Asian Journal of Mycology 7(2): 11–20 (2024) ISSN 2651-1339
www.asianjournalofmycology.org

Article
Doi 10.5943/ajom/7/2/2

Diversity and seasonal variations of endophytic fungi associated with *Terminalia chebula* Retz. (Combretaceae) of Tripura, India

Tripura S[1*], Debbarma P[1, 2], Paul S[3], Saha R[1], Datta BK[3] and Saha AK[1]

[1]Mycology and Plant Pathology Laboratory, Department of Botany, Tripura University, Suryamaninagar, Tripura – 799022, India
[2]Department of Botany, Netaji Subhash Mahavidyalaya, Dhwajanagar – 799120, Udaipur, Tripura, India
[3]Plant Taxonomy and Biodiversity Laboratory, Department of Botany, Tripura University, Suryamaninagar, Tripura – 799022, India

Tripura S, Debbarma P, Paul S, Saha R, Datta BK, Saha AK. 2024 – Diversity and seasonal variations of endophytic fungi associated with *Terminalia chebula* Retz. (Combretaceae) of Tripura, India. Asian Journal of Mycology 7(2), 11–20, Doi 10.5943/ajom/7/2/2

Abstract

Endophytic fungi are microbes living within the interior organs of plants without showing any symptoms of the diseases. These fungi are recognized for their bioactivities, including antimicrobial, anticancer, antifungal, and antioxidant properties, etc. Despite having such importance, less research has been conducted to uncover the fungal endophytes colonizing different tissues of many important medicinal plants. Thus, the current study was undertaken to isolate and determine the diversity of culturable endophytic fungi associated with the medicinal plant *Terminalia chebula* Retz., collected from five different sites representing three different seasons (summer, monsoon and winter) in Tripura, Northeast, India. A total of 697 culturable endophytic fungal isolates were represented by sixteen genera, and a few sterile forms were recorded from 1,125 tissue segments of *Terminalia chebula*. The phylum Ascomycota dominated the endophytic fungal composition. The fungal endophytes like *Diaporthe* sp., *Penicillium exsudans*, *Colletotrichum* sp., and *Corynespora torulosa* were predominantly isolated from the host plant. The colonization and relative frequency of endophytic fungi were maximum in the leaf tissues, followed by barks and roots. The monsoon season harbored the maximum number of fungal isolates (285) compared to the summer (234) and winter (178) seasons. Significant differences were noted in the diversity of endophytic colonization among different study sites and various plant organs in different seasons. The diversity indices revealed maximum fungal diversity in the bark tissues compared to leaf and root tissues. The highest species richness was at the Jalaya site in comparison to the other sites while species diversity of fungal endophytes was highest during the summer season and lowest in winter. The results indicated that the study site, type of tissues and season influenced the endophytic fungal communities of the host plant, *Terminalia chebula*. These diverse endophytic fungi probably possess the ability to secrete bioactive compounds for curing several diseases, bioinoculants for plant protection, drug discovery in pharmaceutical sectors and biofertilizers in agricultural fields.

Keywords – Ascomycota – Chebulic myrobalan plant – Fungal diversity – Seasons

Introduction

Several studies have clearly stated the presence of approximately 390,900 plant species, and

Submitted 04 May 2024, Accepted 21 August 2024, Published 23 September 2024
Corresponding Author: Samrat Tripura – e-mail – samrat.botany@tripurauniv.in

as estimated by the Royal Botanic Gardens, Kew, the majority of them are in symbiotic associations with endophytes in nature (Arnold & Lutzoni 2007, Li et al. 2007, Kumaresan et al. 2013, Zhang et al. 2013). Endophytic fungi are organisms that live within the healthy tissues of the host plants with no sign of disease symptoms (Petrini 1991). Fossil records indicated that for about >400 million years, endophytic fungi have evolved with the plants in the plant kingdom (De Bary 1866, Hardoim et al. 2015, Chetia et al. 2019). Fungal endophytes colonized all types of plant tissues, including leaves, stems, bark, roots and fruits (Shoeb et al. 2012, Rajeswari et al. 2017, Vemireddy et al. 2020). Generally, in a mutualistic relationship, the host plant provides shelter and nourishment to the endophytic fungi, while the endophytes, in return, fight against several pathogenic microbes as well as herbivorous animals (Schardl et al. 2004, Singh et al. 2011), increase the growth and development of the host plant (Hamayun et al. 2010), withstand heavy metal and salt stress/toxicity (Khan et al. 2014), helps in solubilising minerals, in synthesizing secondary metabolites, manufacturing different phytohormones, and even withstanding the adverse environmental conditions (Hallmann et al. 1997, Suman et al. 2016). Endophytic fungi obtained from medicinal plants were found to assist the host plant in secreting biologically active secondary metabolites and enzymes that are crucial for the discovery of various lifesaving drugs (Zou et al. 2000, Strobel et al. 2004, Krishnamurthy et al. 2008). The bioactive secondary products generated by endophytic fungi colonized in medicinal plants were reported to be applied for treating several infections (Tejesvi et al. 2007).

Globally, the genus *Terminalia* (Combretaceae), possesses approximately 250 species and is distributed mainly in South Asia, Australia, and South Africa (Fan et al. 2015). They are widely applied as herbal folk medicines and are reported to possess biological activities such as antitumor, anti-inflammatory, antibacterial, antifungal and antiviral properties (Zhang et al. 2019). Very recently, more than 47 fungal genera and 40 compounds were investigated in the *Terminalia* species having bioactivities such as anticancer, anti-hypercholesterolemic, anti-inflammatory, antimicrobial, antimalarial, antioxidant, biocontrol properties etc. (Kouipou Toghueo & Boyom 2019). In addition, *Terminalia chebula* Retz. (commonly known as Chebulic myrobalan) medicinal plant harbors diversified fungal species and are distributed in the leaf, stem and barks of the plant tissues (Vemireddy et al. 2020). Furthermore, *T. chebula* has been extensively used in traditional medicine for its antibacterial, anti-carcinogenic, anti-inflammatory, antimicrobial, antioxidant, and anti-tumor activities (Fan et al. 2015, Zhang et al. 2016). The host plant has been extensively used in the treatment of dementia, constipation, and diabetes in Indian and Iranian traditional medicine (Jokar et al. 2016). Several fungal endophytes associated with *T. chebula* are known for their actions as an antimicrobial (Kesting et al. 2009), anticancer (Shoeb et al. 2012), antifungal (Phaopongthai et al. 2013), and antioxidant (Shoeb et al. 2014) etc. Despite having several medicinal properties and bioactive potential of associated fungal endophytes of this plant, a limited number of research has been performed to investigate the diversity and richness of these fungal endophytes from this medicinally important plant species, especially from this Indo-Burma biodiversity hotspot region of Tripura, Northeast, India. Thus, the current research was designed to study the fungal diversity and the effect of seasonal fluctuations in endophytic fungal compositions of *T. chebula,* which might contribute to documenting the novel endophytic fungi from this plant that might exhibit their potential to be used in new drug discoveries and sustainable agricultural practices.

Materials & Methods

Study site and collecting the plant samples

Mature and disease-free tissues of the *Terminalia chebula* plants were collected from five sampling sites in Tripura, Northeast India (Table 1). The plant parts were collected during the summer (April-May), monsoon (June-September), and winter seasons (January-February) (Tomar et al. 2017) from August 2021 to July 2022. The average distance between the collection sites was approximately 110.2 km (Fig. 1). The plant organs, such as leaves (375 segments), barks (375

segments), and roots (375 segments), were collected in this study. All the plant samples were kept in zipped polythene bags and brought to the department for further processing. The voucher specimen (Collection No. TUH-2387) of the plant sample was deposited at the Herbarium, Department of Botany, Tripura University for future reference (Fig. 2).

Surface sterilization and recovery of fungal endophytes

The tissue segments were surface sterilized by cleansing in running tap water for 5–7 min. to remove the debris and dust lying on the surface of the samples. Subsequently, the leaves (5 × 5 mm), barks (6 × 6 mm), and root segments (4 × 4 mm) were excised with autoclaved scalpel and surgical blades. These tissue pieces were subsequently washed in 70% ethanol for 2 min., followed by washing in 3% sodium hypochlorite for 1 min. and finally washed thrice using sterile distilled water. The tissue segments were dried in a laminar airflow chamber (Hallmann et al. 2006) before placing onto the petri dish containing malt extract agar (MEA) nutrient medium amended with streptomycin (100 µg mL^{-1}). The petri dish was sealed with Parafilm and kept at 27 ± 2°C in the BOD (biological oxygen demand) incubator and monitored regularly for the emergence of fungal isolates for up to seven days (Bhattacharya et al. 2020). The endophytic fungi emerging from their respective Petri dish were transferred onto the fresh MEA plates for further processing.

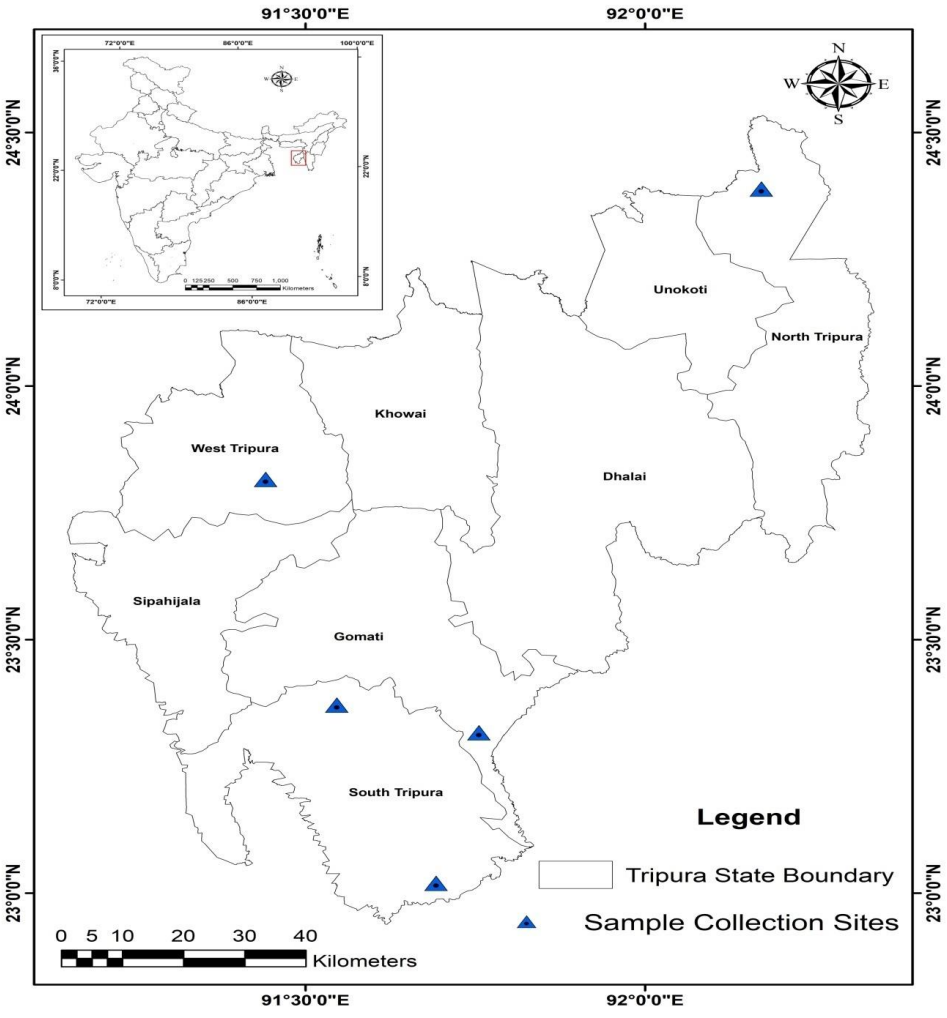

Fig. 1 – Map of Tripura showing the sampling sites of the *Terminalia chebula* plants.

Morphological identification of isolated endophytic fungi

The lactophenol cotton blue technique was applied to observe microscopic structures such as hyphae and spores using the Trinocular Light Microscope (Model No.: Leica DM 750, Germany). The standard manual and textbooks (Ellis 1971, Domsch et al. 1980, Watanabe 2002) were

employed for the identification of the isolates. Many of the endophytic fungi that failed to sporulate *in-vitro* in subculture conditions were categorized as sterile mycelium.

Table 1 – Locations, GPS tract, and the soil characteristics of the collection sites of *Terminalia chebula*

Locations	Latitude	Longitude	Elevation (m)	Soil pH	Soil temperature
Jirania (JRN)	23°48'57"N	91°26'29"E	63 m	7	26°C
Tuikarmaw (TKM)	23°22'15"N	91°32'47"E	75 m	7	27°C
Jalefa (JLF)	23°01'10"N	91°41'33"E	49 m	6.5	26.5°C
Jalaya (JLA)	23°18'59"N	91°45'19"E	91 m	7	28°C
Madhyapara (MDP)	24°23'19"N	92°10'17"E	50 m	6	22°C

Fig. 2 – *Terminalia chebula* plant. a Habitat of the plant sample. b, e Fruits. c Bark. d Roots. f Herbarium specimen (TUH-2387).

Statistical analysis

To understand the effect of tissue types, study sites and sampling seasons on the fungal endophytic compositions within the internal tissues of *T. chebula*, the fungal endophyte isolation and colonization rates, colonization frequency, and relative frequency of fungal species were determined by the formulae listed below:

a. Colonization rate (Petrini et al. 1982)

$$CR = \frac{\text{Total number of plant tissue segments infected by one or more fungi}}{\text{Total number of inoculated segments}} \times 100$$

b. Isolation rate (De Siqueira et al. 2017)

$$IR = \frac{\text{Number of isolates obtained from plant segments}}{\text{Total number of segments}} \times 100$$

c. Colonization frequency (Suryanarayanan et al. 2003)

$$CF = \frac{\text{Number of segments colonized by the fungi}}{\text{Total number of segments observed}} \times 100$$

d. Relative frequency (Huang et al. 2008)

$$RF = \frac{\text{Number of isolates of a species}}{\text{Total number of isolates}} \times 100$$

Various diversity indices like Shannon index (*H'*), Simpson's dominance (*D*), Simpson's index (1-*D*), Fisher's alpha index (*a*), Pielou's evenness (*J*), Berger-Parker dominance (*B*) and Brillouin index (*HB*) were calculated for the estimation of fungal diversity using R statistical software version 4.2.0 (R Core Team 2022).

Results

Isolation and identification of fungal isolates

Totally, 697 fungal endophytes were recovered from 1,125 tissue segments of *Terminalia chebula* from different locations in Tripura during the summer (234), monsoon (285), and winter (178) seasons (Table 2). Of these, 259, 212, and 226 endophytic fungi were recovered from the healthy tissues, viz., leaves, barks and roots of the host plant (Table 3). During this study, overall, 16 genera, 28 fungal species, and a few sterile mycelial forms were obtained. Some of the most frequent fungal strains were *Colletotrichum* sp., *Corynespora torulosa*, *Diaporthe* sp., *Lasiodiplodia* sp., *Penicillium exsudans*, *Trichoderma harzianum* and *Trichoderma* sp. (Fig. 3). The Ascomycota phylum was found to be the most dominant with 91 %, sterile mycelia with 8% and the Mucoromycota was lowest with 1% of fungal isolates (Supplementary Table 1). In addition, the Dothideomycetes class was found to be the class with the highest number of isolates (Fig. 4). The analysis of variance (ANOVA) showed a significant difference of fungal endophytes classes as given in Fig. 4.

Effects of tissues, sampling locations and seasons on isolation and frequency of endophytes

Among the tissue types subjected to the isolation of fungal endophytes, the highest isolation rate (IR) was recorded in leaves (69.07%), followed by root tissues (60.27%) and bark (56.53%) (Table 3). The highest colonization rate (CR) of endophytic fungi was observed in the leaves (94.67%) and then by the barks (89.87%) and roots (88.53%) (Table 3 & Fig. 5). In the case of relative frequency (RF), it was noted that the RF value was recorded highest from the leaves (37.16%) and the lowest was found in the bark (30.42%) (Table 3). In regard to the season (Table 2), IR (76%), CR (96.53%), and RF (40.89%) of fungal isolates were exhibited higher during the monsoon season as compared to summer and winter (Fig. 5). According to the study sites, the fungal endophytes were obtained highest in the Jalaya (JLA) site (152). The maximum IR (67.56%), CR (98.67%) and RF (21.81%) of fungal endophytes were also recorded from the JLA site compared to the other sites (Table 4). The sampling season, different tissues and sampling locations have greatly influenced the relative frequency (RF) of endophytic fungal distribution and composition in *T. chebula*. The highest RF value of endophytic fungi was shown by the *Diaporthe* sp. (10.47%), followed by the *Penicillium exsudans* (9.47%), but *Aspergillus fumigatus* (0.29%) exhibited the lowest RF (Table 5 & Fig. 6). The analysis of variance (ANOVA) showed a

significant difference of fungal endophytes in terms of colonization rate and relative frequency as provided in Fig. 5 and Fig. 6.

Table 2 Isolation rate, Colonization rate and Relative frequency of endophytic fungi associated with the *Terminalia chebula* according to seasons.

Seasons	Summer	Monsoon	Winter	Total
Number of tissues studied	375	375	375	1125
Number of tissues infected	337	362	325	1024
Number of isolates obtained	234	285	178	697
Isolation rate (%)	62.4	76	47.47	185.87
Colonization rate (%)	89.87	96.53	86.67	273.07
Relative frequency (%)	33.57	40.89	25.54	100

Table 3 Isolation rate, Colonization rate and Relative frequency of fungal endophytes associated with the various tissues of *Terminalia chebula.*

Tissue types	Leaf	Bark	Root	Total
Number of tissues studied	375	375	375	1125
Number of tissues infected	355	337	332	1024
Number of isolates obtained	259	212	226	697
Isolation rate (%)	69.07	56.53	60.27	185.87
Colonization rate (%)	94.67	89.87	88.53	273.07
Relative frequency (%)	37.16	30.42	32.42	100

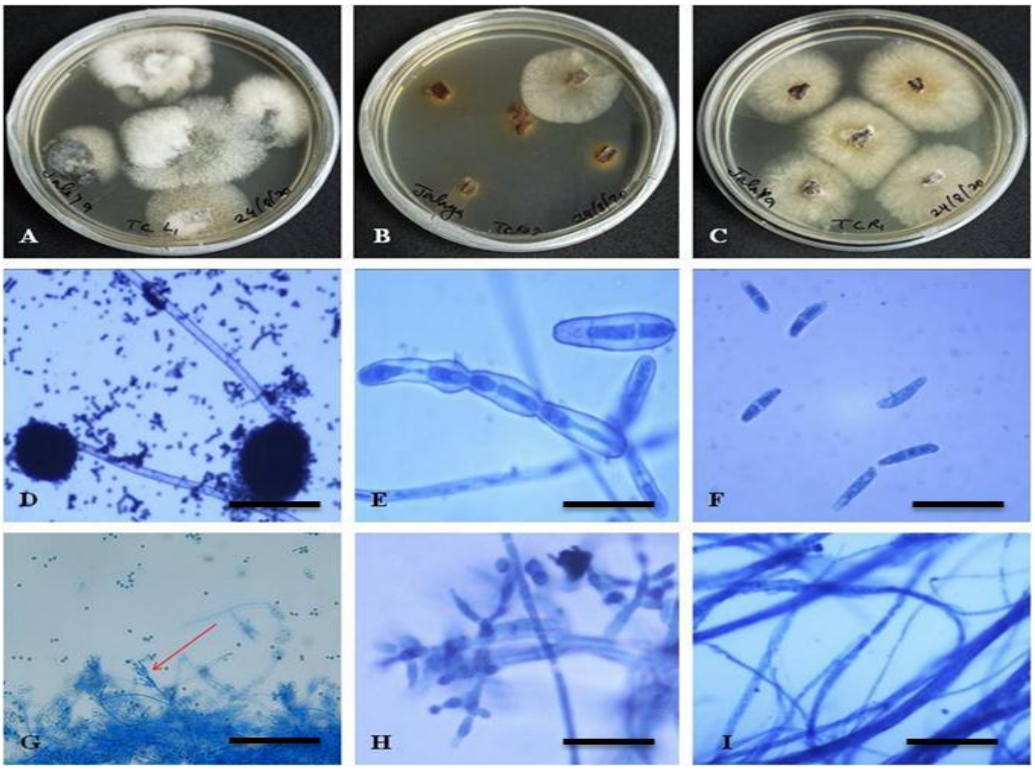

Fig. 3 – Emergence of fungal isolates colonies from the leaf. A bark. B and root segments. C of *Terminalia chebula.* Microscopic features of the isolated endophytic fungi. D *Aspergillus niger*. E *Corynespora torulosa*. F *Fusarium* sp. G *Penicillium* sp. 1, H *Trichoderma* sp. I White sterile 1. Scale bars: D = 50 µm, E–I = 100 µm.

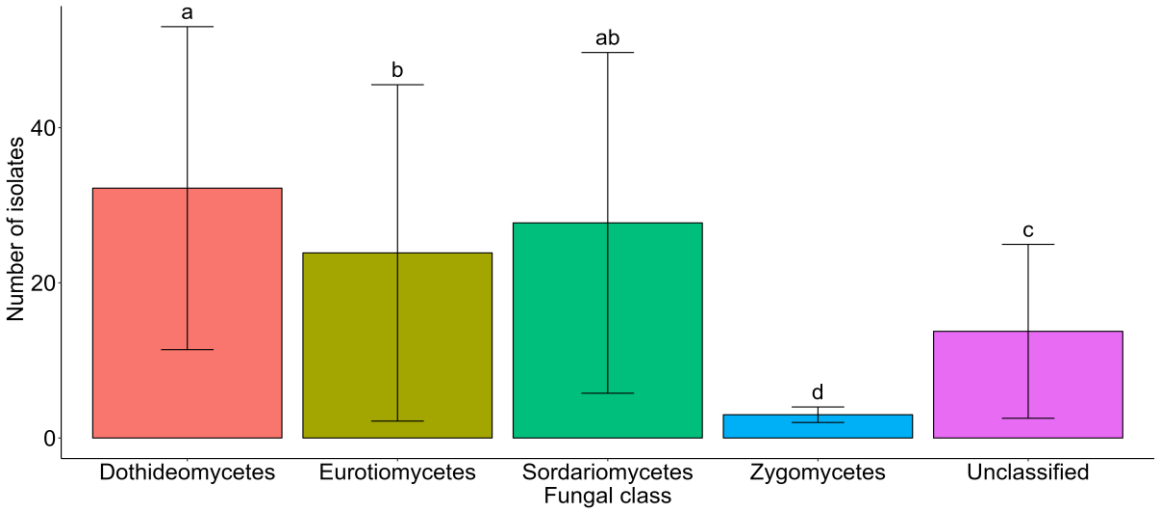

Fig. 4 – Fungal class of fungal endophytes in *Terminalia chebula*. Different letters showed a significant difference at p<0.05. Statistical analysis: One-way analysis of variance (ANOVA; Df = 10, F value = 6.732, p<0.05).

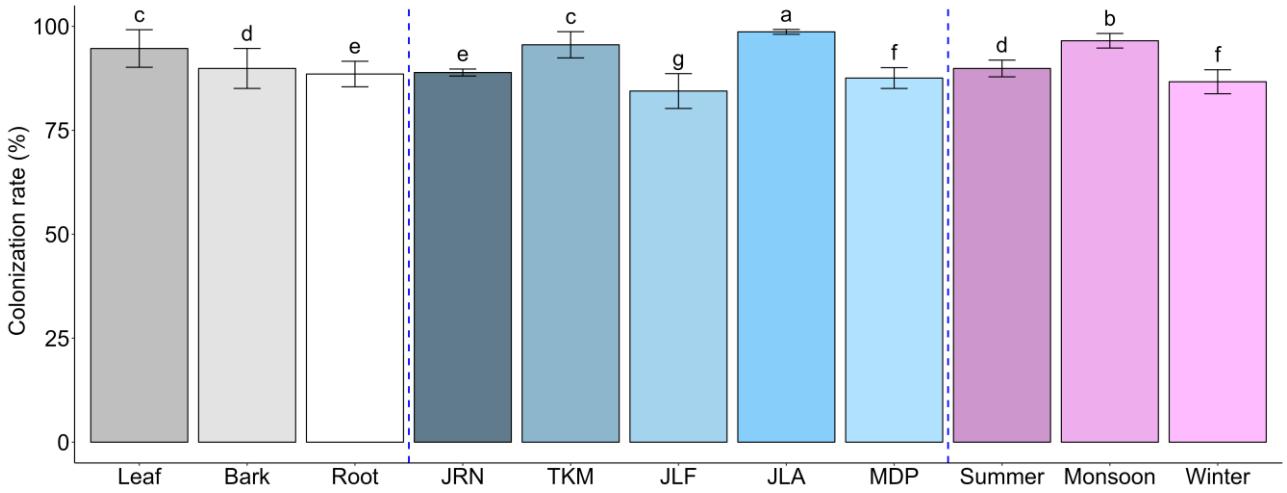

Fig. 5 – Colonization rate of fungal endophytes associated with *Terminalia chebula* according to seasons, tissues and locations. JRN- Jirania, TKM- Tuikarmaw, JLF- Jalefa, JLA- Jalaya, MDP- Madhyapara. Different letters showed a significant difference at p<0.05. Statistical analysis: One-way analysis of variance (ANOVA; Df = 10, F value = 6.732, p<0.05).

Table 4 Isolation rate, Colonization rate and Relative frequency of endophytic fungi associated with the *Terminalia chebula* in different locations.

Locations	JRN	TKM	JLF	JLA	MDP	Total
Number of tissues studied	225	225	225	225	225	1125
Number of tissues infected	200	215	190	222	197	1024
Number of isolates obtained	140	148	129	152	128	697
Isolation rate (%)	62.22	65.78	57.33	67.56	56.89	309.78
Colonization rate (%)	88.89	95.56	84.44	98.67	87.56	455.11
Relative frequency (%)	20.09	21.23	18.51	21.81	18.36	100

Note: JRN- Jirania, TKM- Tuikarmaw, JLF- Jalefa, JLA- Jalaya, MDP- Madhyapara.

Table 5 The relative frequency (RF %) of fungal endophytes colonized in various tissues of *Terminalia chebula* in several locations and seasons.

Endophytic fungal isolates	Tissue type			Location					Season			Total	RF
	L	B	R	JRN	TKM	JLF	JLA	MDP	Summer	Monsoon	Winter		
Alternaria alternata	6	3	3	1	2	3	2	4	5	7	-	12	1.72
Aspergillus flavus	2	-	4	1	3	2	-	-	4	2	-	6	0.86
Aspergillus fumigatus	-	1	1	-	1	-	-	1	1	1	-	2	0.29
Aspergillus niger	12	14	9	7	13	6	5	4	12	17	6	35	5.02
Chaetomium sp.	9	1	7	2	9	2	4	-	6	6	5	17	2.44
Cladosporium sp.	6	12	16	5	12	5	8	4	10	11	13	34	4.88
Colletotrichum sp.	20	28	6	14	3	7	14	16	17	18	19	54	7.75
Corynespora torulosa	40	10	4	16	13	4	11	10	18	22	14	54	7.75
Curvularia sp.	1	2	7	3	3	1	1	2	5	3	2	10	1.43
Diaporthe sp.	62	10	1	13	18	17	13	12	24	32	17	73	10.47
Fusarium falciforme	-	3	-	-	-	-	3	-	3	-	-	3	0.43
Fusarium oxysporum	-	4	21	10	3	2	8	2	5	11	9	25	3.59
Fusarium solani	-	1	6	2	-	-	4	1	2	2	3	7	1.00
Fusarium sp.	2	5	1	-	2	4	2	-	4	4	-	8	1.15
Lasiodiplodia sp.	18	13	20	11	8	18	7	7	15	14	22	51	7.32
Mucor sp.	4	1	4	-	-	-	2	7	6	2	1	9	1.29
Nigrospora sp.	6	7	-	4	1	4	3	1	2	5	6	13	1.87
Penicillium exsudans	21	7	38	13	14	18	16	5	26	24	16	66	9.47
Penicillium sp. 1	9	4	12	2	7	5	7	4	5	16	4	25	3.59
Penicillium sp. 2	1	6	7	2	-	5	2	5	3	8	3	14	2.01
Pestalotiopsis sp.	9	13	3	9	6	2	5	3	5	11	9	25	3.59
Talaromyces australis	2	1	16	5	-	1	8	5	8	11	-	19	2.73
Trichoderma harzianum	2	23	16	5	3	10	7	16	18	17	6	41	5.88
Trichoderma sp.	10	21	8	2	11	7	11	8	11	22	6	39	5.60
White sterile 1	12	12	6	6	9	3	6	6	12	9	9	30	4.30
White sterile 2	4	4	4	3	6	-	2	1	3	3	6	12	1.72
Black sterile	1	4	3	2	1	2	1	2	3	4	1	8	1.15
Yellow sterile	-	2	3	2	-	1	-	2	1	3	1	5	0.72

Note: L- Leaf, B- Bark, R- Root, JRN- Jirania, TKM- Tuikarmaw, JLF- Jalefa, JLA- Jalaya, MDP- Madhyapara.

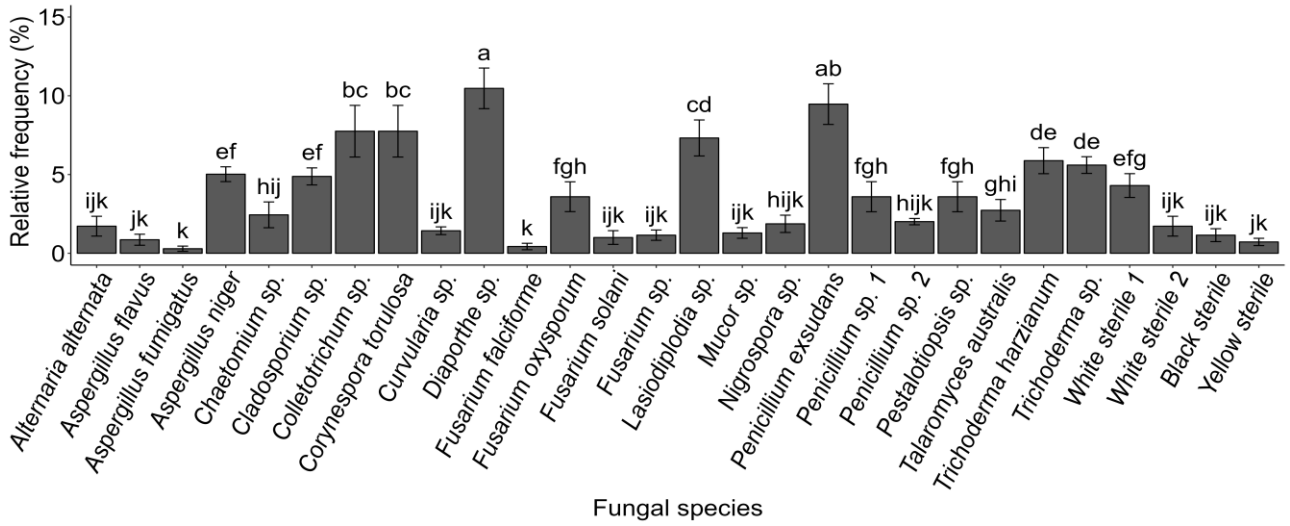

Fig. 6 – Relative frequency of fungal endophytes in *Terminalia chebula* plant. Different letters showed a significant difference at $p<0.05$. Statistical analysis: One-way analysis of variance (ANOVA; Df = 27, F value = 38.8, $p<0.05$).

Influence of seasons on the colonization of fungal isolates

The colonization frequency (CF) differed strongly in different sampling sites. During the monsoon season, the root segments (104 isolates) showed the highest colonization of endophytic fungi, followed by the leaves (93 isolates) and barks (88 isolates), whereas, during summer and winter seasons, the maximum fungal endophytes were recorded from the leaves (90 and 75 isolates), followed by bark (66 and 58 isolates) and the root tissue segments (77 and 45 isolates). The highest number of fungal isolates (60) were noticed in Jalaya (JLA) site and the lowest in Madhyapara (MDP) site with 32 isolates. During the summer season, the maximal CF was recorded for *Penicillium exsudans* (34.67%), followed by the *Diaporthe* sp. (32%), *Corynespora torulosa* and *Trichoderma harzianum* (24%), and the minimal CF was shown by *Aspergillus fumigatus* and yellow sterile (1.33%) as depicted in Table 6 and Fig. 7.

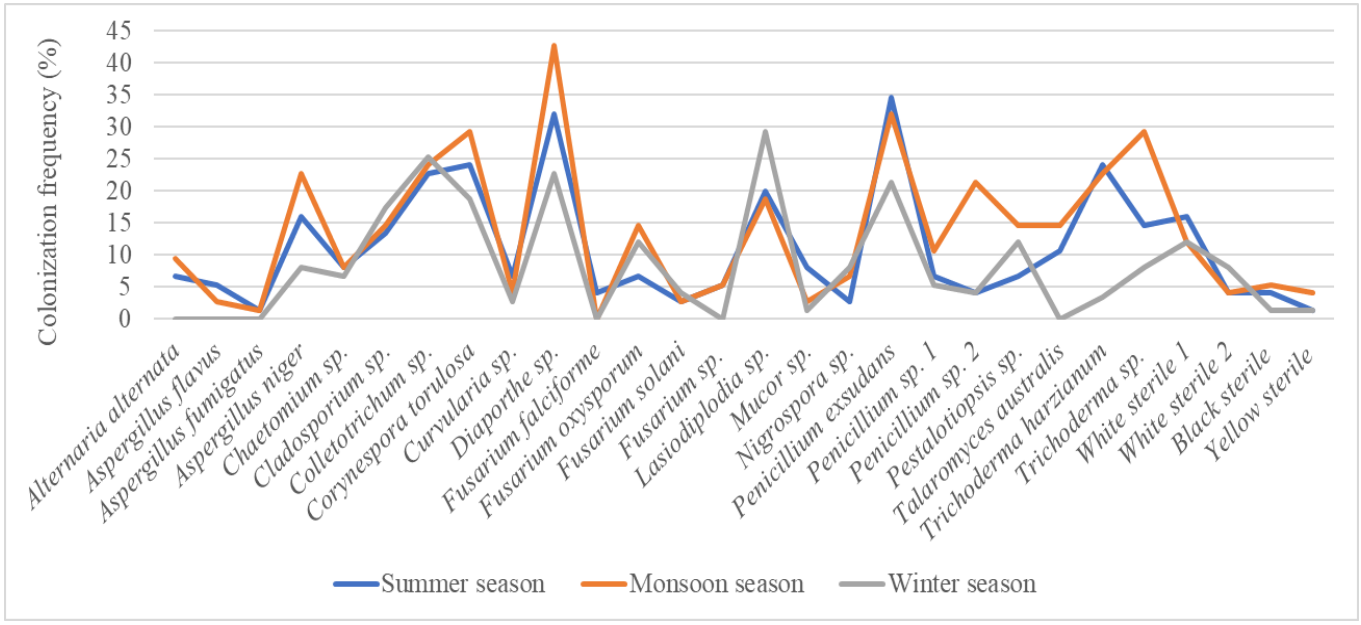

Fig. 7 – Colonization frequency of fungal endophytes in association with tissue types and locations during the three different seasons of *Terminalia chebula*.

Madhyapara (MDP) site (68), which exhibited the highest fungal isolates among the five sites during the monsoon season, was presented in Table 7 and Fig. 7. Among all the fungal isolates, *Diaporthe* sp. (42.67%) showed the highest CF and the least was shown by *A. fumigatus* (1.33%). *Fusarium falciforme* was absent in the monsoon season. During the winter season, the highest number of fungal isolates (43) were equally obtained from the two sites, viz., Jirania (JRN) and Jalaya (JLA). *Lasiodiplodia* sp. (29.33%) showed the maximum CF, followed by the *Colletotrichum* sp. (25.33%), while the minimum CF was shown by the black and yellow sterile (1.33%). Some of the common fungal species recovered from several tissues of *T. chebula* during summer, such as *Alternaria alternata*, *Aspergillus flavus, A. fumigatus, Fusarium falciforme, Fusarium* sp. and *Talaromyces australis*, could not be isolated during the winter season (Table 8 & Fig. 7).

Diversity of endophytic fungi

Tissue specificity

The tissue types had a prominent influence on the endophytic fungal diversity of *T. chebula* collected from various locations. Most of the fungal isolates were detected in the barks in contrast to leaves and roots (Table 9). The impacts of tissue concerning sites on the colonization frequency of fungal endophytes were represented in Tables 6, 7 & 8. The results showed that the diversity indices such as species richness (27), Shannon index (2.92), Simpson index (0.93), Pielou's evenness index (0.30), Fisher alpha index (8.20), and Brillouin index (2.71) were highest in the bark tissues. However, Dominance (0.10) and Berger-Parker index (0.23) were recorded maximum in the leaf tissues compared to barks and the roots (Table 9).

Present study showed that *Nigrospora* sp. was recovered from both the leaves and bark tissues of *T. chebula*. *Aspergillus flavus* was found in both the leaves and roots, whereas *A. fumigatus, F. oxysporum, F. solani*, and yellow sterile were recovered from the internal barks and roots. *Fusarium falciforme* was solely detected in the barks of this plant.

Effects of geographical location

Different kinds of fungal species were isolated from various locations. The fungal richness was noticed highest at the Jalaya (JLA) site with 25 isolates compared to the other locations. The diversity indices, namely the Shannon index (2.98), Simpson index (0.94), and Brillouin index (2.72), showed maximum values in the JLA site, whereas the lowest Dominance index (0.05) and Berger-Parker index (0.10) was also recorded from JLA site. On the other side, the maximum Pielou's evenness index (0.88) and Fisher's alpha index (8.71) were noted from the MDP site (Table 9).

Effects of season

The endophytic fungal diversity was strongly influenced by the sampling season in this study (Table 9). The highest colonization of endophytes occurred in the summer season (28 species) and the minimum in the winter season (22 species) (Table 6). The fungal isolates were shown maximum during the monsoon season (285), immediately followed by summer (234) and winter (178) seasons (Table 2). The diversity indices values of fungal endophytes confirmed the influence of season on the diversity of fungal species. The Shannon index (3.02) and Fisher alpha index (8.29) were recorded maximum in the summer season, while the highest Simpson (0.94) and Brillouin index (2.84) were recorded in the monsoon season. Pielou's evenness index (0.30), Dominance index (0.06), and Berger-Parker index (0.12) showed the highest value for the winter season (Table 9). *Fusarium falciforme* was exclusively isolated during the summer season. Moreover, fungal isolates such as *Alternaria alternata, Aspergillus flavus, A. fumigatus, Fusarium* sp. and *Talaromyces australis* were found both in summer and monsoon seasons except for the winter season.

Table 6 Colonization frequency (CF %) of fungal isolates in various tissues and locations of *Terminalia chebula* during the summer season.

Endophytic fungi isolates	JRN			TKM			JLF			JLA			MDP			Total	CF
	L	B	R	L	B	R	L	B	R	L	B	R	L	B	R		
Alternaria alternata	-	-	-	-	-	-	1	-	-	1	1	-	2	-	-	5	6.67
Aspergillus flavus	-	-	1	-	-	1	-	-	2	-	-	-	-	-	-	4	5.33
Aspergillus fumigatus	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	1.33
Aspergillus niger	-	2	1	2	2	-	1	-	-	-	1	-	2	-	1	12	16
Chaetomium sp.	-	-	-	1	-	2	-	-	-	3	-	-	-	-	-	6	8
Cladosporium sp.	-	-	3	3	1	1	-	-	2	-	-	-	-	-	-	10	13.33
Colletotrichum sp.	3	2	-	-	-	-	1	-	2	4	1	-	-	2	2	17	22.67
Corynespora torulosa	4	2	-	-	-	3	-	-	-	7	-	1	1	-	-	18	24
Curvularia sp.	-	-	2	1	-	1	-	-	1	-	-	-	-	-	-	5	6.67
Diaporthe sp.	3	1	-	4	-	-	8	2	-	5	-	1	-	-	-	24	32
Fusarium falciforme	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	3	4
Fusarium oxysporum	-	-	2	-	-	-	-	-	-	-	-	3	-	-	-	5	6.67
Fusarium solani	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	2	2.67
Fusarium sp.	-	-	-	1	-	-	1	-	-	-	2	-	-	-	-	4	5.33
Lasiodiplodia sp.	1	-	3	-	-	1	-	4	3	-	-	2	-	1	-	15	20
Mucor sp.	-	-	-	-	-	-	-	-	-	-	-	-	4	-	2	6	8
Nigrospora sp.	-	-	-	-	-	-	1	-	-	-	1	-	-	-	-	2	2.67
Penicillium exsudans	-	-	2	5	-	-	4	4	5	-	-	6	-	-	-	26	34.67
Penicillium sp. 1	-	-	-	1	1	2	-	-	-	-	-	1	-	-	-	5	6.67
Penicillium sp. 2	-	-	-	-	-	-	1	2	-	-	-	-	-	-	-	3	4
Pestalotiopsis sp.	-	2	-	2	-	-	-	-	-	-	-	1	-	-	-	5	6.67
Talaromyces australis	-	1	2	-	-	-	-	-	1	-	-	3	-	-	1	8	10.67
Trichoderma harzianum	-	-	-	-	-	-	2	1	2	-	5	-	-	6	2	18	24
Trichoderma sp.	-	-	-	2	-	-	5	1	-	-	3	-	-	-	-	11	14.67
White sterile 1	-	2	-	2	1	-	-	-	1	1	2	-	-	3	-	12	16
White sterile 2	-	1	-	-	1	-	-	-	-	-	-	-	1	-	-	3	4
Black sterile	-	-	-	-	1	-	-	-	2	-	-	-	-	-	-	3	4
Yellow sterile	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	1.33
Total	11	13	16	24	7	11	24	14	21	21	19	20	10	13	9	234	

Note: JRN- Jirania, TKM- Tuikarmaw, JLF- Jalefa, JLA- Jalaya, MDP- Madhyapara, L- Leaf, B- Bark, R- Root.

Table 7 Colonization frequency (CF %) of fungal isolates in various tissues and locations of *Terminalia chebula* during the monsoon season

Endophytic fungi isolates	JRN			TKM			JLF			JLA			MDP			Total	CF
	L	B	R	L	B	R	L	B	R	L	B	R	L	B	R		
Alternaria alternata	-	-	1	1	1	-	-	-	2	-	-	-	1	1	-	7	9.33
Aspergillus flavus	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	2	2.67
Aspergillus fumigatus	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1	1.33
Aspergillus niger	-	2	1	5	2	1	-	-	2	2	2	-	-	-	-	17	22.67
Chaetomium sp.	-	-	2	3	-	-	-	1	-	-	-	-	-	-	-	6	8
Cladosporium sp.	-	-	2	-	-	-	2	-	1	-	-	2	1	-	3	11	14.67
Colletotrichum sp.	2	4	-	-	1	1	-	-	-	1	4	-	3	2	-	18	24
Corynespora torulosa	3	-	-	7	-	-	2	2	-	-	3	-	5	-	-	22	29.33
Curvularia sp.	-	-	1	-	-	-	-	-	-	-	-	-	-	2	-	3	4
Diaporthe sp.	4	-	-	10	-	-	3	2	-	1	2	-	10	-	-	32	42.67
Fusarium falciforme	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
Fusarium oxysporum	-	-	3	-	-	2	-	-	1	-	4	-	-	-	1	11	14.67
Fusarium solani	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	2	2.67
Fusarium sp.	-	-	-	-	-	1	-	3	-	-	-	-	-	-	-	4	5.33
Lasiodiplodia sp.	-	2	1	1	-	2	1	-	3	1	-	2	-	-	1	14	18.67
Mucor sp.	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1	2	2.67
Nigrospora sp.	-	-	-	-	1	-	2	1	-	-	-	-	-	1	-	5	6.67
Penicillium exsudans	-	3	5	-	-	4	-	-	3	2	-	2	-	-	5	24	32
Penicillium sp. 1	-	-	2	-	-	-	-	-	1	-	-	-	-	4	1	8	10.67
Penicillium sp. 2	-	-	1	-	-	1	3	2	-	3	-	2	-	-	4	16	21.33
Pestalotiopsis sp.	-	3	-	-	-	2	-	2	-	-	2	-	2	-	-	11	14.67
Talaromyces australis	-	-	2	-	-	-	-	-	-	2	-	3	-	-	4	11	14.67
Trichoderma harzianum	-	2	3	-	3	-	-	2	2	-	-	2	-	-	3	17	22.67
Trichoderma sp.	-	1	-	-	5	2	-	-	1	3	3	-	-	2	5	22	29.33
White sterile 1	-	-	2	1	2	-	-	1	-	-	-	-	2	1	-	9	12
White sterile 2	-	-	-	2	1	-	-	-	-	-	-	-	-	-	-	3	4
Black sterile	-	1	1	-	-	-	-	-	-	-	-	-	-	2	-	4	5.33
Yellow sterile	-	-	1	-	-	-	-	1	-	-	-	-	-	1	-	3	4
Total	9	18	30	32	16	17	13	17	16	15	21	13	24	16	28	285	

Note: JRN- Jirania, TKM- Tuikarmaw, JLF- Jalefa, JLA- Jalaya, MDP- Madhyapara, L- Leaf, B- Bark, R- Root.

Table 8 Colonization frequency (CF %) of fungal isolates in various tissues and locations of *Terminalia chebula* during the winter season

Endophytic fungi isolates	JRN			TKM			JLF			JLA			MDP			Total	CF
	L	B	R	L	B	R	L	B	R	L	B	R	L	B	R		
Alternaria alternata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
Aspergillus flavus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
Aspergillus fumigatus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
Aspergillus niger	-	1	-	-	1	-	-	-	3	-	-	-	-	1	-	6	8
Chaetomium sp.	-	-	-	2	-	1	-	-	1	-	-	1	-	-	-	5	6.67
Cladosporium sp.	-	-	-	-	6	1	-	-	-	-	5	1	-	-	-	13	17.33
Colletotrichum sp.	1	2	-	1	-	-	2	2	-	-	3	1	2	5	-	19	25.33
Corynespora torulosa	5	2	-	2	1	-	-	-	-	-	-	-	4	-	-	14	18.67
Curvularia sp.	-	-	-	-	-	1	-	-	-	-	-	1	-	-	-	2	2.67
Diaporthe sp.	4	1	-	4	-	-	-	2	-	4	-	-	2	-	-	17	22.67
Fusarium falciforme	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
Fusarium oxysporum	-	-	5	-	-	1	-	-	1	-	-	1	-	-	1	9	12
Fusarium solani	-	-	-	-	-	-	-	-	-	-	-	2	-	1	-	3	4
Fusarium sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
Lasiodiplodia sp.	3	1	-	2	1	1	5	2	-	-	1	1	4	1	-	22	29.33
Mucor sp.	-	-	-	-	-	-	-	-	-	-		1	-	-	-	1	1.33
Nigrospora sp.	3	1	-	-	-	-	-	-	-	-	2	-	-	-	-	6	8
Penicillium exsudans	-	-	3	5	-	-	-	-	2	5	-	1	-	-	-	16	21.33
Penicillium sp. 1	-	1	-	1	-	1	-	-	-	1	-	-	-	-	-	4	5.33
Penicillium sp. 2	-	-	-	-	-	-	-	-	1	-	-	2	-	-	-	3	4
Pestalotiopsis sp.	-	4	-	2	-	-	-	-	-	2	-	-	1	-	-	9	12
Talaromyces australis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
Trichoderma harzianum	-	-	-	-	-	-	-	1	-	-	-	-	-	3	2	6	3.37
Trichoderma sp.	-	1	-	-	2	-	-	-	-	-	2	-	-	1	-	6	8
White sterile 1	2	-	-	1	-	2	1	-	-	2	-	1	-	-	-	9	12
White sterile 2	-	-	2	-	1	1	-	-	-	1	-	1	-	-	-	6	8
Black sterile	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1	1.33
Yellow sterile	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	1	1.33
Total	18	14	11	20	12	9	8	7	8	16	13	14	13	12	3	178	

Note: JRN- Jirania, TKM- Tuikarmaw, JLF- Jalefa, JLA- Jalaya, MDP- Madhyapara, L- Leaf, B- Bark, R- Root.

Table 9 Diversity of endophytic fungi colonized in the tissues of *Terminalia chebula* across the locations and seasons.

Diversity indices	Tissue types			Locations					Seasons		
	Leaf	Bark	Root	JRN	TKM	JLF	JLA	MDP	Summer	Monsoon	Winter
Species richness (*S*)	23	27	26	24	22	23	25	24	28	27	22
Shannon (*H*')	2.5831	2.9214	2.899	2.8879	2.8187	2.7859	2.9868	2.8883	3.0268	3.0128	2.8143
Simpson (1-*D*)	0.8897	0.9326	0.9276	0.9329	0.9303	0.9198	0.9411	0.9315	0.9404	0.9417	0.9292
Pielou's evenness (*J*)	0.2772	0.3001	0.2987	0.2961	0.2841	0.284	0.288	0.3048	0.2842	0.2992	0.3039
Fisher alpha (*a*)	6.0971	8.208	7.5864	8.3368	7.1488	8.1458	8.5126	8.7199	8.2985	7.3235	6.6062
Brillouin (*HB*)	2.4335	2.7158	2.7052	2.6269	2.5851	2.5216	2.7282	2.6101	2.8238	2.8422	2.6101
Dominance (*D*)	0.1069	0.063	0.0683	0.0604	0.0633	0.073	0.0526	0.0611	0.0555	0.055	0.0656
Berger-Parker (*B*)	0.2394	0.1321	0.1681	0.1143	0.1216	0.1395	0.1053	0.125	0.1111	0.1123	0.1236

Discussion

Overall, a total of 697 fungal endophytes were recorded from 1,125 segments of leaf, barks, and root tissues of *Terminalia chebula*. The isolation and colonization rate (IR & CR), as well as relative frequency (RF) of fungal endophytes, were shown to be highest in the leaves compared to the bark and root tissues of *T. chebula*. The leaf segments harbored the maximum fungal isolates than barks and roots, which coincides with the previous study (Vemireddy et al. 2020). This result may be due to the exposure of larger surface regions of leaves available to the external atmosphere, and the presence of stoma provides the fungal spores an easy pathway to enter the leaf tissues. Previous studies have shown that most of the endophytic fungi exhibited higher colonization in leaf tissues than the rest of the tissues (Lebron et al. 2001, Gond et al. 2012).

During the monsoon season, the colonization rate (CR) of fungal endophytes obtained from *T. chebula* was found to be the highest, with a CR of 96.53%, and the winter season showed the lowest CR of 86.67%. It was similar to previous findings (Naik et al. 2008), suggesting that endophytic fungal spores are prone to grow and localized in host tissues more effortlessly when the moisture and temperature increase (Arnold et al. 2001, Hilarino et al. 2011). During the monsoon season, variations in the CR of fungal endophytes were noticed due to the fact that the fungal spores are well scattered by rainfall, and climatic factors affect the development of conidia (Wilson & Carroll 1994). Furthermore, the dispersal and frequency of endophytic fungi may be affected by the distinction in tissue chemistry of the host plants (Arnold et al. 2001). Our results further indicated that the IR, CR, and RF values of fungal endophytes were comparatively higher at the Jalaya (JLA) site. This may be due to site-specific environments that caused the differences in the CF across different sites (Naik et al. 2008).

The endophytic fungi recovered from three types of tissues were identified (28 isolates) morphologically at the generic or species level. *Colletotrichum* sp., *Corynespora torulosa*, *Diaporthe* sp., *Lasiodiplodia* sp., *Penicillium exsudans*, *Trichoderma harzianum* and *Trichoderma* sp. etc. were the most dominant ones in this study (Table 6–8). They showed a greater occurrence and may present as host-specific species with *T. chebula*, which was incorporated in a previous report (Kouipou Toghueo & Boyom 2019). This may be due to the large spore dispersion capability of these fungal endophytes and their universal nature (Schulthess & Faith 1998, Raviraja 2005, Verma et al. 2007).

Our study revealed that most of the fungal endophytes belonged to the phylum Ascomycota (91%), and the lowest was represented by Mucoromycota (1%) (Supplementary Table 1). Previous research has also reported that the majority of the endophytes colonized in medicinal plants represented the Ascomycota phylum (Jahromi et al. 2021, Kumar & Prasher 2022, Crasta & Anandrao 2024). Here, the fungal endophytes belonged to 11 different orders, representing four classes in the phylum Ascomycota, i.e., Dothideomycetes (Botryosphaeriales, Capnodiales, Pleosporales), Eurotiomycetes (Eurotiales), Sordariomycetes (Amphisphaeriales, Diaporthales, Glomerellales, Hypocreales,

Sordariales, Trichosphaeriales). However, Zygomycetes (Mucorales) were recorded from Mucoromycota, and a few isolates were considered sterile mycelia. Among the classes, the dominant class was Dothideomycetes, followed by Sordariomycetes, and the lowest was Zygomycetes (Aleynova et al. 2022, Crasta & Anandrao 2024). The fungal orders Eurotiales and Hypocreales have shown the highest diversity in the present study.

Our result suggests that endophytic fungal colonization varies across the tissue types in *T. chebula*. In this study, it was noted that certain fungal isolates were confined to a particular vegetative part. For instance, *Aspergillus flavus* exhibited only in the leaves and roots. *Nigrospora* sp. was recovered only from the leaf and bark tissues, while *Aspergillus fumigatus, Fusarium oxysporum, F. solani,* and yellow sterile mycelia were isolated only from the barks and roots. *Fusarium falciforme* was confined only to the barks. Several studies showed that endophytic fungi belonging to the genera such as *Alternaria* (Phaopongthai et al. 2013), *Colletotrichum* (Suryanarayanan et al. 2018), *Lasiodiplodia* (Suryanarayanan et al. 2018), and *Penicillium* (Shoeb et al. 2014) were recovered from the leaf tissues of *T. chebula*, whereas *Pestalotiopsis* was recovered from the leaves and barks of this plant. Our result also correlates with the previously reported findings (Tejesvi et al. 2006, 2007, Suryanarayanan et al. 2018). Host tissue specificity of endophytes has been proven in many plants (Sadeghi et al. 2019). It could be due to the high dependence of specific endophytes on a particular plant tissue for a specific action (Photita et al. 2001).

Geographical locations influenced the endophytic distributions in this medicinal plant. The maximum fungal species was recovered from the Jalaya (JLA) site in our study. Tuikarmaw (TKM) and Jirania (JRN) locations were subsequent locations with an identical frequency in fungal isolates recovered. On the other hand, Jalefa (JLF) showed the lowest fungal isolates recovered. *Fusarium falciforme* was restricted to the JLA site only. *Mucor* sp. was detected only at JLA and Madhyapara (MDP) sites, whereas *Aspergillus fumigatus* was noted at Tuikarmaw (TKM) and MDP sites only. Some of the exclusive isolates were *Chaetomium* sp. in the MDP site, *Talaromyces australis* in the TKM site, and white sterile 2 in the JLF site. The current study indicated that the variations of fungal endophytes across the different sites could be due to environmental conditions at the study sites. It was also to be noted that certain endophytic fungi, at the species or genus level, have location-specific distribution (Massimo et al. 2015). The likelihood of recovering many endophytic fungal species increases with site distance.

The various sampling sites showed significant differences in endophytic fungal diversity. Our results indicate that the bark tissues harbored rich diversity of endophytes, and the richness of fungal species was slightly higher than the roots and leaves, which were in congruence with the former studies (Stone et al. 2000, Wang & Guo 2007). However, our data contradicts the former report on *T. chebula,* which showed higher diversity in the leaf tissues (Vemireddy et al. 2020). These results clearly demonstrated that the endophytes have the tendency to colonize specific tissues (Bettucci et al. 1997). Jalaya (JLA) site has the highest fungal diversity, and Tuikarmaw (TKM) has the lowest among the sampling sites. This may be due to the ecological characteristics of sampling locations, such as altitude, soil temperature, and soil pH (Table 1), that could affect the endophytic fungal communities within the sites. Studies have also revealed that the differences in the endophytic fungal diversity among study sites may be due to variations in soil physical-chemical properties, plant features (Jan et al. 2022), and environmental conditions, including plant botanical distribution, climatic conditions of each location (Jahromi et al. 2021), lower annual rainfall and low annual temperature (Sadeghi et al. 2019).

Our results indicated that the diversity of endophytic fungal communities was affected by the seasons. The endophytic fungal composition varied across different sampling seasons. The fungal diversity was maximum during the summer season. The incidence of fungal species was significantly higher in the summer season, which could be due to the survivability of fungal spores at even low water contents (Naik et al. 2008). Our study displayed that the high temperature and pH of the soil

favour the endophytic fungal growth in the dispersal of spores, as depicted in Table 1. The main factor in the seasonal diversity of endophytic fungi was temperature, as indicated by Manna & Kim (2018). Seasonal variations in endophytic fungal species composition were due to selection pressure variations on fungal strains residing within living tissues at different time periods of the respective year, according to Guo et al. (2008) and Kamalraj & Muthumary (2013). These variable selection pressures highly affect the germination capacity of endophytic fungal spores (Schulthess & Faeth 1998). Fang et al. (2013) reported that variations in the secondary metabolite concentration level in the plant are the primary determinant of the seasonal variation of endophytic fungal diversity. These factors could impact the endophytic fungal spores' ability to germinate and propagate. According to Oita et al. (2021), both seasons and climatic factors influence the fungal endophyte richness and diversity in tropical countries. Singh et al. (2017) stated that tissue type, location, and seasons together promptly influenced the compositions of endophytic fungi in *Tectona grandis*.

Conclusion

The study sheds light on the endophytic fungal diversity in many *Terminalia chebula* tissues during various study seasons and locations in Tripura, Northeastern India. Most of the fungal endophytes represented phylum Ascomycota, in which Dothideomycetes was the predominant class. Although leaves reported more fungal endophytes than barks and roots, the maximum fungal diversity was reported in the bark tissues at the Jalaya site. The high diversity indices of fungal species were noted during the summer compared to the monsoon and winter seasons. Tissue type, site and season affected the entire composition of the endophytic fungal community on *T. chebula*. Our study indicated that the fungal endophytes colonized in this medicinal plant can further be applied to discover their role in plant growth and development, antimicrobial activity and synthesis of natural products for new drug design.

Acknowledgements

The authors are thankful to the Head of the Department of Botany, Tripura University, for utilizing the laboratory facilities in the department. Tripura S (first author) is grateful to the DBT Twinning Project, Govt. of India (Sanctioned Order No.: BT/PR25260/NER/95/1102/2017) for providing the fellowship and the equipment to bring out the outcome of the research. The authors would like to thank Mr. Biplab Banik, Research Scholar, Plant Taxonomy and Biodiversity Laboratory, Department of Botany, Tripura University, Tripura, for making the study area map of Tripura, India.

References

Aleynova OA, Nityagovsky NN, Suprun AR, Ananev AA et al. 2022 – The diversity of fungal endophytes from wild grape *Vitis amurensis* Rupr. Plants 11(21), 1–16.

Arnold AE, Lutzoni F. 2007 – Diversity and host range of foliar fungal endophytes: are tropical leaves biodiversity hotspots?. Ecology 88(3), 541–549.

Arnold AE, Maynard Z, Gilbert GS. 2001 – Fungal endophytes in dicotyledonous neotropical trees: patterns of abundance and diversity. Mycological Research 105(12), 1502–1507.

Bhattacharya S, Debnath S, Saha AK. 2020 – Diversity and endophytic fungal composition in *Ananas comosus* (L.) Merr. var queen cultivar isolated from three different growing conditions. Studies in Fungi 5(1), 310–319.

Bettucci L, Alonso R, Fernandez LM. 1997 – A comparative study of fungal populations in healthy and symptomatic twigs and seedlings of *Eucalyptus globulus* in Uruguay. Sydowia 49(2), 109–17.

Chetia H, Kabiraj D, Bharali B, Ojha S et al. 2019 – Exploring the benefits of endophytic fungi via omics. Advances in endophytic fungal research: present status and future challenges, Fungal Biology, Springer, Cham., 51–81.

Crasta GL, Anandrao RK. 2024 – Seasonal diversity & spaciotemporal distribution of fungal endophytes associated with the medicinal plant *Coleus forskohlii* Briq. Plant Science Today 11(1), 223–233.

De Bary A. 1866 – Morphologie und physiologie der pilze, flechten und myxomyceten. Engelmann, 1831–1888

De Siqueira KA, Brissow ER, Santos JL, White JF et al. 2017 – Endophytism and bioactivity of endophytic fungi isolated from *Combretum lanceolatum* Pohl ex Eichler. Symbiosis 71, 211–222.

Domsch KH, Gams W and Anderson TH. 1980 – Compendium of soil fungi. Volume 1. Academic Press (London) Ltd., 7–859.

Ellis MB. 1971 – Dematiaceous hyphomycetes. CAB International Mycological Institute, Kew, England, 28–580.

Fan C, Dong Y, Xie Y, Su Y et al. 2015 – Shikonin reduces TGF-β1-induced collagen production and contraction in hypertrophic scar-derived human skin fibroblasts. International Journal of Molecular Medicine 36(4), 985–991.

Fang W, Yang L, Zhu X, Zeng L et al. 2013 – Seasonal and habitat dependent variations in culturable endophytes of *Camellia sinensis*. Journal of Plant Pathology & Microbiology 4(3), 2157–2471.

Gond SK, Mishra A, Sharma VK, Verma SK et al. 2012 – Diversity and antimicrobial activity of endophytic fungi isolated from *Nyctanthes arbor-tristis*, a well-known medicinal plant of India. Mycoscience 53(2), 113–121.

Guo LD, Huang GR, Wang Y. 2008 – Seasonal and tissue age influences on endophytic fungi of *Pinus tabulaeformis* (Pinaceae) in the Dongling Mountains, Beijing. Journal of Integrative Plant Biology 50(8), 997–1003.

Hallmann J, Berg G, Schulz B. 2006 – Isolation procedures for endophytic microorganisms. In Microbial root endophytes. Springer Berlin Heidelberg, 299–319.

Hallmann J, Quadt-Hallmann A, Mahaffee WF, Kloepper JW. 1997 – Bacterial endophytes in agricultural crops. Canadian Journal of Microbiology 43(10), 895–914.

Hamayun M, Khan SA, Khan AL, Rehman G et al. 2010 – Gibberellin production and plant growth promotion from pure cultures of *Cladosporium* sp. MH-6 isolated from cucumber (*Cucumis sativus* L.). Mycologia 102(5), 989–995.

Hardoim PR, Van Overbeek LS, Berg G, Pirttilä AM et al. 2015 – The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. Microbiology and Molecular Biology Reviews 79(3), 293–320.

Hilarino MP, Silveira FA, Oki Y, Rodrigues L et al. 2011 – Distribution of the endophytic fungi community in leaves of *Bauhinia brevipes* (Fabaceae). Acta Botanica Brasilica 25, 815–821.

Huang WY, Cai YZ, Hyde KD, Corke H et al. 2008 – Biodiversity of endophytic fungi associated with 29 traditional Chinese medicinal plants. Fungal Diversity 33, 61–75.

Jahromi MS, Azizi A, Soltani J. 2021 – Diversity and spatiotemporal distribution of fungal endophytes associated with *Salvia multicaulis*. Current Microbiology 78(4), 1432–1447.

Jan B, Reshi ZA, Mohiddin FA. 2022 – Site and organ-specific culture-dependent endophytic diversity of *Crocus sativus* L.(saffron) in Kashmir Himalaya, India. Microbial Ecology 83(4), 989–1006.

Jokar A, Masoomi F, Sadeghpour O, Nassiri-Toosi M et al. 2016 – Potential therapeutic applications for *Terminalia chebula* in Iranian traditional medicine. Journal of Traditional Chinese Medicine 36(2), 250–254.

Kamalraj S, Muthumary J. 2013 – Prevalence and seasonal periodicity of endophytic coelomycetous fungi in Tamil Nadu, India. International Journal of Biodiversity Conservation 5(8), 469–477.

Kesting JR, Staerk D, Tejesvi MV, Kini KR et al. 2009 – HPLC-SPE-NMR identification of a novel metabolite containing the Benzo [c] oxepin skeleton from the endophytic fungus *Pestalotiopsis virgatula* culture. Planta medica 75(10), 1104–1106.

Khan AL, Waqas M, Hussain J, Al-Harrasi A et al. 2014 – Fungal endophyte *Penicillium janthinellum* LK5 can reduce cadmium toxicity in *Solanum lycopersicum* (Sitiens and Rhe). Biology and Fertility of Soils 50, 75–85.

Kouipou Toghueo RM, Boyom FF. 2019 – Endophytic fungi from *Terminalia* species: a comprehensive review. Journal of Fungi 5(2), 1–20.

Krishnamurthy YL, Naik SB, Jayaram S. 2008 – Fungal communities in herbaceous medicinal plants from the Malnad region, Southern India. Microbes and Environments 23(1), 24–28.

Kumar V, Prasher IB. 2022 – Seasonal variation and tissues specificity of endophytic fungi of *Dillenia indica* L. and their extracellular enzymatic activity. Archives of Microbiology 204(6), 1–14.

Kumaresan V, Veeramohan R, Bhat MM, Sruthi K et al. 2013 – Fungal endophyte assemblages of some Pteridophytes from Mahe, India. World Journal of Science and Technology 3(1), 7–10.

Lebron L, Lodge DJ, Laureano S, Bayman P. 2001 – Where is the gate to the party? Phytopathology 91(1), 91–116.

Li WC, Zhou J, Guo SY, Guo LD. 2007 – Endophytic fungi associated with lichens in Baihua mountain of Beijing, China. Fungal Diversity 25, 69–80.

Massimo NC, Nandi Devan MM, Arendt KR, Wilch MH et al. 2015 – Fungal endophytes in aboveground tissues of desert plants: infrequent in culture, but highly diverse and distinctive symbionts. Microbial Ecology 70, 61–76.

Naik BS, Shashikala J, Krishnamurthy YL. 2008 – Diversity of fungal endophytes in shrubby medicinal plants of Malnad region, Western Ghats, Southern India. Fungal Ecology 1(2–3), 89–93.

Oita S, Ibáñez A, Lutzoni F, Miadlikowska J et al. 2021 – Climate and seasonality drive the richness and composition of tropical fungal endophytes at a landscape scale. Communications Biology 4(1), 1–11.

Petrini O, Stone J, Carroll FE. 1982 – Endophytic fungi in evergreen shrubs in western Oregon: a preliminary study. Canadian Journal of Botany 60(6), 789–796.

Petrini O. 1991 – Fungal endophytes of tree leaves. In: Microbial ecology of leaves. New York, NY: Springer, 179–197.

Phaopongthai J, Wiyakrutta S, Meksuriyen D, Sriubolmas N et al. 2013 – Azole-synergistic anti-candidal activity of altenusin, a biphenyl metabolite of the endophytic fungus *Alternaria alternata* isolated from *Terminalia chebula* Retz. Journal of Microbiology 51, 821–828.

Photita W, Lumyong S, Lumyong P, Hyde KD. 2001 – Endophytic fungi of wild banana (*Musa acuminata*) at Doi Suthep Pui National Park, Thailand. Mycological Research 105(12), 1508–1513.

Rajeswari S, Revathi R, Arvind Prasanth D. 2017 – Isolation and identification of endophytic fungi from *Terminalia chebula* of Eastern Ghats, Tamil nadu. Asian Journal of Pharmaceutical and Clinical Research 10(4), 21–23.

Raviraja NS. 2005 – Fungal endophytes in five medicinal plant species from Kudremukh Range, Western Ghats of India. Journal of Basic Microbiology: An International Journal on Biochemistry, Physiology, Genetics, Morphology, and Ecology of Microorganisms 45(3), 230–235.

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

Sadeghi F, Samsampour D, Seyahooei MA, Bagheri A et al. 2019 – Diversity and spatiotemporal distribution of fungal endophytes associated with *Citrus reticulata* cv. Siyahoo. Current Microbiology 76, 279–289.

Schardl CL, Leuchtmann A, Spiering MJ. 2004 – Symbioses of grasses with seedborne fungal endophytes. Annual Review of Plant Biology 55, 315–340.
Schulthess FM, Faeth SH. 1998 – Distribution, abundances, and associations of the endophytic fungal community of Arizona fescue (*Festuca arizonica*). Mycologia 90(4), 569–578.
Shoeb M, Kong Thoo Lin P, Nahar N. 2012 – Anti-colon cancer activity of endophytic fungal strains from *Terminalia chebula* Rezt. Bangladesh Journal of Pharmacology12(1), 47–49.
Shoeb M, Haque M, Nahar N. 2014 – Bioactive compounds from endophytic fungus *Penicillium thiomii* isolated from *Terminalia chebula* Retz. Journal of Natural Products and Plant Resources 4(3), 65–70.
Singh DK, Sharma VK, Kumar J, Mishra A et al. 2017 – Diversity of endophytic mycobiota of tropical tree *Tectona grandis* Linn. f.: Spatiotemporal and tissue type effects. Scientific Reports 7(1), 1–14.
Singh LP, Gill SS, Tuteja N. 2011 – Unraveling the role of fungal symbionts in plant abiotic stress tolerance. Plant Signaling & Behavior 6(2), 175–191.
Stone JK, Bacon CW, White Jr JF. 2000 – An overview of endophytic microbes: endophytism defined. Microbial Endophytes, 17–44.
Strobel G, Daisy B, Castillo U, Harper J. 2004 – Natural products from endophytic microorganisms. Journal of Natural Products 67(2), 257–268.
Suman A, Yadav AN, Verma P. 2016 – Endophytic microbes in crops: diversity and beneficial impact for sustainable agriculture. Microbial inoculants in sustainable agricultural productivity: Vol. 1: research perspectives, Springer, 117–143.
Suryanarayanan TS, Devarajan PT, Girivasan KP, Govindarajulu MB et al. 2018 – The host range of multi-host endophytic fungi. Current Science 115(10), 1963–1969.
Suryanarayanan TS, Venkatesan G, Murali T. 2003 – Endophytic fungal communities in leaves of tropical forest trees: diversity and distribution patterns. Current Science 85, 489–493.
Tejesvi MV, Mahesh B, Nalini MS, Prakash HS et al. 2006 – Fungal endophyte assemblages from ethnopharmaceutically important medicinal trees. Canadian Journal of Microbiology 52(5), 427–435.
Tejesvi MV, Kini KR, Prakash HS, Subbiah V et al. 2007 – Genetic diversity and antifungal activity of species of *Pestalotiopsis* isolated as endophytes from medicinal plants. Fungal Diversity 24(3), 1–8.
Tomar CS, Saha D, Das S, Shaw S et al. 2017 – Analysis of temperature variability and trends over Tripura. Mausam 68(1), 149–160.
Vemireddy B, Madasi A, Ajmeera A, Vanteru KR. 2020 – Distribution and diversity of endophytic fungi associated with three medicinal tree species from Eturnagaram Wildlife Sanctuary, TS, India. Journal of Applied Biology and Biotechnology 8(6), 7–12.
Verma VC, Gond SK, Kumar A, Kharwar RN et al. 2007 – The endophytic mycoflora of bark, leaf, and stem tissues of *Azadirachta indica* A. Juss (Neem) from Varanasi (India). Microbial Ecology 54, 119–125.
Wang Y, Guo LD. 2007 – A comparative study of endophytic fungi in needles, bark, and xylem of *Pinus tabulaeformis*. Botany 85(10), 911–917.
Watanabe T. 2002 – Pictorial Atlas of Soil and Seed fungi: Morphologies of cultured fungi and key to species. 2nd Ed. CRC Press Florida US, 1–486.
Wilson D, Carroll GC. 1994 – Infection studies of *Discula quercina*, an endophyte of *Quercus garryana*. Mycologia 86(5), 635–647.
Zhang T, Zhang YQ, Liu HY, Wei YZ et al. 2013 – Diversity and cold adaptation of culturable endophytic fungi from bryophytes in the Fildes Region, King George Island, maritime Antarctica. FEMS Microbiology Letters 341(1), 52–61.

Zhang XR, Kaunda JS, Zhu HT, Wang D et al. 2019 – The genus *Terminalia* (Combretaceae): An ethnopharmacological, phytochemical and pharmacological review. Natural Products and Bioprospecting 9, 357–392.

Zhang XJ, He LJ, Lu Q, Li DY. 2016 – Pharmacological activity of *Terminalia chebula*. Zhongguo Zhong yao za zhi= Zhongguo zhongyao zazhi (China Journal of Chinese Materia Medica) 41(4), 619–623.

Zou WX, Meng JC, Lu H, Chen GX et al. 2000 – Metabolites of *Colletotrichum gloeosporioides*, an endophytic fungus in *Artemisia mongolica*. Journal of Natural Products 63(11), 1529–1530.