the plant species.

Authors' contributions

ST and AKS conceived the idea. ST carried out the experiments and prepared the initial draft. SP and ST conducted the data analysis. RS, SP and PD edited and revised the manuscript. All authors have read and approved the final manuscript.

Compliance with ethical standards

Declaration : The authors declare no competing interests.

Ethical issues: None.

References

1. Petrini O. Fungal endophytes of tree leaves. In Microbial Ecology of Leaves (ed. j. H. Andrews & S. S. Hirano), Springer Verlag: New York. 1991;pp. 179-97. https://doi.org/10.1007/978-1-4612-3168-4_9
2. Sadeghi F, Samsampour D, Seyahooei MA, Bagheri A, Soltani J. Diversity and spatiotemporal distribution of fungal endophytes associated with *Citrus reticulata* cv. Siyahoo. Curr Microbiol. 2019 Mar;76(3):279-89. doi:10.1007/s00284-019-01632-9
3. Krings M, Taylor TN, Hass H, Kerp H, Dotzler N, Hermsen EJ. Fungal endophytes in a 400-million-yr-old land plant: Infection pathways, spatial distribution and host responses. New Phytol. 2007;174(3):648-57. http://dx.doi.org/10.1111/j.1469-8137.2007.02008.x
4. Guevara-Araya MJ, Vilo C, Urzúa A. Differences in community composition of endophytic fungi between above and below ground tissues of *Aristolochia chilensis* in an arid ecosystem. Rev Chil de Hist Nat. 2020;93:3. https://doi.org/10.1186/s40693-020-00091-y
5. Malinowski DP, Belesky DP. Ecological importance of *Neotyphodium* spp. grass endophytes in agroecosystems. Grassland Science. 2006;52:1-14. https://doi.org/10.1111/j.1744-697X.2006.00041.x
6. Rather RA, Srinivasan V, Anwar M. Seasonal deviation effects foliar endophyte assemblage and diversity in *Asparagus racemosus* and *Hemidesmus indicus*. BMC Ecol. 2018;18:52. https://doi.org/10.1186/s12898-018-0211-y
7. Soltani J. Endophytism in Cupressoideae (Coniferae): A model in endophyte biology and biotechnology. In: Maheshwari, D. (eds) Endophytes: Biology and Biotechnology. Sustainable Development and Biodiversity. Springer, Cham. 2017;vol 15. https://doi.org/10.1007/978-3-319-66541-2_6
8. Kumar V, Prasher IB. Seasonal variation and tissues specificity of endophytic fungi of *Dillenia indica* L. and their extracellular enzymatic activity. Arch Microbiol. 2022;204:341. https://doi.org/10.1007/s00203-022-02933-7
9. Rodriguez RJ, White JF Jr, Arnold AE, Redman RS. Fungal endophytes: Diversity and functional roles. New Phytol. 2009;182:314-30. https://doi.org/10.1111/j.1469-8137.2009.02773.x

10. Ming Q, Su C, Zheng C, Jia M, Zhang Q, Zhang H *et al*. Elicitors from the endophytic fungus *Trichoderma atroviride* promote *Salvia miltiorrhiza* hairy root growth and tanshinone biosynthesis. J Exp Bot. 2013;64:5687-94. https:// doi.org/ 10. 1093/ jxb/ ert342

11. Panaccione DG, Beaulieu WT, Cook D. Bioactive alkaloids in vertically transmitted fungal endophytes. Funct Ecol. 2014;28:299-314. https:// doi.org/ 10. 1111/ 1365- 2435

12. Griffin EA, Traw MB, Morin PJ, Pruitt JN, Wright SJ, Carson WP. Foliar bacteria and soil fertility mediate seedling performance: A new and cryptic dimension of niche differentiation. Ecology. 2016 Nov;97(11):2998-3008. doi:10.1002/ecy.1537

13. Liu H, Carvalhais LC, Crawford M, Singh E, Dennis PG, Pieterse CMJ, Schenk PM. Inner plant values: Diversity, colonization and benefits from endophytic bacteria. Front Microbiol. 2017 Dec 19;8:2552. doi:10.3389/fmicb.2017.02552

14. Hirakue A, Sugiyama S. Relationship between foliar endophytes and apple cultivar disease resistance in an organic orchard. Biological Control. 2018;Vol. 127:139-44. https://doi.org/10.1016/j.biocontrol.2018.09.007

15. Yan L, Zhu J, Zhao X, Shi J, Jiang C, Shao D. Beneficial effects of endophytic fungi colonization on plants. Appl Microbiol Biotechnol. 2019 Apr;103(8):3327-40. doi: 10.1007/s00253-019-09713-2

16. Gupta S, Chaturvedi P, Kulkarni MG, Van Staden J. A critical review on exploiting the pharmaceutical potential of plant endophytic fungi. Biotechnol Adv. 2020 Mar-Apr;39:107462. doi:10.1016/j.biotechadv.2019.107462

17. Petrini O, Sieber TN, Toti L, Viret O. Ecology, metabolite production and substrate utilization in endophytic fungi. Nat Toxins. 1992;1(3):185-96. doi:10.1002/nt.2620010306

18. Coleman-Derr D, Desgarennes D, Fonseca-Garcia C, Gross S, Clingenpeel S, Woyke T *et al*. Plant compartment and biogeography affect microbiome composition in cultivated and native Agave species. New Phytol. 2016 Jan;209(2):798-811. doi:10.1111/nph.13697

19. Maggini V, Mengoni A, Gallo ER, Biffi S, Fani R, Firenzuoli F, Bogani P. Tissue specificity and differential effects on *in vitro* plant growth of single bacterial endophytes isolated from the roots, leaves and rhizospheric soil of *Echinacea purpurea*. BMC Plant Biol. 2019 Jun 28;19(1):284. doi:10.1186/s12870-019-1890-z

20. Marag PS, Suman A. Growth stage and tissue specific colonization of endophytic bacteria having plant growth promoting traits in hybrid and composite maize (*Zea mays* L.). Microbiol Res. 2018 Sep;214:101-13. doi:10.1016/j.micres.2018.05.016

21. Li JL, Sun X, Chen L, Guo LD. Community structure of endophytic fungi of four mangrove species in Southern China. Mycology. 2016;7:180-90. https://doi.org/10.1080/21501203.2016.1258439

22. Collado J, Platas G, González I, Peláez F. Geographical and seasonal influences on the distribution of fungal endophytes in *Quercus ilex*. New Phytol. 1999 Dec;144(3):525-32. doi:10.1046/j.1469-8137.1999.00533.x

23. Göre ME, Bucak C. Geographical and seasonal influences on the distribution of fungal endophytes in *Laurus nobilis*. Forest Pathology. Aug 2007;37(4):281-88. https://doi.org/10.1111/j.1439-0329.2007.00509.x

24. Naik BS, Shashikala J, Krishnamurthy YL. Study on the diversity of endophytic communities from rice (*Oryza sativa* L.) and their antagonistic activities *in vitro*. Microbiol Res. 2009;164(3):290-96. https://doi.org/10.1016/j.micres.2006.12.003

25. Singh DK, Sharma VK, Kumar J. Diversity of endophytic mycobiota of tropical tree *Tectona grandis* Linn.f.: Spatiotemporal and tissue type effects. Sci Rep. 2017;**7**:3745 https://doi.org/10.1038/s41598-017-03933-0

26. Stace CA. Combretaceae. In: Kubitzki, K. (eds) flowering plants· Eudicots. The Families and Genera of Vascular Plants. Springer, Berlin, Heidelberg. 2007;9:67-82. https://doi.org/10.1007/978-3-540-32219-1_11

27. Li K, Diao Y, Zhang H, Wang S, Zhang Z, Yu B *et al*. Tannin extracts from immature fruits of *Terminalia chebula* Fructus Retz. promote cutaneous wound healing in rats. BMC Complement Altern Med. 2011 Oct 7;11:86. doi:10.1186/1472-6882-11-86

28. Tejesvi MV, Kini KR, Prakash HS, Subbiah V, Shetty HS. Genetic diversity and antifungal activity of species of Pestalotiopsis isolated as endophytes from medicinal plants. Fungal Divers. 2007 Jan 31;24(3):1-8.

29. Felber AC, Orlandelli RC, Rhoden SA, Garcia A, Costa AT, Azevedo JL, Pamphile JA. Bioprospecting foliar endophytic fungi of *Vitis labrusca* Linn., Bordô and Concord cv. Annals of Microbiology. 2016 Jun;66(2):765-75. https://doi.org/10.1007/s13213-015-1162-6

30. KouipouToghueo RM, Boyom FF. Endophytic fungi from *Terminalia* species: A comprehensive review. J Fungi (Basel). 2019 May 24;5(2):43. doi:10.3390/jof5020043

31. Jena SK, Tayung K. Endophytic fungal communities associated with two ethno-medicinal plants of Similipal Biosphere Reserve, India and their antimicrobial prospective. J App Pharm Sci. 2013;3(4 Suppl 1):S7-S12.

32. Bhattacharya S, Debnath S, Saha AK. Diversity of foliar endophytic fungi in *Artocarpus heterophyllus* Lam. and *Citrus reticulata* Blanco of Tripura. Journal of Tropical Life Science. 2021;11(2):251-58. doi:10.11594/jtls.11.02.15

33. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning a laboratory manual. New York: Cold Spring Harbor Laboratory Press;1989.

34. Boratyn GM, Camacho C, Cooper PS, Coulouris G, Fong A, Ma N *et al*. BLAST: A more efficient report with usability improvements. Nucleic Acids Res. 2013;41:W29-W33. https://doi.org/10.1093/nar/gkt282

35. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990;215:403-10. https:// doi.org/ 10. 1016/ S0022- 2836(05) 80360-2

36. Tamura K, Stecher G, Kumar S. MEGA 11: Molecular evolutionary genetics analysis version 11. Molecular Biology and Evolution. 2021. https://doi.org/10.1093/molbev/msab120

37. Maheswari S, Rajagopal K. Biodiversity of endophytic fungi in *Kigelia pinnata* during two different seasons. Curr Sci. 2013;104:515-18.

38. Sunayana N, Nalini MS, Sampath KKK, Prakash HS. Diversity studies on the endophytic fungi of *Vitex negundo* L. Mycosphere. 2014;5(4):578-90. https:// doi.org/10.5943/mycosphere/5/ 4/ 10

39. Suryanarayanan TS, Venkatesan G, Murali TS. Endophytic fungal communities in leaves of tropical forest trees: Diversity and distribution patterns. Current Science. 2003;85 (4):489-93. http://www.jstor.org/stable/24108544

40. Huang WY, Cai YZ, Hyde KD, Corke H, Sun M. Biodiversity of endophytic fungi associated with 29 traditional Chinese medicinal plants. Fungal Diversity. 2008;33:61-75.

41. R Core Team 2022. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

42. Al-Harthi HF, Elgorgan AM, Ahmed B, Bahkali AH, ElSheshtawi M, Shaik JP *et al*. Identification, molecular characterization and plant growth promoting activities of endophytic fungi of *Jasminum sambac*, *Camellia sinensis* and *Ocimum basilicum*. Journal of King Saud University – Science. 2023;Volume 35 (3):102558.https://doi.org/10.1016/j.jksus.2023.102558

43. Ming Q, Huang X, Guo L, Liu D, Qin L, He Y *et al*. Diversity of endophytic fungi in *Coptis chinensis* Franch. and their activity against methicillin-resistant *Staphylococcus aureus*. Folia Microbiol (Praha). 2022 Dec;67(6):965-74. doi:10.1007/s12223-022-00994-1

44. Kharwar RN, Gond SK, Kumar A, Mishra A. A comparative study of endophytic and epiphytic fungal association with leaf of *Eucalyptus citriodora* Hook. and their antimicrobial activity. World Journal of Microbiology and Biotechnology. 2010 Nov;26:1941-48. https://doi.org/10.1007/s11274-010-0374-y

45. Esposito E, Azevedo JL. Fungos: umaintroduc¸ ão à biologia, bioquímica e biotecnologia. 2nd ed Caxias do Sul: Edusc; 2004.

46. Saikkonen K, Saari SS, Helander M. Defensive mutualism between plants and endophytic fungi? Fungal Diversity. 2010;41:101-13. http://dx.doi.org/10.1007/s13225-010-0023-7

47. Du W, Yao Z, Li J, Sun C, Xia J, Wang B. Diversity and antimicrobial activity of endophytic fungi isolated from *Securinega suffruticosa* in the Yellow River Delta. PLoS ONE. 2020;15(3):e0229589. https://doi.org/10.1371/journal.pone.0229589

48. Suryanarayanan TS, Devarajan PT, Girivasan KP, Govindarajulu MB, Kumaresan V, Murali TS *et al*. The host range of multi-host endophytic fungi. Current Science. 2018 Nov 25;115(10):1963-69. https://doi.org/10.18520/cs/v115/i10/1963-1969

49. Tejesvi MV, Mahesh B, Nalini MS, Prakash HS, Kini KR, Subbiah V, Shetty HS. Fungal endophyte assemblages from ethnopharmaceutically important medicinal trees. Can J Microbiol. 2006 May;52(5):427-35. doi:10.1139/w05-143

50. Wu L, Han T, Li W, Jia M, Xue L, Rahman K, Qin L. Geographic and tissue influences on endophytic fungal communities of *Taxus chinensis* var. *mairei* in China. Current Microbiology. 2013 Jan;66:40-48. https://doi.org/10.1007/s00284-012-0235-z

51. Jahromi MS, Azizi A, Soltani J. Diversity and spatiotemporal distribution of fungal endophytes associated with *Salvia multicaulis*. Current Microbiology. 2021 Apr;78:1432-47. https://doi.org/10.1007/s00284-021-02430-y