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Isolation, characterization, and diversity of fungal endophytes from *Albizia lebbeck* in Jammu & Kashmir, India

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ABSTRACT

Endophytes are microorganisms that reside within various plant parts and cause no damage to the host plant. Fungal endophytes from various medicinal plants have been isolated and characterized due to their beneficial role; however, their diversity in the *Albizia lebbeck* plant remains unexplored. This study aims to isolate and represent the diversity of endophytic fungi associated with the leaves and stem of *A. lebbeck*, commonly known as "Siris" or Shirish" in Ayurvedic medicine. This medicinal plant is widely distributed in India, including the Jammu and Kashmir region. Endophytes were isolated and characterized using a combination of morphological and molecular approaches, employing internal transcribed spacer markers. A total of 210 endophytic fungi were successfully isolated (111 from leaves and 99 from stems) from 52 plant samples collected across 13 sites in Jammu and Kashmir. Most of the isolates (99.5%) belonged to the phylum Ascomycota, while only 0.5% were identified as Basidiomycota. Around 82 distinct species were identified, with greater diversity and species richness in leaf samples, highlighting the ecological significance of endophytes in plant systems. These findings inspire us to further investigate the role of fungal endophytes in maintaining plant health and their potential applications in pharmaceuticals.

1. INTRODUCTION

Medicinal plants play an important role in both traditional and modern medicine because of their abundance of bioactive compounds. These plants are fundamental to traditional healing practices in many cultures, and they frequently serve as the cornerstone for current pharmacological studies and drug development [1,2]. The importance of medicinal plants like *Albizia lebbeck,* commonly known as Shirish, belonging to the family Fabaceae, lies in their ability to support endophytes that reside inside plant tissues and produce secondary metabolites. These metabolites are critical to the plant's defense systems, especially against pests, diseases, and environmental stresses [3]. Their ability to promote plant growth and survival under extreme circumstances emphasizes their ecological significance and prospective uses in agriculture and horticulture [4].

Some reports show the production of bioactive compounds like saponins, flavonoids, and alkaloids derived from *A. lebbeck* that have demonstrated remarkable therapeutic potential, including anti-inflammatory, antibacterial, and anticancer activities [5]. Endophytic fungi in *A. lebbeck* were reported as highly important, suggesting that they may be used to produce distinct secondary metabolites through complex metabolic processes that have developed over millions of years [6]. These fungi may be cultured under normal laboratory conditions to produce vast amounts of these bioactive compounds potentially leading to the discovery of novel drugs [7–9]. Mathur *et al.* (2022) explored the bioactive metabolites produced by the endophytes of *A. lebbeck*, emphasizing their potential in sustainable agriculture [10]. The importance of *A. lebbeck* in both traditional medicine and current drug development highlights the need for more research on its endophytes. These diverse fungal endophytes offer insights into biotechnology, ecosystem function, and environmental protection [2,11]. Previous studies have shown that endophytic fungi can exhibit significant diversity in various plant species, including medicinal plants but specific data on *A. lebbeck* remains limited [12–16]. Our study provides new insights into the fungal communities associated with this plant.

The endophytic fungus can strengthen the plant's immune system by producing a variety of secondary metabolites that protect it from biotic and abiotic threats [17,18]. These metabolites frequently reproduce or enhance the plant's natural defense systems, providing an extra layer of protection necessary for plant health and vitality [19]. Furthermore, these metabolites are produced by complicated biochemical pathways

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that have evolved over millions of years, allowing these fungi to manufacture unique and potent compounds that are typically difficult to synthesize chemically [6]. The ability of endophytic fungi under controlled conditions, such as bioreactors, allows for the production of large quantities of these valuable compounds avoiding the seasonal and geographical limitations that frequently reduce the isolation of plant-derived metabolites. The therapeutic potential of endophytic fungi is also increased by their ability to undergo genetic modification, which may be utilized to enhance the production of some metabolites or to form the production of new compounds [20,21]. This makes endophytic fungus not only important for plant health but also a promising resource for the discovery of novel and effective drugs, particularly in fighting against diseases that are resistant to traditional therapy [22].

Our research group is focused on drug discovery and has already reported bioactive molecules from various microbes [20,21,23–26]. This is the first report exploring the diversity of endophytic fungi associated with *A. lebbeck,* a traditional medicinal plant, collected from various regions of Jammu (Jammu & Kashmir, India). It advances our understanding and promotes sustainable use for future generations.

2. MATERIALS AND METHODS

2.1. Collection and Sampling of *A. lebbeck*

Plant samples of *A. lebbeck* were collected from 12 different regions of Jammu (Jammu & Kashmir, India). The sampling sites included Talab-Tillo Bohri, Domana, Mishriwala, Akhnoor, Palanwala, Chatha, Samba, Kathua, Udhampur, Katra, and Reasi. Plant material (leaves and stem) was placed in sterile polyethylene bags, packed in the icebox, and brought carefully to the laboratory followed by the isolation of endophytic fungi within 24 hours of collection (Fig. 1a) [10,27].

2.2. Isolation of Fungal Endophytes and Pure Cultures

The collected samples were first washed several times under running tap water to remove adhered soil and dirt particles. The washed sample was surface sterilized to all surface-adhered microbial epiphytes [28]. Surface sterilization was performed by sequentially rinsing with double distilled water for 2–3 minutes, then with 1% Sodium Hypochlorite (NaOCl) for 1 minute followed by double distilled water for 2–3 minutes then with 75% ethanol (C_2H_5OH) for 30 seconds and finally with double distilled water for 2–3 minutes. After that, the plant sample was allowed to air dry between the sterile filter paper folds. The margins of the plant sample were cut with a sterilized scalpel to expose the inner tissue and cut into small pieces of size 5 mm. Pieces were then inoculated on petri plates of three different media, potato dextrose agar (PDA), yeast malt agar (YMA), and water agar (WA) media supplemented with streptomycin sulphate (HiMedia Laboratories) (0.2 mg/ml), an antibiotic to suppress bacterial growth [29]. The media encourages the endophytic fungi to develop and exit the plant tissue. Plates were sealed with parafilm and incubated at 28°C in an incubator under dark conditions, observation was taken from the second day as some fastidious fungi may cause overlapping if plates remain unobserved. Hyphal tips from the inoculated sample were transferred to fresh PDA plates. Subculturing was done several times until pure culture was obtained and coded as ALE-X, (x represents the number of strains), a voucher of each endophytic fungus was deposited at Col. Sir R.N Chopra, Microbial Resource Center Jammu (MRCJ), India in 20% glycerol and mineral oil and stored at −20°C and 4°C, respectively (Table S1).

2.3. Identification of Fungal Endophytes

2.3.1. Molecular characterization

As none of the fungal endophytes were sporulating for identification purposes, we followed a more reliable internal transcribed spacer (ITS) method. For the past 15 years, the ITS of nuclear DNA has been targeted for analyzing the diversity of fungal endophytes in environmental samples [30]. It has recently been used as a standard marker for fungal DNA barcoding. For the isolation of fungal genomic DNA, the zymo research Fungal/Bacterial DNA MiniPrepTM kit was used according to the manufacturer's protocol. The qualitative and quantitative analysis of genomic DNA was assessed using the NanoDrop 2000. Polymerase chain reaction (PCR) was performed using Taq PCR Reaction Mix (Sigma Aldrich) and the universal ITS region primer pair ITS5: 5'-GGAAGTAAAAGTCGTAACAAGG-3' and ITS4: 5'-TCCTCCGCTTATTGATATGC-3' (Integrated DNA Technologies) in a thermal cycler (Eppendorf, Mastercycler pro) according to the following protocol: initial denaturation (95°C for 5 minutes), denaturation (94°C for 30 seconds), annealing (56°C for 45 seconds), extension (72°C for 1 minutes), and final extension (72°C for 10 minutes) [31]. The last three steps of denaturation, annealing, and extension were repeated for 35 cycles. The PCR cocktail comprised 25 µl of Taq PCR Reaction Mix, 2X, 1 µl of each primer (12 µM), 2.5 µl of the template DNA, and 20.5 µl of nuclease-free water (Promega). After amplification, the PCR product was purified using HiPurA™ PCR product purification kit (HiMedia Laboratories), and the purity of the amplified product was analyzed using 5 µl of the sample loaded onto a 1.8% agarose in 1X TAE containing 4 µl of ethidium bromide (0.5 µg/ml). Gel electrophoresis was then carried out at 80 V for 50–60 minutes to visualize the PCR product (approx. size of amplified product, 550 bp). The PCR product was sequenced with an ABI Prism 377 DNA sequencer in both directions using the application of the BigDye Terminator v3.1 cycle sequencing kit as mentioned in the manufacturer's protocol.

2.3.2. DNA sequence assembly and phylogenetic analysis

To taxonomically designate the endophytic fungi, all the sequences generated in this study were used as query sequences and compared with those available in GenBank at the National Center for Biotechnology Information (NCBI) with Basic Local Alignment Search Tool (BLAST) algorithm, and the sequences showing similarity of ≥99% to the sequence of ITS region (600 bp) were aligned by Clustal W software [32]. A phylogenetic tree was constructed using MEGA 6.0 software, followed by the analysis of evolutionary distances using neighbor-joining agglomerative clustering. The number of bootstrap analyses was 1,000 replications. All 210 sequences were submitted in the GenBank database using the SEQUIN program (Table S1) [33].

2.4. Data Analysis

The diversity of endophytic fungi was assessed by calculating their colonization frequency (%CF) by using the formula given below [34]. The dominance of specific endophytes was calculated as the proportion of the colony frequency of an individual endophyte relative to the total colony frequency of all endophytes expressed as a percentage.

$$CF\ (\%) = \left(\frac{Nf}{Nt}\right) \times 100$$

where Nf = Number of segments of plant tissue colonized by each isolate and Nt = Total number of segments of plant tissue.

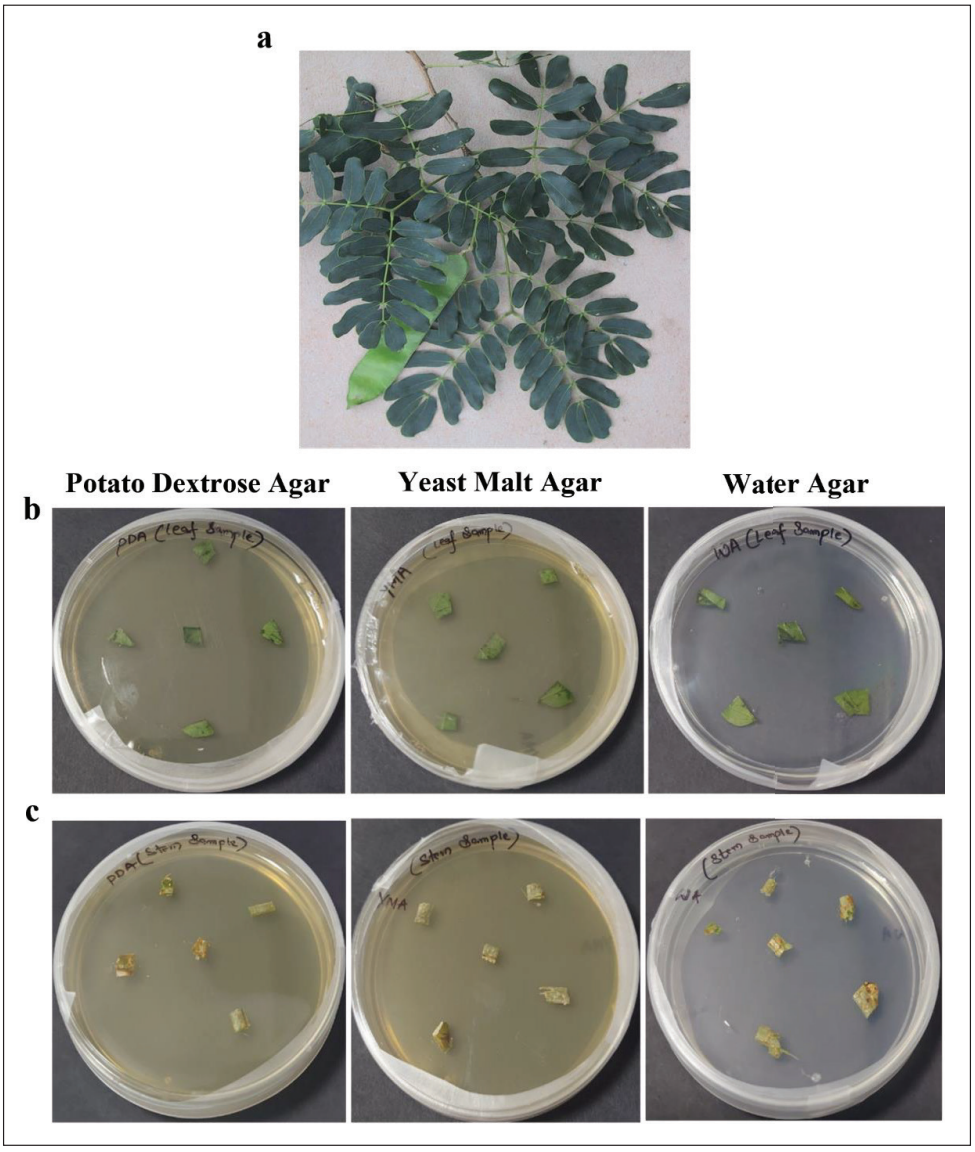

Figure 1. *Albizia lebbeck* and inoculated explant. (a) whole plant, (b and c) leaf and stem explant cultured on potato dextrose agar, yeast malt agar and water agar media.

All the statistical analyses were performed using the software package PAST3 and MS Excel [35].

3. RESULTS

3.1. Endophyte Isolation and Characterization

3.1.1. Isolation of endophytes

Two hundred and ten fungal endophytes were isolated from *A. lebbeck* samples collected from different regions of Jammu, J&K, India. Three different media viz. PDA, YMA, and WA were used for both the leaf and stem explants (Fig. 1b). 111 endophytic fungi were isolated from leaves and 99 from stems. The details of fungal endophytes, incubation time, and number of colonies recovered are provided in Table 1. The percentage recovery was higher in PDA followed by YMA and WA. This may be due to optimal nutrient availability in PDA to facilitate the growth of endophytes. Pure cultures were sub-cultured on conventional media (PDA) for 7 days at 28°C.

Table 1. Recovery of endophytes from *Albizia lebbeck* on different culture media.

Plant tissue	Media	Incubation time (in days)	No. of colonies recovered/720 segments
Leaves	PDA	4	51
	YMA	4	33
	WA	5	25
Stem	PDA	5	44
	YMA	5	31
	WA	6	16

PDA = potato dextrose agar, YMA = yeast malt agar, WA = water agar.

3.1.2. Taxonomic identification of the fungal endophytes

Two hundred and ten endophytic fungi were isolated from *A. lebbeck*. All the fungi were not sporulating and therefore difficult to identify and classify in different taxa based on morphological characters

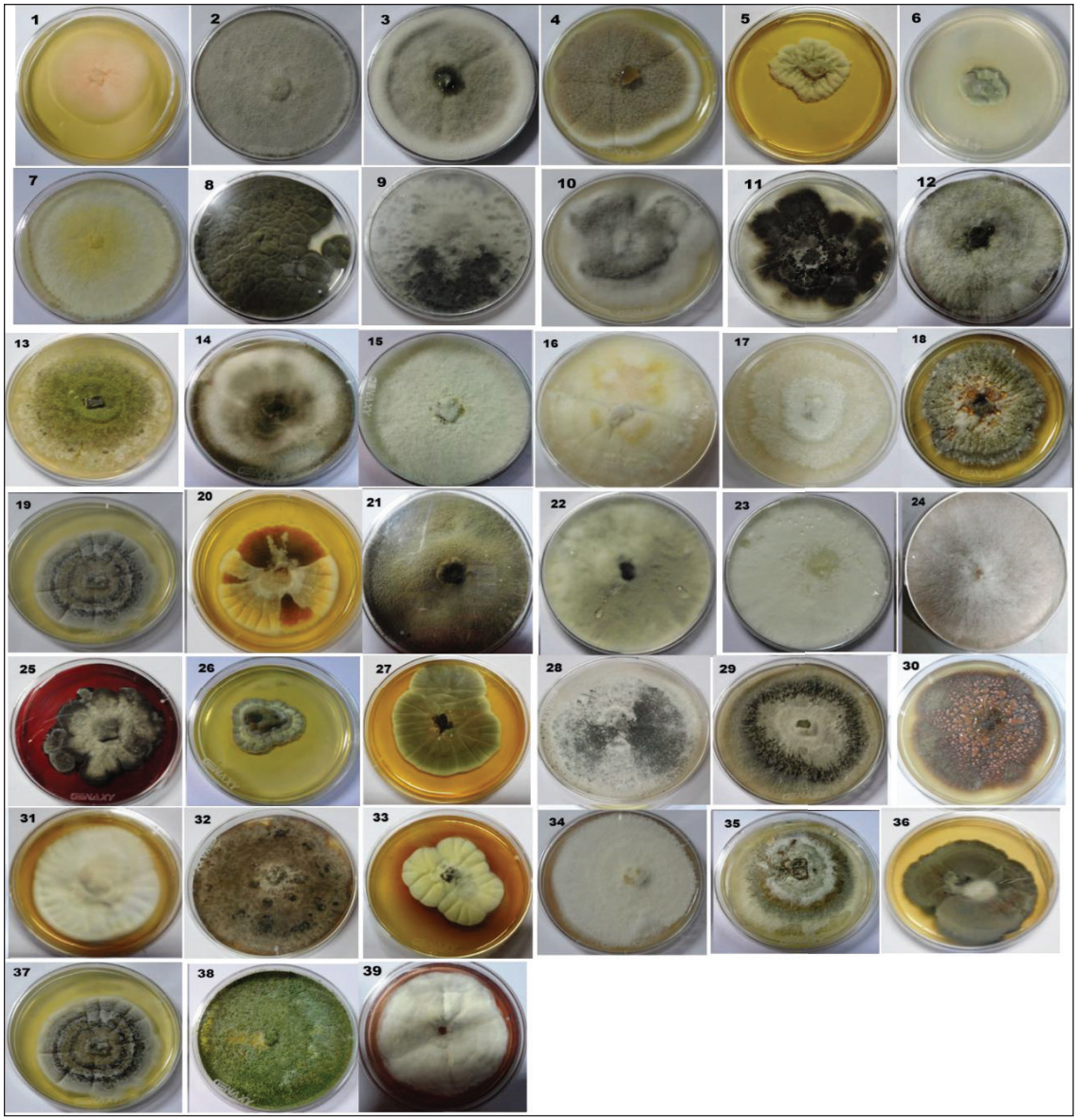

Figure 2. Endophytic Fungi isolated from the leaves and stem of *Albizia lebbeck* representing to each genus: 1. ALE-19 (*Acremonium sp.*), 2. ALE- 2 (*Alternaria sp.*), 3. ALE-167 (*Ampelomyces sp.*), 4. ALE-4 (*Aspergillus sp.*), 5. ALE-77 (*Bipolaris sp.*), 6. ALE-206 (*Cercospora sp.*), 7. ALE-81 (*Chaetomium sp.*), 8. ALE-145 (*Cladosporium sp.*), 9. ALE-84 (*Cochliobolus sp*), 10. ALE-87 (*Colletotrichum sp.*), 11. ALE-123 (*Curvularia sp.*), 12. ALE-214 (*Daldinia sp.*), 13. ALE-3 (*Diaporthe sp.*), 14. ALE-84 (*Epicoccum sp.*), 15. ALE-79 (*Eutypella sp.*), 16. ALE-54 (*Fusarium sp.*), 17. ALE-160 (*Fusicoccum sp.*), 18. ALE-109 (*Glomerella sp.*), 19. ALE-213 (*Hamigera sp.*), 20. ALE-94 (*Hansfordia sp.*), 21. ALE-14 (*Hypoxylon sp.),* 22. ALE-143 (*Lasiodiplodia sp.*), 23. ALE-207 (*Lenzites sp.*), 24. ALE-26 (*Nectria sp.*), 25. ALE-134 (*Nigrospora sp.*), 26. ALE-74 (*Paraphoma sp.*), 27. ALE-217 (*Penicillium sp.*), 28. ALE-168 (*Pestalotiopsis sp.*), 29. ALE-163 (*Peyronellaea sp.*), 30. ALE-114 (*Phoma sp.*), 31. ALE-15 (*Purpureocillium sp.*), 32. ALE-29 (*Phomopsis sp.*), 33. ALE-42 (*Preussia sp.*), 34. ALE-5 (*Rosellinia sp.*), 35. ALE-75 (*Saccharicola sp.*), 36. ALE-185 (*Setosphaeria sp.*), 37. ALE-119 (*Thielavia sp.*), 38. ALE-129 (*Trichoderma sp.*), 39. ALE-210 (*Xylaria sp.*).

(Fig. 2). Thus, we relied on the identification and characterization of endophytic fungi by analyzing the sequences of the amplified ITS region. The sequenced data obtained was used to find the closest matches by doing the BLAST against databases of sequences present in the Genbank (NCBI database). The endophytes were identified by the closest matches of the sequence with the sequences of the cultures that were already identified by ITS sequence homology (>95%) (Table S1). The phylogenetic tree was generated based on ITS4 and ITS5 sequences analysis with a bootstrap value of 1,000 by the maximum likelihood method. The analysis shows that the isolates from the plant samples comprise 25 orders of fungal class (Fig. 3).

3.2. Classification, Tissue Distribution, and Isolation Frequency (%IF) of Endophytic Fungi

A comprehensive analysis yielded 210 endophytic fungal isolates from leaves and stems of the traditional medicinal plant *A. lebbeck*. Specifically, 111 were obtained from the leaves and 99 from the stem tissues.

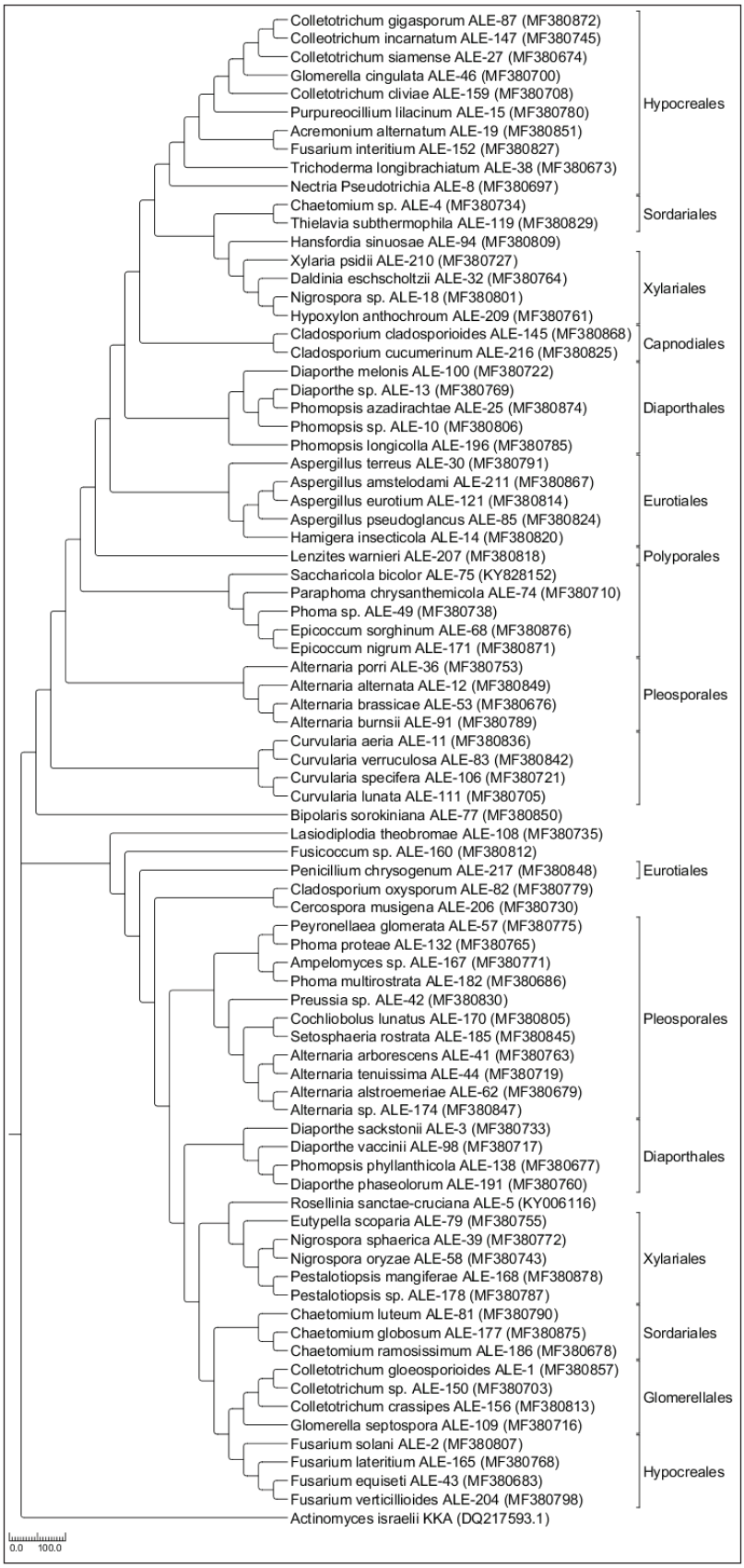

Figure 3. Phylogenetic tree showing the relationship among the fungal endophyte isolates of *Albizia lebbeck*. The tree was constructed based on rDNA sequence (ITS4 and ITS5) by using maximum likelihood method with 1000 bootstrap analyses and genetic distances were generated using Tamura-Nei model.

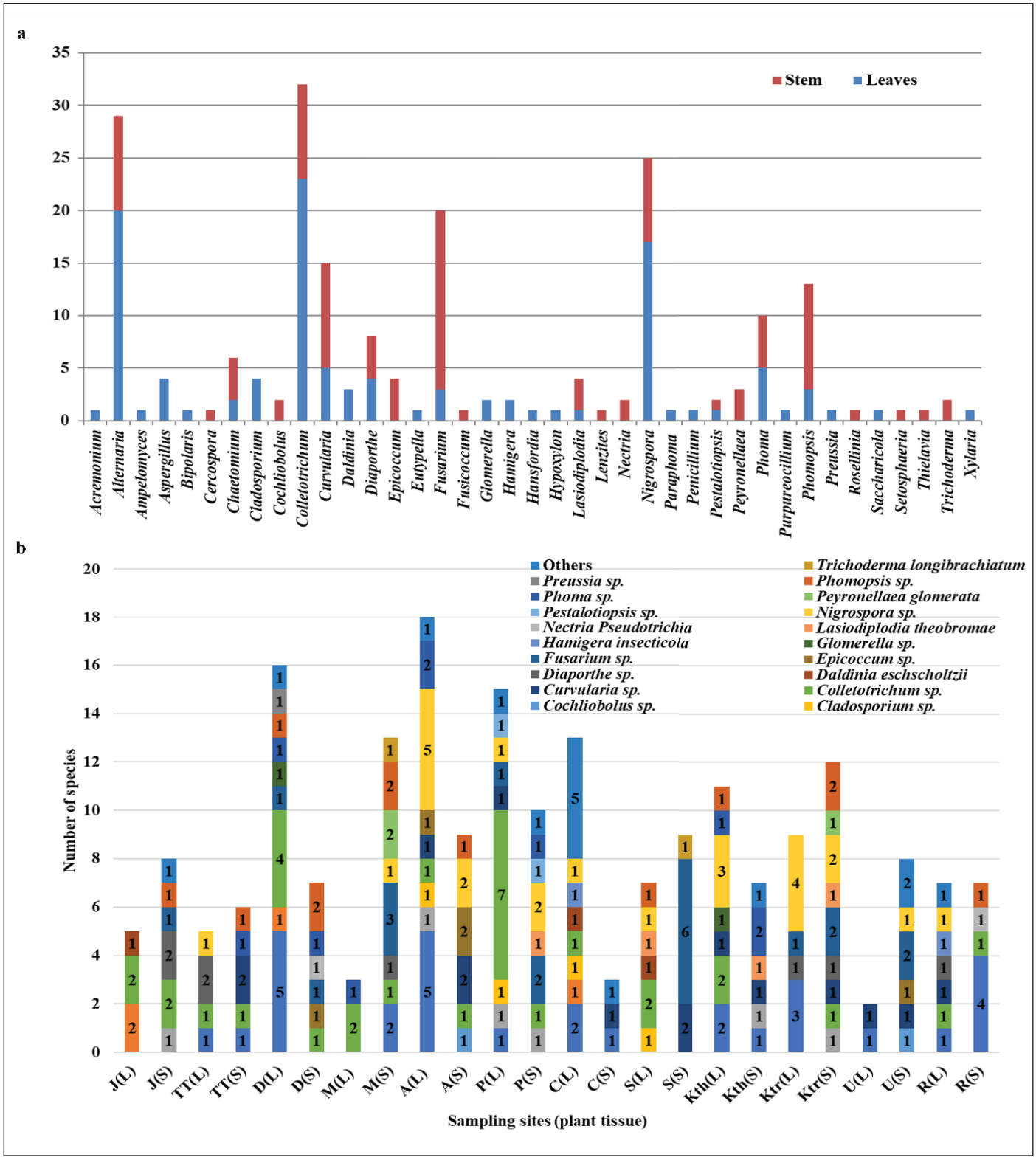

Figure 4. Fungal endophytes from *Albizia lebbeck* leaves and stem of different sampling sites. **(**a) Genera wise (39 genera), (b) species distribution, *X*-axis represents the sampling sites, and the letters representing plant tissue, leaves (L), and stem (S). Jammu: J(L), J(S), Talab tillo: TT(L), TT(S), Domana: D(L), D(S), Mishriwala: M(L), M(S), Akhnoor: A(L), A(S), Palanwala: P(L), P(S), Chatha: C(L), C(S), Samba: S(L), S(S), Kathua: Kth(L), Kth(S), Katra: Ktr(L), Ktr(S), Udhampur: U(L), U(S), Reasi: R(L), R(S). Others includes: *Ampelomyces sp., Bipolaris sorokiniana, Cercospora musigena, Eutypella scoparia, Hansfordia sinuosae, Hypoxylon anthochroum, Lasiodiplodia theobromae, Lenzites warnieri, Nectria Pseudotrichia, Rosellinia sanctae-cruciana, Saccharicola bicolor, Setosphaeria rostrata, Thielavia subthermophila, Trichoderma longibrachiatum,* and *Xylaria psidii*.

Out of 210 isolates, 209 fungal endophytes belonged to Ascomycota and 1 belonged to Basidiomycota. Sordariomycetes (57.61%) was the prominent class followed by Dothideomycees (38.09%), Eurotiomycetes (3.33%), Pezizomycetes (0.47%), and Agariomycetes (0.47%). In class Sordariomycetes, the order Glomerales (27.86%), Hypocreales (21.31%), Trichosphaeriales (20.66%), and Diaporthales (17.21%) were the most abundant followed by Sordariales (4.91%) and Xylariales (4.09%). In Dothideomycetes, the order Pleosporales (87.5%) was the most abundant followed by Capnodiales (6.25%) and Botryosphaeriales (6.25%). Peizomycetes and Agariomycetes with only one order Pezizates and Polyporales, respectively. A maximum of 32 fungal endophytes belonged to the genera *Colletotrichum* followed by *Alternaria* (29), *Nigrospora* (25), *Fusarium* (20), *Curvularia* (15), *Phomopsis* (13), *Phoma* (10),

Table 2. Distribution of the endophytic fungi in different tissues and their isolation frequency in percentage (%IF) in *Albizia lebbeck*.

Fungal Taxa	Leaves	Stem	Isolates number	Phylum, Class, Order	%IF
Acremonium	1	0	1	Ascomycota; Sordariomycetes; Hypocreales.	0.48
Alternaria	20	9	29	Ascomycota; Dothideomycetes; Pleosporales.	13.81
Ampelomyces	1	0	1	Ascomycota; Dothideomycetes; Pleosporales.	0.48
Aspergillus	4	0	4	Ascomycota; Eurotiomycetes; Eurotiales.	1.90
Bipolaris	1	0	1	Ascomycota; Dothideomycetes; Pleosporales.	0.48
Cercospora	0	1	1	Ascomycota; Dothideomycetes; Capnodiales.	0.48
Chaetomium	2	4	6	Ascomycota; Sordariomycetes; Sordariales.	2.86
Cladosporium	4	0	4	Ascomycota; Dothideomycetes; Capnodiales.	1.90
Cochliobolus	0	2	2	Ascomycota; Dothideomycetes; Pleosporales.	0.95
Colletotrichum	23	9	32	Ascomycota; Sordariomycetes; Glomerellales.	15.24
Curvularia	5	10	15	Ascomycota; Dothideomycetes; Pleosporales.	7.14
Daldinia	3	0	3	Ascomycota; Sordariomycetes; Xylariales.	1.43
Diaporthe	4	4	8	Ascomycota; Sordariomycetes; Diaporthales.	3.81
Epicoccum	0	4	4	Ascomycota; Dothideomycetes; Pleosporales.	1.90
Eutypella	1	0	1	Ascomycota; Sordariomycetes; Xylariales.	0.48
Fusarium	3	17	20	Ascomycota; Sordariomycetes; Hypocreales.	9.52
Fusicoccum	0	1	1	Ascomycota; Dothideomycetes; Botryosphaeriales.	0.48
Glomerella	2	0	2	Ascomycota; Sordariomycetes; Glomerellales.	0.95
Hamigera	2	0	2	Ascomycota; Eurotiomycetes; Eurotiales.	0.95
Hansfordia	1	0	1	Ascomycetes; Pezizomycetes; Pezizales.	0.48
Hypoxylon	1	0	1	Ascomycota; Sordariomycetes; Xylariales.	0.48
Lasiodiplodia	1	3	4	Ascomycota; Dothideomycetes; Botryosphaeriales.	1.90
Lenzites	0	1	1	Basidiomycota; Agaricomycetes; Polyporales.	0.48
Nectria	0	2	2	Ascomycota; Sordariomycetes; Hypocreales.	0.95
Nigrospora	17	8	25	Ascomycota; Sordariomycetes; Trichosphaeriales.	11.90
Paraphoma	1	0	1	Ascomycota; Dothideomycetes; Pleosporales.	0.48
Penicillium	1	0	1	Ascomycota; Eurotiomycetes; Eurotiales.	0.48
Pestalotiopsis	1	1	2	Ascomycota; Sordariomycetes; Xylariales.	0.95
Peyronellaea	0	3	3	Ascomycota; Dothideomycetes; Pleosporales.	1.43
Phoma	5	5	10	Ascomycota; Dothideomycetes; Pleosporales.	4.76
Purpureocillium	1	0	1	Ascomycota; Sordariomycetes; Hypocreales.	0.48
Phomopsis	3	10	13	Ascomycota; Sordariomycetes; Diaporthales.	6.19
Preussia	1	0	1	Ascomycota; Dothideomycetes; Pleosporales.	0.48
Rosellinia	0	1	1	Ascomycota; Sordariomycetes; Xylariales.	0.48
Saccharicola	1	0	1	Ascomycota; Dothideomycetes; Pleosporales.	0.48
Setosphaeria	0	1	1	Ascomycota; Dothideomycetes; Pleosporales.	0.48
Thielavia	0	1	1	Ascomycota; Dothideomycetes; Pleosporales.	0.48
Trichoderma	0	2	2	Ascomycota; Sordariomycetes; Hypocreales.	0.95
Xylaria	1	0	1	Ascomycota; Sordariomycetes; Xylariales.	0.48
Total no. of isolates	111	99	210	-	-

Table 3. Diversity indices to show the diversity among isolated fungal endophytes from different sampling sites.

Diversity indices	Sampling sites											
	J	TT	D	M	A	P	C	S	Kth	Ktr	U	R
Taxa S	5	5	8	5	8	9	6	4	6	7	4	5
Individuals	7	6	12	8	14	12	8	8	9	10	5	7
Dominance D	0.273	0.251	0.185	0.351	0.185	0.196	0.270	0.344	0.178	0.229	0.343	0.265
Simpson 1-D	0.948	1	0.948	0.966	0.968	0.983	0.991	0.908	0.980	0.942	1	0.923
Shannon H	2.204	2.397	2.510	2.426	2.833	2.941	2.685	2.182	2.659	2.529	2.302	2.143
Margalef d	3.508	4.170	4.146	3.967	5.461	5.902	5.049	3.606	4.843	4.926	3.908	3.410
Pielou's evenness j	0.957	1	0.951	0.875	0.962	0.981	0.991	0.910	0.982	0.912	1	0.931

J = Jammu, TT = Talab tillo, D = Domana, M = Mishriwala, A = Akhnoor, P = Palanwala, C = Chatha, S = Samba, Kth = Kathua, Ktr = Katra,U = Udhampur, R = Reasi.

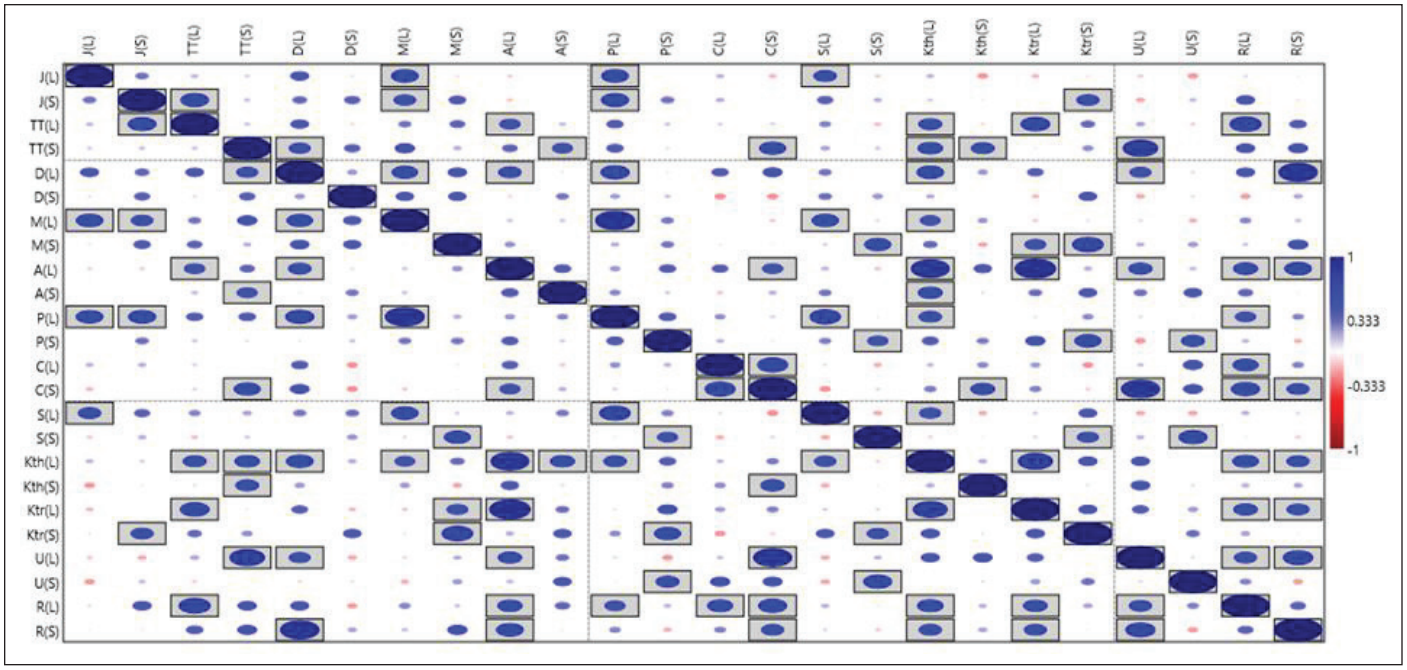

Figure 5. The correlation plot representing the strength and direction of relationship between the multiple species. The cells within the grid represent the correlation coefficient between the pair of variables at the intersection of the rows and column. Color gradients depict the strength of the correlation (range from −1 to 1) representing positive (one species increase, the other also increase), negative (one species increases, the other decreases), and no correlation (no relationship between the species). The axis shows sampling sites and in bracket plant tissue: leaves (L) and stem (S).

Table 4. ANOVA table of different endophytic fungi group from *Albizia lebbeck* of twelve different sites.

	Sum of square	Degree of freedom	Mean^2	*F*	Significance
Between groups	17.1522	23	0.745747	1.05	0.3992
Within groups	374.957	528	0.710145	-	-
Total	392.109	551	0.3947	-	-

Diporthe (8), and *Chaetomium* (6). Four from *Cladosporium* and *Epicoccum*, three from *Lasiodiplodia* and P*eyronellaea*, and two each from *Trichoderma*, *Cochliobolus*, *Nectria*, and *Pestalotiopsis*. One each from *Acremonium, Ampelomyces, Cercospora, Eutypella, Fusicoccum, Hansfordia, Hypoxylon, Lenzites, Paraphoma, Penicillium, Purpureocillium*, *Preussia, Rosellinia, Saccharicola, Setosphaeria, Thielavia,* and *Xylaria*. Endophytes belonging to genera *Alternaria*, *Chaetomium*, *Colletotrichum, Curvularia, Diporthe, Fusarium, Lasiodiplodia, Nigrospora, Phoma*, and *Phomopsis* were found in both stem and leaves. Whereas some of the endophytes belonging to genera viz. *Ampelomyces, Bipolaris, Daldinia, Eutypella, Glomerella, Hamigera, Hansfordia, Hypoxylon Paraphoma, Penicillium, Purpureocillium, Preussia, Saccharicola, and Xylaria* were found exclusively in leaves and isolates of genera *Cercospora, Cochliobolus, Fusicoccum, Lenzites, Nectria, Rosellinia, Setosphaeria, Thielavia, and Trichoderma* in stem only (Fig. 4a, Table 2).

3.3. Diversity Indices

Biological diversity was quantified using different indices. The Shannon–Wiener index was used to calculate the diversity of species within a community or habitat. Simpson's index was used to quantify the biodiversity of the habitat by taking into account the number of species present as well as the abundance of each species in the sample, Margalef's index was used to measure the species richness in the sample, Pielou's evenness index measures the relative abundance of the different species making up the richness of an area. Biodiversity was highest in Udhampur whereas species diversity was highest in Palanwala. Species richness was highest in Palanwala whereas evenness among species was found in Udhampur. Data on various indices of fungal endophytes from different locations are provided in Table 3. The species diversity in the plant tissue among the sampling sites was presented as a graph plot (Fig. 4b). A correlation plot was also drawn to assess and understand the relationships among multiple species in the plant tissues of different sampling sites to detect potential multicollinearity (Fig. 5). Differences in the number of isolates and colonization frequency differed non-significantly between the groups ($p > 0.05$), possibly due to environmental variation or physiological conditions such as stress levels affecting endophytes' colonization (Table 4).

4. DISCUSSION

In this study, 210 fungal strains were isolated from 720 fragments of *A. leebeck*, indicating its rich diversity of fungal taxa. The plant exhibited significant colonization frequency by *Colletotrichum* sp., which accounted for 6.4% of the total isolates. Specifically, 111 fungal isolates were obtained from leaf samples, while 99 were from the stem samples. This suggests that leaves may provide a more favorable environment for fungal colonization compared to stems. Similar observations were reported by Verma *et al.* (2007) in *Azadirachta indica*, where endophytic colonization was highest in leaf segments (45.5%) compared to bark (31.5%) and the stem tissues. This trend may result from increased environmental exposure and microbial interactions in leaves [36]. Wezowicz et al. (2014) also noted a significant difference in fungal endophyte diversity in the leaf tissues of *Verbascum lychnitis* [37].

Our analysis showed that 58.57% of the isolated fungi belonged to the class Sordarimycetes, with *Alternaria*, *Colletotrichum*, *Nigrospora*, and *Phoma* being the dominant genera in stem samples. Interestingly, certain fungal species showed tissue specificity with genera such as *Cercospora*, *Cochliobolus*, *Epicoccum*, *Lenzites*, *Nectria*, *Peyronellaea*, *Setosphaeria*, *Thielavia*, and *Trichoderma* being confined to stem tissues, while others being found in leaves. This highlights unique interactions between endophytic fungi and plant tissues. A total of 57 species were isolated from leaves and 44 from stems with no significant differences in species richness between the two tissues. Diversity indices including Simpson and Shannon–Wiener showed that leaf samples had more diversity than stem samples. This could be because leaves are usually exposed to more light, moisture, and air, which could make the environment more conductive to a variety of fungus species. Most of the endophytic fungi associated with *A. leebeck* predominantly belonged to the subphylum Ascomycotina with only one member from Basidiomycota, *Lenzites warnieri*, isolated from stem samples indicating the dominance of Ascomycetes in the endophytic community of *A. leebeck.* Previous studies also reported diverse endophytic fungi in *A. leebeck,* emphasizing the need to explore their colonization patterns and interactions across different species and environments [38–41].

Endophytic fungi have become a focal point of research due to their remarkable biological activities and wide-ranging applications. Investigation into their distribution and diversity across various plant species including *Securinega suffruticosa* and *Vanda cristata* reveal their ability to produce bioactive metabolites with antimicrobial properties and plant growth-promoting effects. These fungi offer a promising alternative source of bioactive compounds that hold significant potential for pharmacological and other applications [42–44].

5. CONCLUSION

The finding of this study underscores the significant role of endophytic fungi in the biodiversity of *A. leebeck.* The high colonization frequency and diversity of endophytic fungi, particularly in leaf tissues suggest that these microorganisms may play a crucial role in plant health and resilience. The predominance of Ascomycetes among the isolated fungi highlights their ecological importance. Additionally, the diverse nature and accessibility of these endophytic fungi make them valuable candidates for screening pharmaceutically important natural products. In the future, we will focus on unraveling the molecular interactions between these fungi and their host, which could enhance their applications in agriculture and medicine. Further studies are needed to understand the origins of tissue specificity and its impact on host plant health and resilience.

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7. AUTHOR CONTRIBUTION

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS:

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY:

All the data is available with the authors and shall be provided upon request.

11. PUBLISHER'S NOTE

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12. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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SUPPLEMENTARY MATERIAL

The supplementary material can be accessed at the link here: [https://jabonline.in/admin/php/uploadss/1343_pdf.pdf]