



New insights into hidden microbiota landscape of *Salvia persepolitana*: evidence of host-specific endophytic fungal communities through a molecular survey

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Abstract

This study investigated the endophytic fungal diversity associated with *Salvia persepolitana*, a medicinal plant endemic to Iran. Plant specimens were collected from Khuzestan province of Iran. Taxonomically verified using morphological keys and herbarium comparisons. Endophytic fungi were isolated from root and lower stem tissues. A total of 35 fungal isolates were obtained, with a majority (74%) derived from root tissues. Fungal genomic DNA was extracted, and internal transcribed spacer and large subunit rRNA regions were amplified and sequenced for molecular identification. Sequence analysis revealed dominance of Ascomycota (97.14%), particularly within the Dothideomycetes and Sordariomycetes classes; Pleosporales and Hypocreales orders; Pleosporaceae and Nectriaceae families. The most common genera were *Fusarium* (40%) and *Alternaria* (37.14%). Phylogenetic analysis based on the Maximum Likelihood approach identified a total of 21 distinct fungal species. Among these, *Alternaria alstroemeriae* and *Alternaria angustiovoidea* were the only taxa recovered from both root and aerial tissues, suggesting a degree of tissue specificity among the endophytic community. Root tissues harbored greater fungal diversity relative to aerial parts, highlighting differential colonization patterns. The dataset encompasses both previously recognized and novel fungal associations, thereby substantially advancing current knowledge of endophytic interactions within the genus *Salvia*. Except for *Fusarium avenaceum*, *Fusarium redolens*, and *Phoma herbarum*, the remaining 18 taxa represent new records for the genus. Moreover, several species including *Fusarium hostae*, *Alternaria conjuncta*, *Alternaria malorum*, *Cladosporium cucumerinum*, *Diaporthe ambigua*, and *Xenodidymella camporesii* are reported here, to the best of our knowledge, for the first time as endophytes in any plant host.

Keywords Plant-microbe interaction · Mycobiome · Lamiaceae · Middle east

1 Introduction

Endophytic fungi are intracellular microorganisms that reside symbiotically within plant tissues without causing discernible harm to their host (Omomowo et al. 2023). Extensive investigations have revealed that nearly all vascular and medicinal plant species harbor diverse endophytic communities (Gao et al. 2025). Throughout their lifecycle,

endophytes colonize plant tissues and interact with a variety of environmental microorganisms, including pathogens, rhizosphere bacteria, mycorrhizal fungi, and other endophytes (Omomowo et al. 2023). These interactions can trigger intricate biochemical networks, ultimately leading to the biosynthesis and accumulation of secondary metabolites (Gao et al. 2025). The symbiotic relationships between endophytic fungi and medicinal plants confer mutual benefits: while the plant supplies nutrients to the endophytes, the fungi enhance the plant's metabolic pathways and biosynthetic capabilities (Adeleke and Babalola 2021). Furthermore, endophytes may acquire genetic material that enables them to synthesize bioactive compounds analogous to those produced by their host plants (Gao et al. 2025). Beyond metabolic enhancement, endophytes contribute significantly to plant health by promoting tolerance to abiotic stresses such as

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heavy metals, drought, and salinity, increasing resistance to herbivores and nematodes, and inducing systemic resistance to pathogens (Omomowo et al. 2023).

Salvia persepolitana, represents a plant of particular medicinal interest (Askari et al. 2021). Among the 57 *Salvia* species identified in Iran, 17 are endemic, with *S. persepolitana* being exclusively distributed across the provinces of Khuzestan, Isfahan, Ilam, and Shiraz (Asgarpanah 2021). The roots of various *Salvia* species have been found to contain structurally diverse diterpenoids, predominantly featuring abietane-type carbon skeletons with biological activities (Jassbi et al. 2016). Labdane-type diterpenoids have demonstrated significant pharmacological potential, particularly in anticancer and antimicrobial contexts (Fronza et al. 2011; Jassbi et al. 2016). These bioactive constituents are believed to be, at least in part influenced by its associated endophytic fungi (Gao et al. 2025). Although identifying these root endophytes is the first step in addressing this hypothesis, significant gaps remain regarding their diversity, tissue specificity, and geographic distribution in *Salvia* species, particularly *S. persepolitana* (Zimowska et al. 2020). Addressing this knowledge gap, the present study

seeks to isolate, identify, and investigate the communities of endophytic fungi associated within roots of *S. persepolitana*. We also evaluate the differences in endophytic fungal communities in the herbaceous axis tissue originating from the root system above the soil, aiming to identify key root fungal endophytes.

2 Materials and methods

2.1 Collection and taxonomic verification of plant specimens

Healthy and asymptomatic plant specimens, exhibiting no signs of biotic or abiotic stress, were obtained from three distinct regions in Iran: Eslamabad Dowreh (33°37'28.4"N 47°52'48.8"E), Dimeh Darb (31°37'02.1"N 49°29'37.9"E), and Tang-e Māgher (31°00'21.0"N 50°06'38.3"E) at the flowering stage (Fig. 1I–J). To maintain aseptic conditions during collection, plants were carefully excavated using a sterile spade treated with 75% ethanol. Residual soil attached to the root systems was gently dislodged by

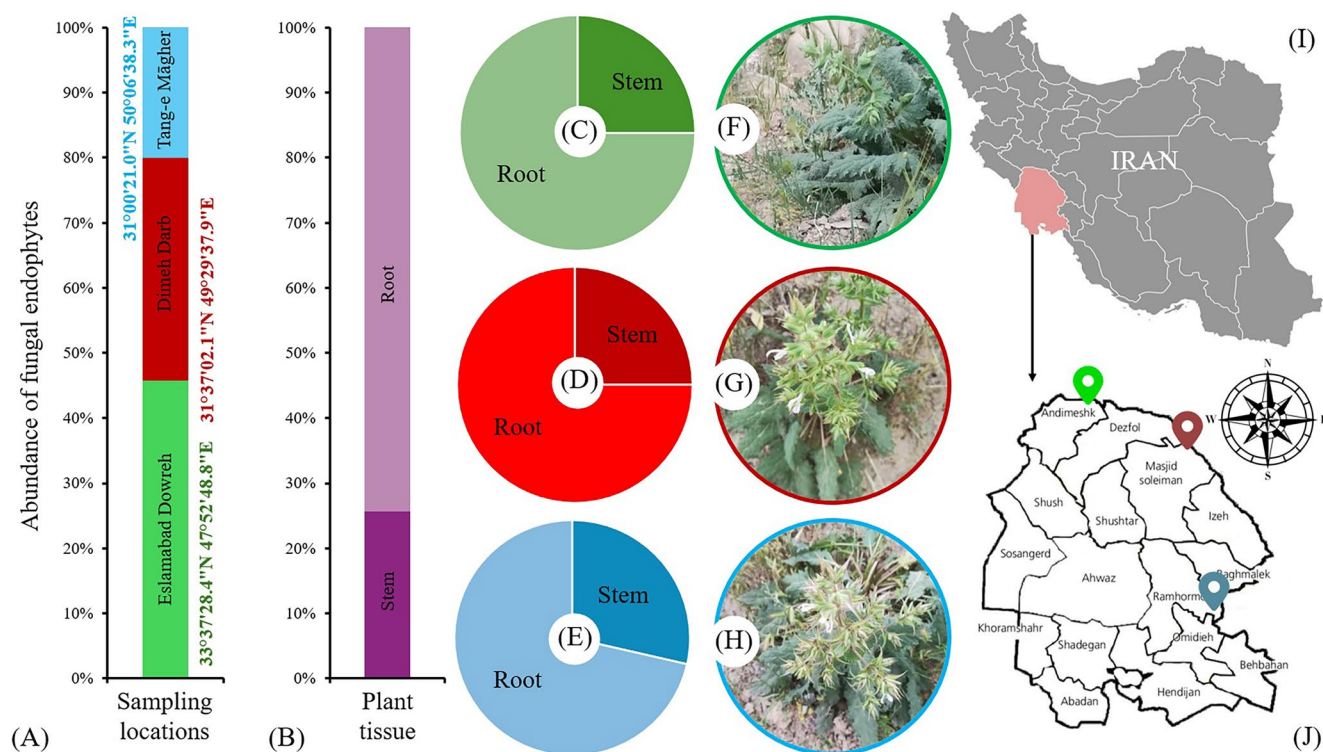


Fig. 1 Geographic origin and tissue-based distribution of endophytic fungi isolated from *Salvia persepolitana*. (A): Frequency of endophytic fungi isolated from each plant sampling location. (B): Frequency of endophytic fungi isolated from different host plant tissues. (C): Tissue-specific distribution of endophytic fungi from the Eslamabad Dowreh sampling site. (D): Tissue-specific distribution of endophytic fungi from the Dimeh Darb sampling site. (E): Tissue-specific distribution

of endophytic fungi from the Tang-e Māgher sampling site. (F): Representative *Salvia persepolitana* plant sampled from the Eslamabad Dowreh site. (G): Representative *Salvia persepolitana* plant sampled from the Dimeh Darb site. (H): Representative *Salvia persepolitana* plant sampled from the Tang-e Māgher site. (I, J): Geographical location of Khuzestan Province within Iran and detailed locations of the sampling sites

minimal agitation to preserve root integrity. Each specimen was promptly enclosed in sterile plastic bags and transported on ice to the laboratory for subsequent analysis. A total of 30 specimens were collected, with 10 plants sampled from each region, to ensure adequate representation of morphological variability. Taxonomic identification at the species level was initially conducted via morphological examination in accordance with the *Flora of Iran*, No. 76 (Jamzad 2012). To validate the morphological assessments, specimens were further compared with authenticated voucher samples maintained in the herbarium of Mashhad University of Medical Sciences. For broader taxonomic confirmation, diagnostic characteristics were also evaluated against the dichotomous key for *Salvia* species across Asia (Turdiboev et al. 2022). Following identification, the plant materials were subjected to standard herbarium preparation procedures, including pressing and drying, and were subsequently accessioned into the herbarium collection of Mashhad University of Medical Sciences, each assigned a unique catalog number for future reference.

2.2 Endophytic fungal isolation from plant tissues

Endophytic fungi were isolated from the herbaceous axis originating from the root system (representing the basal segment of the shoot approximately 5 cm above the soil surface), and root tissues of the collected plant specimens. From each of the 30 plants, one segment of basal stem tissue and one segment of root tissue were excised, yielding a total of 20 tissue samples per region (10 stem and 10 root) and 60 tissue samples overall. All samples were subjected to a standardized surface-sterilization protocol, beginning with immersion in 70% ethanol for 1 min, followed by three successive rinses in sterile distilled water. Root tissues received an additional sterilization step involving treatment with 2.5% sodium hypochlorite solution containing Tween-20 for five minutes, succeeded by five successive rinses in sterile distilled water to eliminate residual disinfectant and surfactant, thereby ensuring comprehensive surface sterilization. To assess the effectiveness of the sterilization protocol, control tests were performed by placing sterilized, unsectioned root fragments onto potato dextrose agar (PDA) medium without disturbing internal tissues (Hatamzadeh et al. 2023). Moreover, 100 µL aliquots of the final rinse water were cultured on PDA plates to detect potential microbial contaminants (Akbari Oghaz et al. 2024). Post-sterilization, plant tissues were aseptically cut into approximately 0.5 × 0.5 cm segments using a sterile scalpel and transferred onto PDA supplemented with streptomycin (100 µg/mL) to suppress bacterial growth. Inoculated plates were incubated at 25 ± 2°C for 1 to 2 weeks, during which fungal outgrowths were monitored. Emergent colonies were isolated and

transferred onto fresh PDA plates to obtain axenic cultures for further analysis.

2.3 Crude DNA extraction from fungal isolates

Fungal isolates were cultivated on PDA medium under dark conditions and incubated at 25 ± 2 °C for a period of six days, in accordance with the methodology outlined by Hatamzadeh et al. (2024). For buffer preparation, 1 mL of 1 M 2-Amino-2-(hydroxymethyl)-propane-1,3-diol, 200 µL of 0.5 M ethylenediaminetetraacetic acid (EDTA), 100 µL of 0.1 M sodium chloride, and 50 µL of glycerol were dissolved in 100 mL of double-distilled water, with the solution adjusted to pH 8. To harvest young mycelium (approximately 72 h post-inoculation), sterile toothpicks were employed to gently transfer surface mycelial material from PDA cultures into 1.5 mL microcentrifuge tubes containing 100 µL of the prepared buffer. Samples were vortexed for one minute at 112 m/s², followed by a heat shock treatment at 83 ± 2 °C in a water bath for 35 min. Immediately thereafter, samples were exposed to cold shock by freezing at −20 °C for 15 min. Subsequently, the tubes were centrifuged at 11,200 m/s² for six minutes at 25 ± 2 °C. From each tube, 50 µL of the resulting supernatant was collected and retained as a source of crude fungal DNA for downstream applications.

2.4 Genomic DNA amplification and sequence-based fungal identification

Amplification of the Internal Transcribed Spacer (ITS) and Large Subunit (LSU) ribosomal RNA gene regions was carried out using the polymerase chain reaction (PCR) technique. For ITS region amplification, primer pair ITS5 (5′-G GAAGTAAAAGTCGTAACAAGG-3′) and ITS4 (5′-TCC TCCGCTTATTGATATGC-3′) was utilized, as outlined by Schoch et al. (2012). The LSU region was similarly amplified using primers LROR (5′-CCCGCTGAACTTAAG C-3′) and LR5 (5′-TCCTGAGGGAACTTCG-3′), following the study of Liu et al. (2012). The PCR reactions were set up in 12.5 µL volumes containing 1× PCR buffer, 10–20 ng of genomic DNA, 0.7 µL of 99.9% dimethyl sulfoxide (DMSO), 0.5 µM of each primer, 25 µM of each dNTP, 2 mM MgCl₂, and 1.0 U of Taq DNA polymerase (NEB). Reactions were performed using a GeneAmp PCR System 9600 (Perkin Elmer, USA) under the following thermocycling parameters: an initial denaturation at 95 °C for 1 min, followed by 40 cycles consisting of denaturation at 95 °C for 30 s, primer annealing at 58 °C for 1 min, and extension at 72 °C for 10 s, applied uniformly to both ITS and LSU targets (Eslami et al. 2025). Resulting amplicons were subjected to sequence analysis via BLASTN against

the NCBI database for initial identification, and corresponding taxonomic classifications were confirmed using the MycoBank database.

2.5 Construction of phylogenetic trees and analysis of evolutionary patterns

Phylogenetic relationships and the evolutionary trajectory of the fungal isolates were reconstructed using the Maximum Likelihood (ML) approach, implemented under the Tamura-Nei nucleotide substitution model. Preliminary tree topologies were generated using the Neighbor-Joining and BioNJ methods, with the topology yielding the highest log-likelihood score selected for final interpretation. The resulting phylogenetic tree was scaled to reflect evolutionary distances, with branch lengths representing the number of substitutions per nucleotide site and illustrating taxonomic clustering. Sequence alignment was carried out prior to ML analysis. The robustness of the phylogenetic inferences was assessed through bootstrap resampling with 1000 replicates. Model selection confirmed the Tamura-Nei model as the most appropriate fit for the dataset, based on the assumption of uniform substitution rates across sites and inclusion of all positions. The heuristic search for the optimal ML tree employed the Nearest-Neighbor Interchange (NNI) algorithm without the application of branch swap constraints. All phylogenetic analyses were conducted using the MEGA12 software, and resulting branch lengths reliably represented the inferred evolutionary divergence.

3 Results

3.1 General morphological description, quantitative morphometric, and taxonomic confirmation

The collected plant species were identified as herbaceous perennials, exhibiting a fragrant aroma and possessing quadrangular, pubescent stems. The foliage appeared dense, with opposite, light green leaves that were thick and exhibited a prominent network of veins, particularly noticeable on the abaxial (lower) surface of the leaf blades. The lower stem leaves were recorded as ovate, measuring 4–5 cm in length, with sharply pointed apices. Leaf margins displayed regular dentition and were attached by petioles. In contrast, the upper stem leaves were noted to be smaller and sessile. The flowers were observed to be white in all sampling locations (Fig. 1F–H). The morphological measurements reported below represent the mean ranges obtained from 10 individuals per site. The plant height varied notably among regions, ranging from 22.23 cm to 38.9 cm, while stem diameter was

observed within a narrower range of 1.63 mm to 2.75 mm. Leaf length and width demonstrated considerable variation, ranging from 18.4 mm to 30.7 mm and 9.6 mm to 19.2 mm, respectively.

The leaf length-to-width ratio fluctuated between 1.5 and 2.1, indicating moderate morphological diversity. Petiole length was recorded in the range of 13.8 mm to 22.7 mm. Inflorescence length ranged from 7.0 cm to 11.1 cm, while node diameter exhibited less variation, measured between 8.0 mm and 11.2 mm. The bract characteristics also showed regional differences, with bract length spanning 9.0 mm to 13.2 mm and bract width from 8.4 mm to 12.3 mm. The bract length-to-width ratio remained relatively stable across regions, with values from 1.0 to 1.4. Corolla length measurements ranged from 12.9 mm to 17.6 mm, and internode distance varied from 9.8 mm to 13.8 mm. Finally, terminal shoot length was found to range between 8.2 mm and 11.9 mm. Based on detailed morphological observations and confirmation through established taxonomic identification keys, all collected specimens were authenticated as *S. persepolitana*. These specimens have been formally documented and deposited in the Medicinal Plant Herbarium of the School of Pharmacy, Mashhad University of Medical Sciences, under the voucher number 13217.

3.2 Distribution patterns of endophytic fungi across tissues and locations

A total of 35 endophytic fungal isolates were obtained from *S. persepolitana*, with colonization detected in both stem (26%) and root (74%) tissues (Fig. 1B). The distribution of isolates varied among the three sampling locations (Fig. 1A). The Eslamabad Dowreh region yielded the highest number of endophytes ($n=16$), with 25% isolated from stem tissues and 75% from root tissues. The Dimeh Darb region yielded a moderate number of isolates ($n=12$), comprising three stem-derived and nine root-derived endophytes. In contrast, the Tang-e Māgher region produced the lowest number of isolates ($n=7$), with 21% originating from the stem and 71% from the root.

The taxonomic analysis of the isolated endophytic fungi revealed a diverse distribution across multiple hierarchical levels (Fig. 2). At the phylum level, Ascomycota was overwhelmingly dominant, representing 97.14% of the total isolates, whereas Basidiomycota accounted for only 2.85%. Within the class level, four fungal classes were identified. Dothideomycetes and Sordariomycetes constituted the majority, comprising 51.42% and 42.85% of the isolates, respectively. Eurotiomycetes and Agaricomycetes were minimally represented, each with a relative abundance of 2.85%. At the order level, six distinct fungal orders were

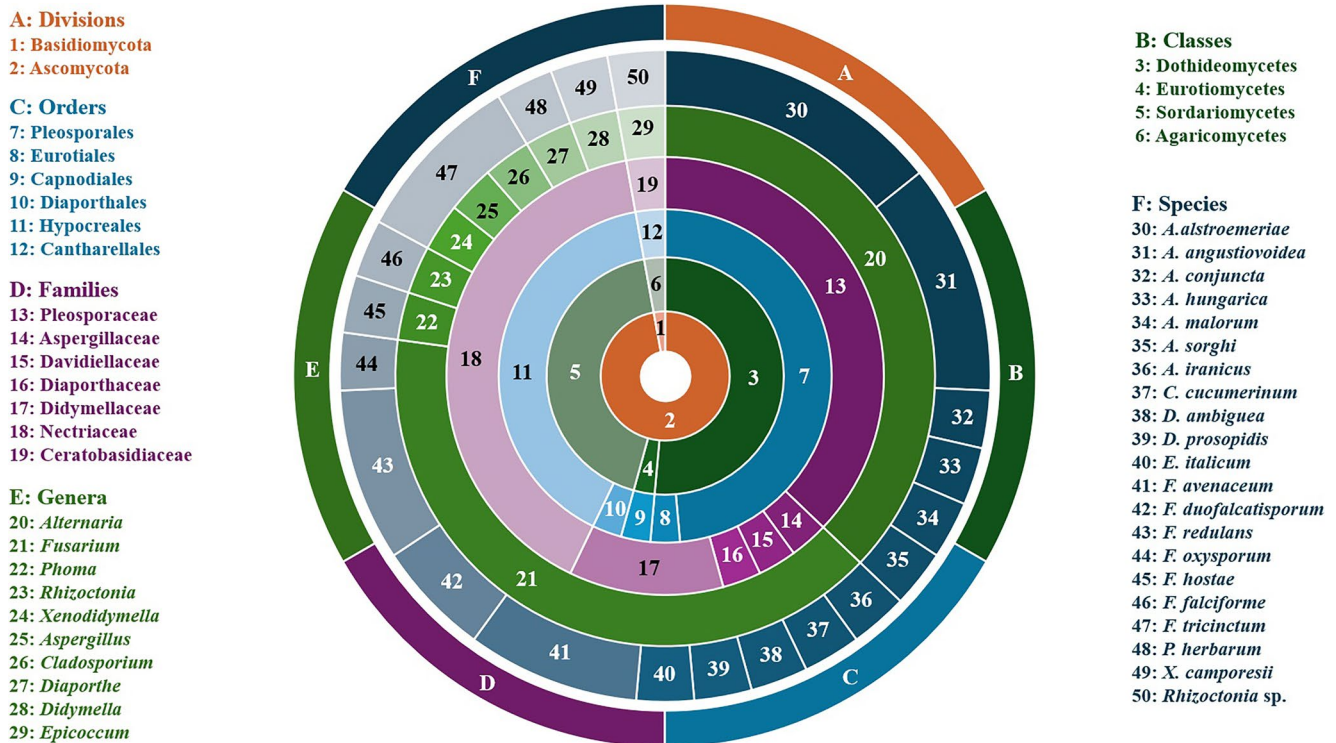


Fig. 2 Taxonomic hierarchy and species composition of endophytic fungi isolated from *Salvia persepolitana*. The concentric rings represent the relative abundance (%) of endophytic fungi across taxonomic

levels. The sectors are scaled by abundance and are not aligned phylogenetically across circles

detected. Pleosporales (48.57%) and Hypocreales (40%) were the most prevalent, indicating their dominant role in the endophytic communities of *S. persepolitana*. The remaining orders Eurotiales, Capnodiales, Diaporthales, and Cantharellales were each represented by a single isolate (2.85%). The family-level distribution included seven families, with Nectriaceae (40%), Pleosporaceae (37.14%), and Didymellaceae (11.42%) encompassing the highest number of isolates. In contrast, Aspergillaceae, Davidiellaceae, Diaporthaceae, and Ceratobasidiaceae each accounted for only one isolate. Finally, ten fungal genera were identified. Among these, *Fusarium* (40%) and *Alternaria* (37.14%) were the most frequently isolated genera. The remaining genera *Phoma*, *Rhizoctonia*, *Xenodidymella*, *Aspergillus*, *Cladosporium*, *Diaporthe*, *Didymella*, and *Epicoccum* were each represented by a single isolate, indicating their limited presence within the endophytic fungal community of *S. persepolitana*.

3.3 Host tissue specificity and relative abundance of identified endophytic fungal species

As illustrated in Figs. 2 and 3 and detailed in Table 1, a total of 21 distinct endophytic fungal species were identified

from *S. persepolitana*, isolated from both stem and root tissues. Among these, *Alternaria alstroemeriae* and *Alternaria angustiovoidea* were the most prevalent, accounting for 14.28% and 11.42% of the total isolates, respectively. These were followed by *Fusarium avenaceum* (8.57%), *Fusarium duofalcatisporum* (5.71%), *Fusarium redolens* (8.57%), and *Fusarium tricinatum* (8.57%), which exhibited moderate levels of abundance. The remaining 15 species were each represented by a relative frequency of 2.85%, indicating a broad but uneven species distribution. Analysis of tissue-specific colonization patterns revealed that only *A. alstroemeriae* and *A. angustiovoidea* were common to both stem and root tissues, constituting 9.52% of the total fungal community, while the remaining 90.47% of species displayed a clear preference for a specific tissue type. In stem tissues, six fungal species were detected, with *Alternaria* spp. dominating the assemblage at 88.88%, and *Xenodidymella camporesii* being the only non-*Alternaria* species, with an abundance of 11.1%. Conversely, the root tissue harbored a more diverse fungal community, comprising 26 isolates. Within this compartment, *Fusarium* spp. were the most prevalent, accounting for 53.84% of the root-associated isolates, while *Alternaria* spp. also exhibited notable representation, comprising 19.23% of the community.

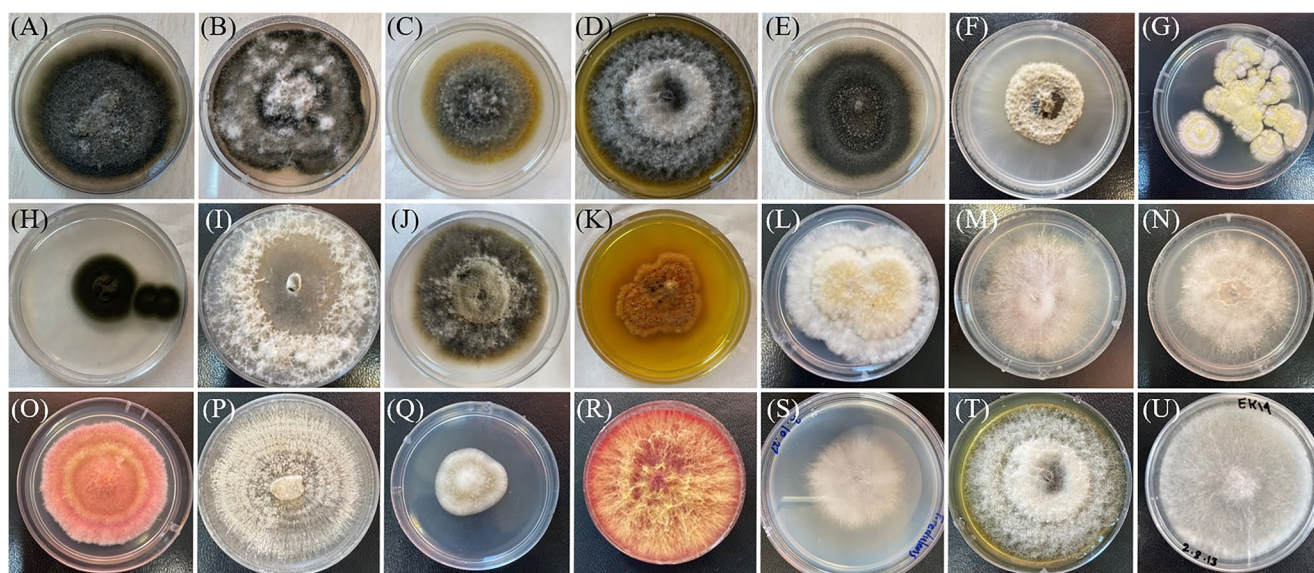


Fig. 3 Colony of endophytic fungal species isolated from *Salvia persepolitana* grown on PDA after 7 days at 25 ± 2 °C. (A): *Alternaria alstroemeriae*. (B): *Alternaria angustiovoidea*. (C): *Alternaria conjuncta*. (D): *Alternaria hungarica*. (E): *Alternaria Sorghi*. (F): *Alternaria malorum*. (G): *Aspergillus iranicus*. (H): *Cladosporium cucumerinum*.

(I): *Diaporthe ambiguae*. (J): *Didymella prosopidis*. (K): *Epicoccum italicum*. (L): *Fusarium duofalcatisporum*. (M): *Fusarium hostae*. (N): *Fusarium oxysporum*. (O): *Fusarium tricinctum*. (P): *Rhizoctonia* sp. (Q): *Xenodidymella camporesii*. (R): *Fusarium avenaceum*. (S): *Fusarium redulans*. (T): *Phoma herbarum*. (U): *Fusarium falciforme*

3.4 Molecular phylogenetic delineation based on ITS and LSU sequence data

All endophytic fungal species examined in this study were sequenced using standard genomic regions (ITS and LSU). Figure 4 illustrates the phylogenetic relationships of the isolates excluding *Alternaria* and *Fusarium*. In Fig. 4A, based on ITS sequences, *Epicoccum italicum* forms the top clade (Fig. 4A-I), where isolate R21 clusters within the *E. italicum* clade together with reference isolates, supported by moderate bootstrap values (78–85%), confirming its species identity. The *Didymella prosopidis* clade (Fig. 4A-II) includes root isolate RE10 clustering with reference isolates with strong support (bootstrap=91%), validating their close phylogenetic placement. In the *Phoma herbarum* clade (Fig. 4A-III), root isolate RE4 groups with reference isolates (bootstrap=68%), supporting its assignment to this species. A distinct pattern is observed in the *X. camporesii* clade (Fig. 4A-IV), where two reference isolates (MFLUCC 17–2309) and the stem isolate SE8 form a highly supported group (bootstrap=99%). The *Diaporthe* clade (identified as *Diaporthe ambiguae* based on LSU data) contains root isolate R26 within a moderately supported branch (bootstrap=58%), indicating affiliation with this species (Fig. 4A-V). In the *Aspergillus iranicus* clade (Fig. 4A-VI), root isolate RB6 forms a well-supported branch (bootstrap=100%), confirming its identification within this species. The *Cladosporium cucumerinum* clade (Fig. 4A-VII) places root isolate RB5 together with reference isolates

with strong support (bootstrap values=100% and 58%), validating species-level placement. At the base of the tree, isolates of an unidentified *Rhizoctonia* species serve as outgroups (Fig. 4A-VIII), encompassing both basidiomycetous and ascomycetous representatives. Isolate RE12 falls within this group, clustering with a reference strain, confirming its classification as *Rhizoctonia* sp.

Figure 4B presents the phylogenetic relationships of the endophytic fungal isolates based on the LSU genomic region. At the top of the tree, the *A. iranicus* clade includes root isolate RB6 clustering with reference isolates (bootstrap=96%), confirming its species-level identity. Directly below, the *C. cucumerinum* clade contains root isolate RB5 grouped with reference isolate CBS 171.52 (bootstrap=65%), validating its taxonomic placement. In the *X. camporesii* clade, two reference isolates (MFLUCC 17–2309 and A4-3) and the stem isolate SE8 form a strongly supported monophyletic group, confirming their close relatedness at the species level. The *E. italicum* clade shows root isolate R21 clustering with reference isolates (bootstrap=92%), supporting its placement within this species. In both the *P. herbarum* and *D. prosopidis* clades, the respective root isolates (RE4 and RE10) group with references in closely aligned branches, indicating high genetic similarity and confirming species-level identification. At the base of the tree, the *Rhizoctonia* sp. clade, serving as the outgroup, includes root isolate RE12 clustering with reference isolate 296–17 (bootstrap=83%), validating its placement within this genus.

Table 1 List of endophytic fungal species isolated from different plant tissues of *Salvia persepoltana* with taxonomic classification and Genbank accession number details

Endophytic fungal species	*MUMS voucher code	Plant tissue	Sampling-location of the host plant	**NCBI accession numbers		ITS fragments (bp)	LSU fragments (bp)
				LSU genomic region	ITS genomic region		
<i>Alternaria alstroemeriae</i>	SE3	Stem	Eslamabad Dowreh	OR008903.1	OR149873.1	525	548
<i>A. alstroemeriae</i>	RE7	Root	Eslamabad Dowreh	OR008904.1	OR149874.1	529	542
<i>A. alstroemeriae</i>	SR2	Stem	Dimeh Darb	OR008926.1	OR149896.1	531	548
<i>A. alstroemeriae</i>	SR10	Stem	Dimeh Darb	OR008927.1	OR149897.1	534	549
<i>A. alstroemeriae</i>	RR10	Root	Dimeh Darb	OR008928.1	OR149898.1	528	545
<i>A. angustiovoidea</i>	SE2	Stem	Eslamabad Dowreh	OR008905.1	OR149875.1	560	529
<i>A. angustiovoidea</i>	RE11	Root	Eslamabad Dowreh	OR008906.1	OR149876.1	561	528
<i>A. angustiovoidea</i>	RE14	Root	Eslamabad Dowreh	OR008907.1	OR149877.1	566	529
<i>A. angustiovoidea</i>	SR1	Stem	Dimeh Darb	OR008929.1	OR149899.1	562	528
<i>A. conjuncta</i>	RE2	Root	Eslamabad Dowreh	OR008908.1	OR149878.1	582	576
<i>A. hungarica</i>	SE1	Stem	Eslamabad Dowreh	OR008909.1	OR149879.1	527	574
<i>A. malorum</i>	SB4	Stem	Tang-e Māgher	OR008919.1	OR149889.1	595	840
<i>A. sorghi</i>	SB5	Stem	Tang-e Māgher	OR008920.1	OR149890.1	553	605
<i>Aspergillus iranicus</i>	RB6	Root	Tang-e Māgher	OR008921.1	OR149891.1	596	809
<i>Cladosporium cucumerinum</i>	RB5	Root	Tang-e Māgher	OR008922.1	OR149892.1	551	864
<i>Diaporthe ambigua</i>	R26	Root	Dimeh Darb	OR008930.1	NS***	528	
<i>Didymella prosopidis</i>	RE10	Root	Eslamabad Dowreh	OR008910.1	OR149880.1	535	584
<i>Epicoccum italicum</i>	R21	Root	Dimeh Darb	OR008931.1	OR149900.1	526	832
<i>Fusarium avenaceum</i>	RB4	Root	Tang-e Māgher	OR008923.1	OR149893.1	523	720
<i>F. avenaceum</i>	RE4	Root	Eslamabad Dowreh	OR008911.1	OR149881.1	522	586
<i>F. avenaceum</i>	RR3	Root	Dimeh Darb	OR008932.1	OR149901.1	521	720
<i>F. duofalcatisporum</i>	RE5	Root	Eslamabad Dowreh	OR008912.1	OR149882.1	544	849
<i>F. duofalcatisporum</i>	RE6	Root	Eslamabad Dowreh	OR008913.1	OR149883.1	542	845
<i>F. redulans</i>	RE9	Root	Eslamabad Dowreh	OR008914.1	OR149884.1	559	867
<i>F. redulans</i>	RE15	Root	Eslamabad Dowreh	OR008915.1	OR149885.1	560	859
<i>F. redulans</i>	RR5	Root	Dimeh Darb	OR008935.1	OR149905.1	558	845
<i>F. oxysporum</i>	RB7	Root	Tang-e Māgher	OR008924.1	OR149894.1	542	865
<i>F. hostae</i>	RR4	Root	Dimeh Darb	OR008933.1	OR149902.1	553	870
<i>F. falciforme</i>	RR7	Root	Dimeh Darb	OR015784.1	OR149906.1	569	810
<i>F. tricinctum</i>	RB2	Root	Tang-e Māgher	OR008925.1	OR149895.1	562	848
<i>F. tricinctum</i>	RR11	Root	Dimeh Darb	OR008934.1	OR149903.1	560	850
<i>F. tricinctum</i>	RR15	Root	Dimeh Darb	OR008936.1	OR149904.1	557	850
<i>Phoma herbarum</i>	RE4	Root	Eslamabad Dowreh	OR008916.1	OR149886	544	881
<i>Rhizoctonia</i> sp.	RE12	Root	Eslamabad Dowreh	OR008917.1	OR149887.1	719	851
<i>Xenodidymella camporesii</i>	SE8	Stem	Eslamabad Dowreh	OR008918.1	OR149888.1	542	830

(*): Microbial culture collection of Mashhad University of Medical Sciences. (**): National Center for Biotechnology Information. (***): Not sequenced

3.4.1 Phylogenetic structure of *Alternaria* species

The ITS-based phylogenetic analysis classified *Alternaria* isolates into six distinct clades (Fig. 5A). At the top of the tree, the *Alternaria conjuncta* clade (Fig. 5A-I) comprises four isolates, including root isolate RE2, which forms an independent branch with strong bootstrap support (100%), confirming its placement within the *A. conjuncta* clade. Similarly, in the *Alternaria malorum* clade (Fig. 5A-II), stem isolate SB4 appears on a strongly supported branch

(bootstrap=100%), supporting its identification as *A. malorum*. In the *Alternaria hungarica* clade (Fig. 5A-III), stem isolate SE1 forms a distinct branch (bootstrap=94%), validating its assignment to this species. The *Alternaria sorghi* clade (Fig. 5A-IV) includes stem isolate SB5 on an individual branch (bootstrap=86%), supporting species-level identification. The two largest clades *A. angustiovoidea* and *A. alstroemeriae* demonstrate more complex internal structures. In the *A. angustiovoidea* clade (Fig. 5A-V), root isolates RE11 and RE14 cluster together (bootstrap=49%),

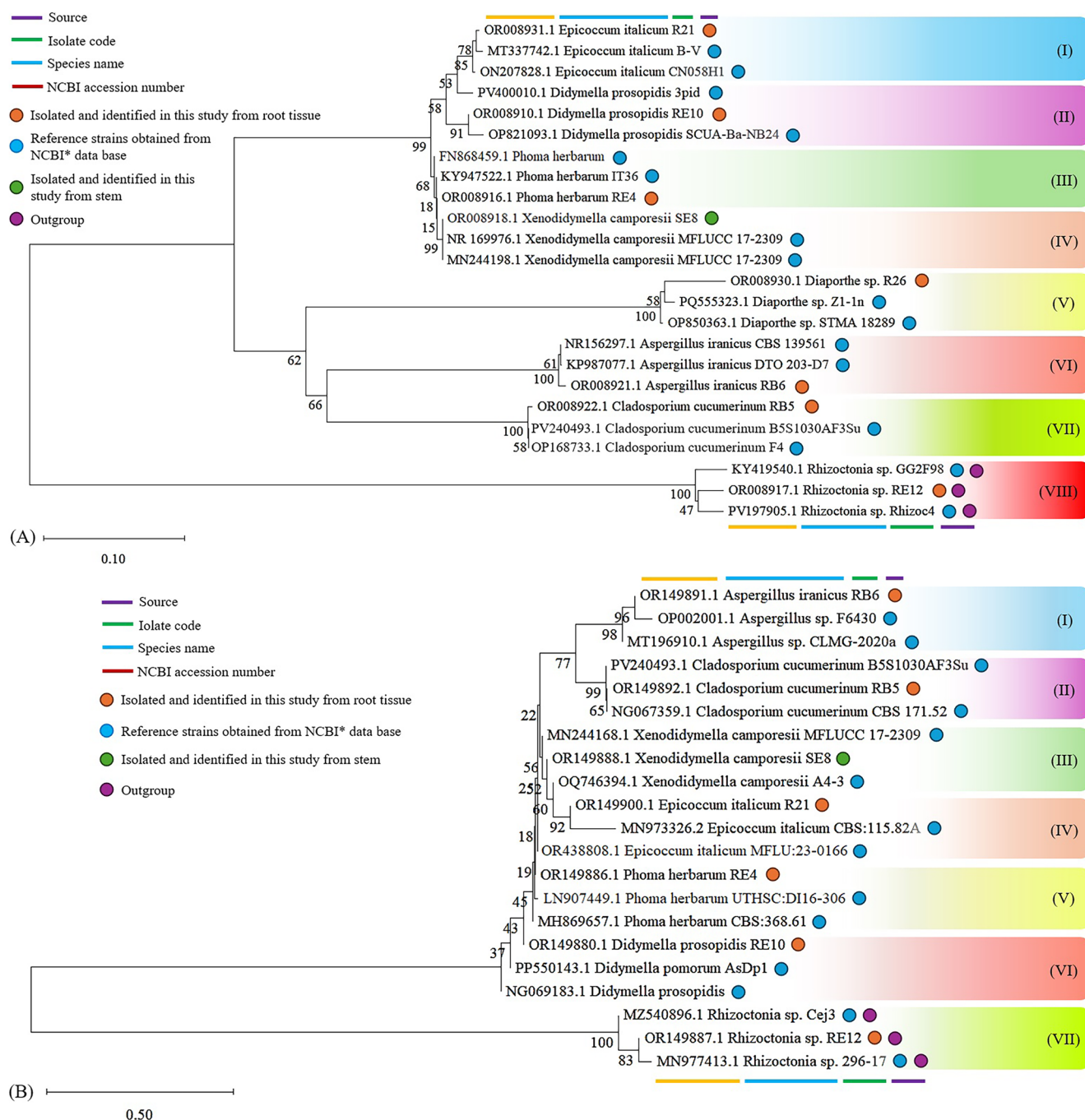


Fig. 4 Phylogenetic analysis and evolutionary insights into endophytic fungi associated with *Salvia persepoltana*. **(A)**: A phylogenetic tree generated using sequences from the internal transcribed spacer (ITS) region, incorporating 24 nucleotide sequences and covering 901

aligned positions in the final dataset. **(B)**: A phylogenetic tree based on the large subunit (LSU) rRNA region, constructed from 21 nucleotide sequences with a total alignment length of 1,738 positions. All bifurcations are shown in Supplementary Fig. S1

while stem isolate SE2 and SR1 occupy neighboring branches, collectively supporting their classification within this species despite weak internal resolution. At the bottom of the tree, the *A. alstroemeriae* clade (Fig. 5A-VI) includes three single-branch isolates (SR10, SR2, and RR10), all positioned within the species clade along with reference isolates, confirming their identity as *A. alstroemeriae*. Root

isolate RE7 clusters with reference isolate WZ-879 (bootstrap = 83%), further validating its placement in this species clade.

Phylogenetic analysis of *Alternaria* species using the LSU region reveals moderate to low bootstrap support across several clades, reflecting varying levels of genetic convergence (Fig. 5B). At the top of the tree, the *A. conjuncta* clade

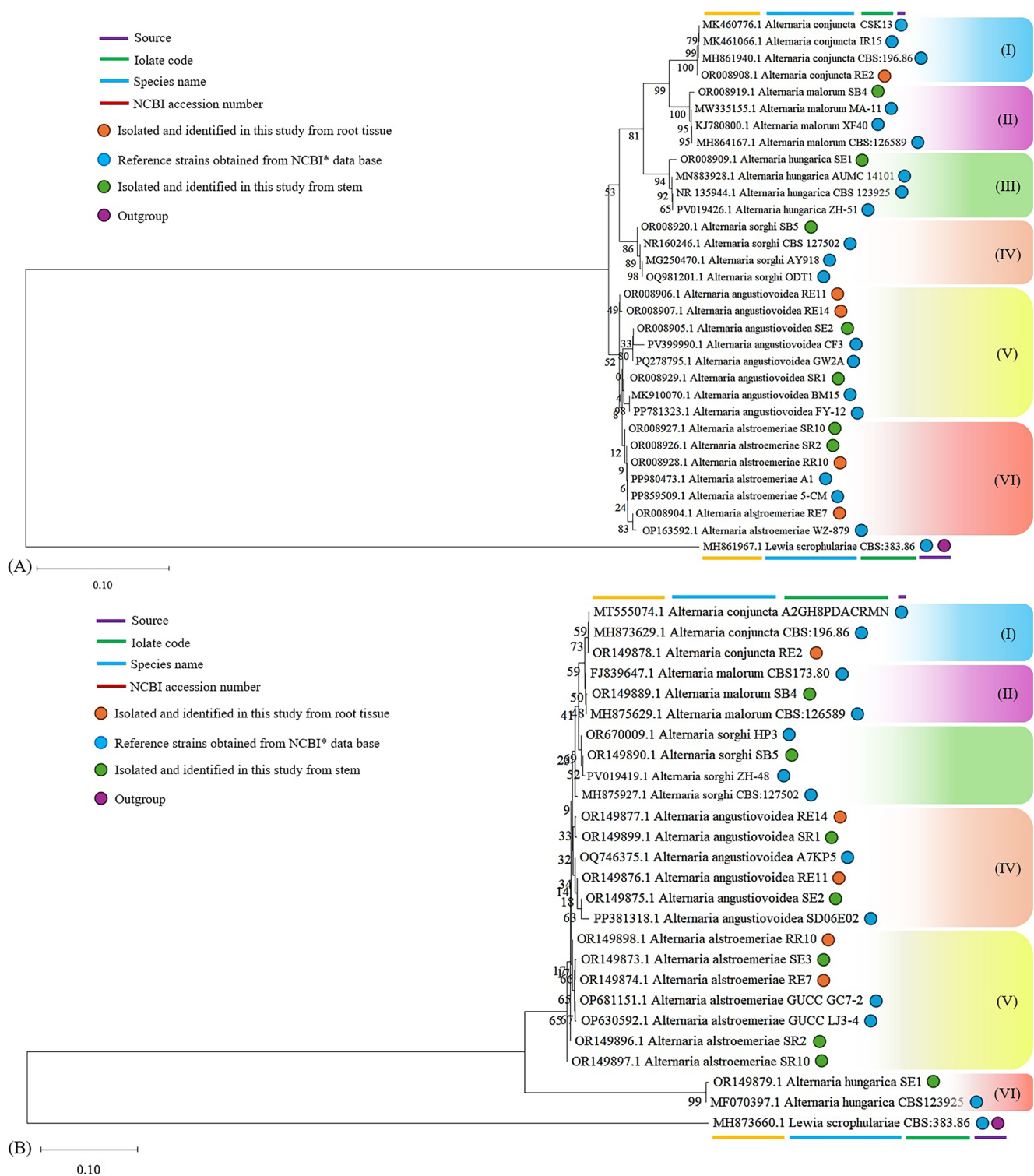


Fig. 5 Phylogenetic reconstruction and evolutionary characterization of *Alternaria* endophytic fungi isolated from *Salvia persepoltana*. **(A)**: Maximum likelihood phylogenetic tree inferred from internal transcribed spacer (ITS) region sequences, comprising 32 fungal isolates and aligned across 881 nucleotide positions. **(B)**: Phylogenetic

tree constructed using sequences from the large subunit (LSU) rRNA region, including 26 isolates with a total alignment length of 1006 nucleotide positions. The complete resolution of bifurcations is provided in Supplementary Fig. S2

(Fig. 5B-I) includes root isolate RE2, which is positioned on an individual branch (bootstrap=73%) within the broader *A. conjuncta* clade, confirming its taxonomic placement. Further down, the *A. malorum* clade (Fig. 5B-II) places stem isolate SB4 within a weakly supported subcluster (bootstrap=50%), nevertheless consistent with its identification as *A. malorum*. In the *A. sorghi* clade (Fig. 5B-III), stem isolate SB5 clusters within the clade with moderate bootstrap support (52%), reinforcing species-level assignment. The *A. angustiovoidea* clade (Fig. 5B-IV) exhibits a more complex topology. Stem isolate SR1 and root isolate RE14 appear in weakly supported subclusters (bootstrap=33%), together with neighboring references, indicating ambiguous but consistent placement within the species clade. Stem isolate SE2 groups with reference isolate SD06E02 (bootstrap=63%), further supporting species-level identification. In the *A. alstroemeriae* clade (Fig. 5B-V), most isolates from this study occupy distinct branches, consistent with their placement within this species. Stem isolate SE3 and root isolate RE7 form a moderately supported subcluster (bootstrap=66%), aligning with reference isolates that cluster separately (bootstrap=65%). Remaining isolates also fall within the clade, reinforcing species-level identification. The *A. hungarica* clade (Fig. 5B-VI) comprises stem isolate SE1 together with the reference strain CBS 123,925 in a strongly supported subcluster (bootstrap=99%), confirming its placement within this species.

3.4.2 Phylogenetic resolution of *Fusarium* species

The ITS-based phylogenetic analysis delineates several *Fusarium* species clades with varying degrees of bootstrap support (Fig. 6A). At the top of the tree, the *F. duofalcatisporum* clade (Fig. 6A-I) includes two root isolates (RE6 and RE5), which form a strongly supported subcluster (bootstrap=93%), confirming their placement within this species. In the *Fusarium oxysporum* clade (Fig. 6A-II), root isolate RB7 is positioned on an individual branch (bootstrap=94%), supporting its classification as *F. oxysporum*. The *Fusarium falciforme* clade (Fig. 6A-III) consists of root isolate RR7 and reference DTO 422-H8 forming a strongly supported group (bootstrap=99%), validating species-level identity. The *F. redolens* clade (Fig. 6A-IV) includes three root isolates (RE15, RE9, and RE5), each occupying separate branches but consistently assigned to this species together with references. In the *Fusarium hostae* clade (Fig. 6A-V), root isolate RR4 groups within the clade, albeit with weak support (bootstrap=34%), suggesting tentative placement. The *F. avenaceum* clade (Fig. 6A-VI) contains root isolates RR3, RB4, and RE4, all positioned on separate branches, but supported within the species clade along with multiple references. The *F. tricinctum* clade (Fig. 6A-VII)

includes root isolates RR15, RR11, and RB2, which cluster with reference isolates, confirming species-level affiliation.

The LSU-based phylogenetic tree (Fig. 6B) provides robust resolution of *Fusarium* species associations. At the top, the *F. avenaceum* clade (Fig. 6B-I) includes root isolate RB4 on an individual branch (bootstrap=72), while RE4 and RR3 cluster together (bootstrap=72%), all falling within the species clade alongside references. In the *F. tricinctum* clade (Fig. 6B-II), root isolates (RR11, RR15, RB2) and reference strains form a highly supported cluster (bootstrap=99%), demonstrating strong taxonomic cohesion. Similarly, in the *F. hostae* clade (Fig. 6B-III), root isolate RR4 groups with references with moderate support (bootstrap=80%), confirming species placement. Within the *F. redolens* clade (Fig. 6B-IV), isolates RR5, RE15, and RE9 occupy separate but supported branches (bootstrap=66–97%), consistently positioned within the species. The *F. oxysporum* clade (Fig. 6B-V) consists of root isolate RB7 and references in a weakly supported subcluster (bootstrap=53%), nevertheless indicating assignment to this species. In the *F. duofalcatisporum* clade (Fig. 6B-VI), root isolates RE5 and RE6 cluster together with references, confirming their taxonomic placement. At the base of the tree, the *F. falciforme* clade (Fig. 6B-VII) includes root isolate RR7 and a reference strain forming a well-supported subcluster (bootstrap=79%), validating its species-level identity.

4 Discussion

The morphological variation observed among populations of *S. persepolitana* appears to reflect ecological differences between sites rather than plant age, as all specimens were sampled at the flowering stage. Plants from cooler, higher-latitude sites tended to be shorter with smaller leaves, while those from warmer regions showed comparatively larger dimensions, consistent with known phenotypic plasticity in *Salvia* species (Turdiboev et al. 2022). Our investigation revealed the coexistence of 21 fungal species as endophytes within *S. persepolitana*. To date, there are no prior reports documenting the endophytic fungal diversity in *S. persepolitana*, highlighting the novelty of our findings. Nevertheless, several of the identified taxa correspond to genera or species previously reported as endophytes in other *Salvia* species, thereby corroborating their endophytic potential within the genus. In line with our findings, *A. iranicus* has previously been reported as an endophyte of *Houttuynia cordata* (Rao et al. 2024). In particular, *Salvia aegyptiaca* leaves from Gebel Elba, Egypt, have yielded multiple *Aspergillus*

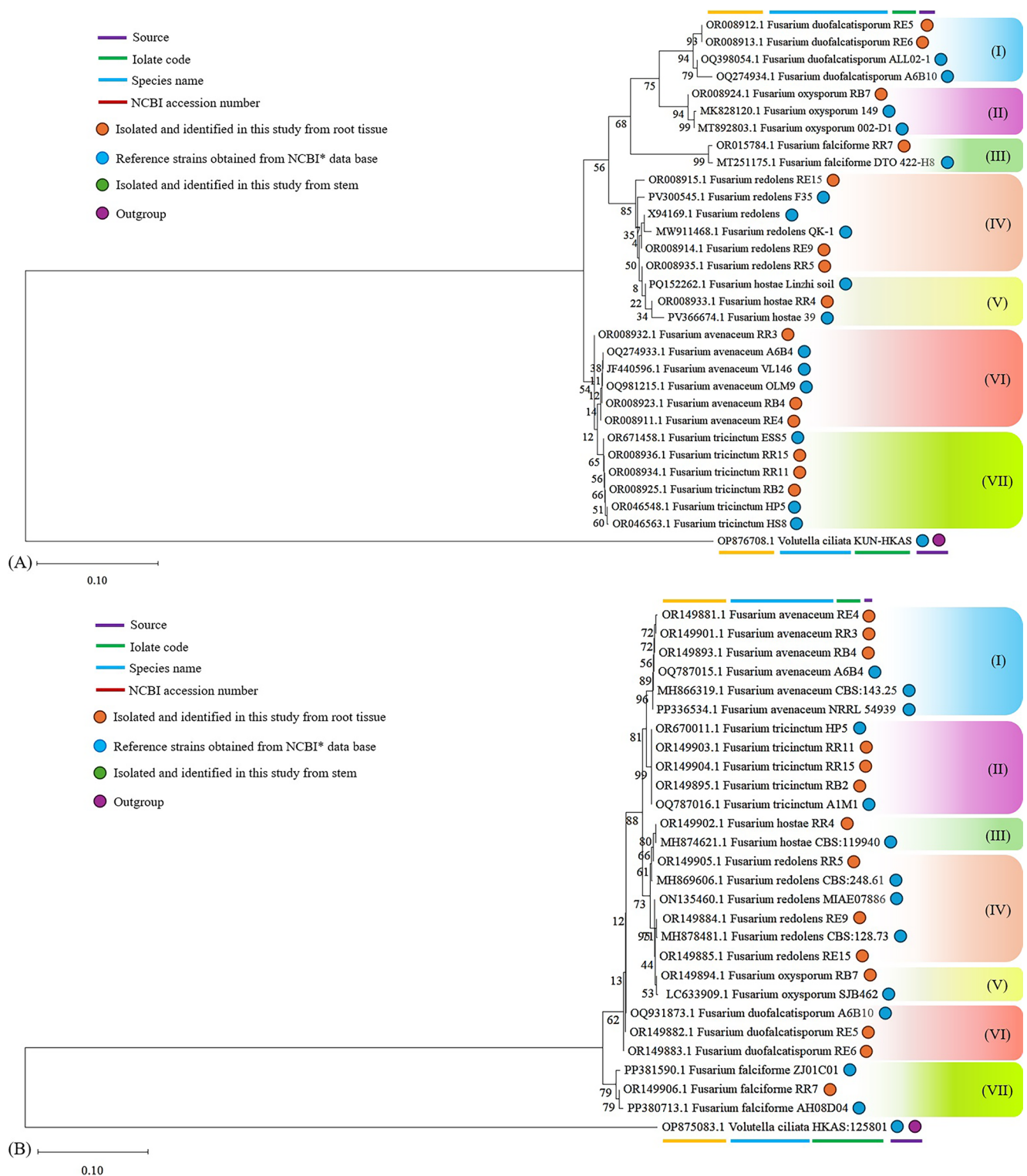


Fig. 6 Phylogenetic inference and evolutionary assessment of *Fusarium* endophytic fungi associated with *Salvia persepoltana*. **(A)**: Phylogenetic tree generated via maximum likelihood analysis based on internal transcribed spacer (ITS) region sequences, incorporating 31

isolates with 1547 aligned nucleotide positions. **(B)**: Tree derived from large subunit (LSU) rRNA region sequences, constructed using 28 fungal isolates and a total sequence alignment of 946 positions. Details of all bifurcations are available in Supplementary Fig. S3

species, including *Aspergillus brasiliensis*, *Aspergillus nidulans*, *Aspergillus niger*, and *Aspergillus terreus* (El-Bondkly et al. 2020). *Aspergillus foeniculicola* was isolated from the root of *Salvia miltiorrhiza* in Shaanxi, China (Ma et al. 2011), while an unidentified *Aspergillus* sp. was reported from *S. miltiorrhiza* roots in Beijing, China (Lou JingFeng et al. 2013), and from *Salvia abrotanoides* leaves in Zoshk, Iran (Teimoori-Boghsani et al. 2020). While the current literature does not establish an endophytic association for *C. cucumerinum* in plants making this the first report of such a relationship only a limited number of species within the genus *Cladosporium* have previously been documented in association with the genus *Salvia*. Notably, *Cladosporium cladosporioides* has been reported from the leaves of *S. aegyptiaca* in the Gebel Elba region of Egypt (El-Bondkly et al. 2020). Although *D. ambigua* has not previously been documented as an endophyte, several unidentified species of the *Diaporthe* genus have been isolated from plant tissues. Specifically, endophytic *Diaporthe* spp. have been reported from the stems of *S. miltiorrhiza* in Sichuan, China (Li et al. 2015), as well as from the stems of *S. abrotanoides* and *Salvia yangii* in Wroclaw, Poland (Zimowska et al. 2020).

In alignment with our findings, *D. prosopidis* has been previously documented as an endophyte in apple (Liu et al. 2023). Additionally, several species within the genus including *Didymella pediae* and *Didymella glomerata*, have been reported as endophytes in the roots and leaves of *S. miltiorrhiza* in China (Li et al. 2016; Lou JingFeng et al. 2013). Consistent with our observations, *E. italicum* has been reported as an endophyte in the leaf tissue of wheat in the Giza Governorate, Egypt (Rashad et al. 2025). Furthermore, a literature review identified *Epicoccum sorghinum* as an endophytic species associated with *S. miltiorrhiza* (Wang et al. 2025). Consistent with our observation of an unidentified *Rhizoctonia* species as an endophyte of *S. persepolitana*, a study by Wang et al. (2021) confirmed the endophytic association of *Rhizoctonia bataticola* with *S. miltiorrhiza*. In this study, we report *X. camporesii* as an endophyte; however, neither this species nor other congeners within the genus *Xenodidymella* have previously been documented in association with *Salvia* species. Furthermore, to the best of our knowledge, no reports exist regarding endophytic relationships involving this species with any other plant hosts. Additionally, *P. herbarum* D603, as reported by Chen et al. (2020), is known to colonize root tissues and establish a stable symbiotic relationship with its host plant *S. miltiorrhiza*. Another species of this genus *Phoma glomerata* D14 has also reported as an endophytic fungus from *S. miltiorrhiza* (Li et al. 2016).

4.1 Diversity and novelty of endophytic *Fusarium* species

Two species warrant specific attention due to their documented endophytic presence in related contexts. *F. oxysporum* was among twenty fungal species from fourteen genera isolated as leaf endophytes of *S. aegyptiaca* collected from Gebel Elba (El-Bondkly et al. 2020). This species has also been reported in association with the roots and stems of *Salvia multicaulis* across its native range in Iran, including the provinces of Qazvin, Alborz, and Mazandaran (Jahromi et al. 2021). Furthermore, *F. redolens* has been found in the root of *S. miltiorrhiza* in Beijing, China (Lou JingFeng et al. 2013). In addition to *F. oxysporum* and *F. redolens*, five additional *Fusarium* species were isolated as endophytes from *S. persepolitana* in the present study. The endophytic behavior of *Fusarium* species within *Salvia* species has been well-documented, with several species isolated from different plant parts and geographic locations.

Fusarium dlamini and *Fusarium solani* were reported from the roots of *S. abrotanoides* in Darrud, Iran (Teimoori-Boghsani et al. 2020), while *Fusarium proliferatum* was isolated from the roots of *S. miltiorrhiza* in both Shandong, China (Li et al. 2015), and Iran (Jahromi et al. 2021). *Fusarium asiaticum* was also detected in the leaf tissue of *S. multicaulis* in Iran (Jahromi et al. 2021). Additionally, *Fusarium equiseti* was previously reported in the hairy roots of *S. miltiorrhiza* and was also recorded in Wenjiang (Li et al. 2024). It is important to note that the *Fusarium* species identified in the present study *F. avenaceum*, *F. duofalcatisporum*, *F. hostae*, *F. falciforme*, and *F. tricinctum* are distinct from those previously reported in association with *Salvia* species. However, their endophytic associations with other plant taxa have been documented, thereby supporting the plausibility of our findings. For instance, endophytic relationships involving *F. avenaceum*, *F. duofalcatisporum*, and *F. tricinctum* have been reported in *Achillea wilhelmsii* and *Achillea arabica* (Eslami et al. 2025). Additionally, *F. falciforme* strain R-423 has been isolated as an endophyte from pigeon pea (*Cajanus cajan*) (Fu et al. 2025). In contrast, no previous reports exist concerning the endophytic behavior of *F. hostae*, making this the first documented instance of its association with a plant host, to the best of our knowledge.

4.2 Established associations and geographic distribution of endophytic *Alternaria* species

Notably, seven *Alternaria* species were isolated as endophytes. Of these, five have previously been reported as

endophytes in other plant taxa, supporting their potential endophytic capacity. For instance, *A. angustiovoidea* was identified as a floral endophyte in *A. arabica* (Eslami et al. 2025), while *A. alstroemeriae* has been reported in association with *Catharanthus roseus* (Li et al. 2025). Similarly, *A. hungarica* has been documented as an endophyte in soybean (*Glycine max*) by Abdelmagid et al. (2023). Furthermore, endophytic interactions involving other *Alternaria* species and *Urtica dioica* (common nettle) have been described by Salmi et al. (2021). Although these findings suggest a potential for broad host adaptability within the genus *Alternaria*, the existence of such a relationship should not be far-fetched in *Salvia* species. The endophytic behavior of *Alternaria* species has been evident in *Salvia* species to date, with various strains reported from diverse geographic regions and plant organs. *Alternaria alternata* has been isolated from the leaf of *S. aegyptiaca* in Gebel Elba, Egypt (El-Bondkly et al. 2020), and from the flower of *S. miltiorrhiza* in Shandong, China (Li et al. 2015) or *Alternaria multi-formis* from *S. multicaulis* in Iran (Jahromi et al. 2021). *Alternaria chlamydosporigena* was found in the root of *S. abrotanoides* in Zoshk, Iran (Teimoori-Boghsani et al. 2020), and in the root of *S. miltiorrhiza* in Beijing, China (Lou JingFeng et al. 2013). Additionally, an unspecified *Alternaria* sp. has been identified in multiple plant parts (root, shoot, and leaf) of *S. miltiorrhiza* in Henan, China (Zhou et al. 2018); in the leaf and stem of *S. abrotanoides*; and in *S. yangii* in Wroclaw, Poland (Zimowska et al. 2020). However, no previous reports were found in the literature documenting the endophytic behavior of *A. conjuncta*, and *A. malorum* as identified in the present study. Thus, their endophytic association appears to be novel and reported here for the first time.

5 Conclusion

This study presents the first comprehensive account of endophytic fungal diversity within *S. persepoltana*, uncovering a total of 21 fungal species across multiple genera. The findings reveal both well-established and previously unreported associations, significantly enriching our understanding of endophytic relationships within the genus *Salvia*. With the exception of *F. avenaceum*, *F. redolens*, and *P. herbarum*, all 18 of the identified endophytic fungal species represent novel records for the genus *Salvia*. Notably, several species such as *F. hostae*, *A. conjuncta*, *A. malorum*, *C. cucumerinum*, *D. ambigua*, and *X. camporesii* are documented here for the first time as plant-associated endophytes. The detection of numerous *Fusarium* and *Alternaria* species, many of which

have been previously identified in diverse plant taxa and geographic contexts, reflects the ecological adaptability and potential functional roles of these fungi within plant microbiomes. Our findings not only reinforce the genus *Salvia* as a reservoir of endophytic fungal diversity but also highlight the importance of underexplored medicinal plants in discovering novel fungal symbionts. These insights provide a valuable foundation for future studies aimed at understanding the ecological significance, functional roles, and potential biotechnological applications of these endophytes.

Appendix A

The ITS and LSU genome sequences of the endophytic fungi, which identified in this research are available online at Nucleotide database of National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov>).

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s13199-025-01094-7>.

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Author contributions Haniyeh Rashedi: performed the experiments, data analysis, wrote and revised the manuscript. Ali Ganjeali and Javad Asili supervised the whole research work, administrated the project, and revised the manuscript. Zahra Tazick: Methodology, Data curation and writing – original draft. Abolfaz Shakeri: Methodology, Data curation. All authors contributed to the editing and approved the final version of the manuscript.

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Data availability The raw data can be made available upon reasonable request from corresponding author.

Declarations

Ethics approval and consent to participate This article reports no studies involving human participants or animals conducted by any of the authors. All authors have provided their approval to participate in this research and to contribute to the manuscript.

Consent for publication All authors approved this manuscript to be published.

Competing interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

- Abdelmagid A, Hou A, Wijekoon C (2023) Endophytic fungi of soybean (*Glycine max* (L.) Merr.) and their potential applications. *Can J Plant Sci* 104(1):32–40. <https://doi.org/10.1139/cjps-2023-0070>
- Adeleke BS, Babalola OO (2021) Pharmacological potential of fungal endophytes associated with medicinal plants: a review. *J Fungi* 7(2):147. <https://doi.org/10.3390/jof7020147>
- Akbari Oghaz N, Rahnama K, Habibi R, Razavi SI, Farias ARG (2024) Endophytic and rhizospheric *Trichoderma* spp. Associated with cucumber plants as potential biocontrol agents of *Fusarium oxysporum* f. sp. *cucumerinum*. *Asian J Mycol* 7(1):31–46. <https://doi.org/10.5943/ajom/7/1/3>
- Asgarpanah J (2021) A review on the essential oil chemical profile of *salvia* genus from Iran. *Nat Volatiles Essent Oils* 8(3):1–28. <https://doi.org/10.37929/nveo.852794>
- Askari SF, Avan R, Tayarani-Najaran Z, Sahebkar A, Eghbali S (2021) Iranian *Salvia* species: a phytochemical and pharmacological update. *Phytochemistry* 183:112619. <https://doi.org/10.1016/j.phytochem.2020.112619>
- Chen HM, Wu HX, He XY, Zhang HH, Miao F, Liang ZS (2020) Promoting Tanshinone synthesis of *Salvia miltiorrhiza* root by a seed endophytic fungus, *Phoma herbarum* D603. *China J Chin Materia Med* 45(1):65–71. <https://doi.org/10.19540/j.cnki.cjcmm.20191113.101>
- El-Bondkly AAM, El-Gendy MMAA, El-Bondkly EAM, Ahmed AM (2020) Biodiversity and biological activity of the fungal microbiota derived from the medicinal plants *Salvia aegyptiaca* L. and *Balanties aegyptiaca* L. *Biocatal Agric Biotechnol* 28:101720. <https://doi.org/10.1016/j.bcab.2020.101720>
- Eslami Z, Tazik Z, Asili J, Soheili V, Akbari Oghaz N, Rezaei T, Motaghipisheh J, Shakeri A (2025) Unveiling the endophytic fungal communities in Iranian *Achillea* species: molecular insights into host specificity and colonization patterns. *Symbiosis* 95:225–240. <https://doi.org/10.1007/s13199-025-01043-4>
- Fronza M, Murillo R, Ślusarczyk S, Adams M, Hamburger M, Heinzmann B, Laufer S, Merfort I (2011) In vitro cytotoxic activity of abietane diterpenes from *Peltodon longipes* as well as *Salvia miltiorrhiza* and *Salvia Sahendica*. *Bioorg Med Chem* 19(16):4876–4881. <https://doi.org/10.1016/j.bmc.2011.06.067>
- Fu JX, Jiao J, Gai QY, Gao J, Wang XQ, Zhang ZY, He J, Wen MN, Fu YJ (2025) A novel endophytic fungus *Fusarium falciforme* R-423 for the control of *Rhizoctonia solani* root rot in pigeon pea as reflected by the alleviation of reactive oxygen species-mediated host defense responses. *J Agric Food Chem* 73(6):3373–3388. <https://doi.org/10.1021/acs.jafc.4c09886>
- Gao Y, Xu Y, Dong Z, Guo Y, Luo J, Wang F, Yan L, Zou X (2025) Endophytic fungal diversity and its interaction mechanism with medicinal plants. *Molecules* 30(5):1028. <https://doi.org/10.3390/molecules30051028>
- Hatamzadeh S, Rahnama K, White JF, Akbari Oghaz N, Nasrollahnejad S, Hemmati K (2023) Investigation of some endophytic fungi from five medicinal plants with growth promoting ability on maize (*Zea Mays* L.). *J Appl Microbiol* 134(1):lxac015. <https://doi.org/10.1093/jambio/lxac015>
- Hatamzadeh S, Akbari Oghaz N, Rahnama K, Noori F (2024) Combination of a hierarchical and thermal shock process with a specific aqueous buffer: a safe, rapid and reliable DNA extraction method for plant endophytic fungi. *Biologia* 79(2):597–604. <https://doi.org/10.1007/s11756-023-01579-0>
- Jahromi MS, Azizi A, Soltani J (2021) Diversity and spatiotemporal distribution of fungal endophytes associated with *Salvia multicaulis*. *Curr Microbiol* 78(4):1432–1447. <https://doi.org/10.1007/s00284-021-02430-y>
- Jamzad Z (2012) Flora of Iran, No. 76: the family lamiaceae Martinov. Forest and Rangeland Research Institute of Iran, Tehran, Iran
- Jassbi AR, Zare S, Firuzi O, Xiao J (2016) Bioactive phytochemicals from shoots and roots of *Salvia* species. *Phytochem Rev* 15:829–867. <https://doi.org/10.1007/s11101-015-9427-z>
- Li YL, Xin XM, Chang ZY, Shi RJ, Miao ZM, Ding J, Hao GP (2015) The endophytic fungi of *Salvia miltiorrhiza* Bge. f. *alba* are a potential source of natural antioxidants. *Bot Stud* 56(5):1–7. <https://doi.org/10.1186/s40529-015-0086-6>
- Li X, Zhai X, Shu Z, Dong R, Ming Q, Qin L, Zheng C (2016) *Phoma glomerata* D14: an endophytic fungus from *Salvia miltiorrhiza* that produces Salvianolic acid C. *Curr Microbiol* 73:31–37. <https://doi.org/10.1007/s00284-016-1023-y>
- Li X, Lin Y, Qin Y, Han G, Wang H, Yan Z (2024) Beneficial endophytic fungi improve the yield and quality of *Salvia miltiorrhiza* by performing different ecological functions. *PeerJ* 12:e16959. <https://doi.org/10.7717/peerj.16959>
- Li YF, Yue F, Wan XR, Li C, Sun Y, Pei YH (2025) Antimicrobial perylenequinones isolated from the endophytic fungus *Alternaria alstroemeriae*. *Phytochem Lett* 65:64–67. <https://doi.org/10.1016/j.phytol.2024.12.003>
- Liu KL, Porras-Alfaro A, Kuske CR, Eichorst SA, Xie G (2012) Accurate, rapid taxonomic classification of fungal large-subunit rRNA genes. *Appl Environ Microbiol* 78(5):1523–1533. <https://doi.org/10.1128/AEM.06826-11>
- Liu Jj, Zheng J, Gao C, Liu H, Zhang Dh, Liu L (2023) Screening of endophytic growth-promoting fungi and their characteristics in Apple. *J Fruit Sci* 40:735–746. <https://doi.org/10.13925/j.cnki.gsxh.20220495>
- Lou JingFeng LJF, Fu LinYun FLY, Luo RuiYa LRY, Wang XiaoHan WX, Luo HaiYu LHY, Zhou LiGang ZL (2013) Endophytic fungi from medicinal herb *Salvia miltiorrhiza* bunge and their antimicrobial activity. *Afr J Microbiol Res* 7(47):5343–5349
- Ma C, Jiang D, Wei X (2011) Mutation breeding of *Emericella foeniculicola* TR21 for improved production of Tanshinone IIA. *Process Biochem* 46(10):2059–2063. <https://doi.org/10.1016/j.procbio.2011.07.012>
- Omomowo IO, Amao JA, Abubakar A, Ogundola AF, Ezediuno LO, Bamigboye CO (2023) A review on the trends of endophytic fungi bioactivities. *Sci Afr* 32(46):e01594. <https://doi.org/10.1186/s41938-022-00547-1>
- Rao J, Tao Z, Chen J, Yang S (2024) Investigation of bioactive metabolites produced by the endophytic fungus *Aspergillus Iranicus* from *Houttuynia cordata* thunb. *Nat Prod Res* 12:1–5. <https://doi.org/10.1080/14786419.2024.2412836>
- Rashad YM, El-Sharkawy HHA, Abdalla SA, Ibrahim OM, Elazab NT (2025) Unraveling the regulating effect of the endophyte *Epicoecum italicum* HE20 on the biosynthetic genes of polyphenols in wheat in response to yellow rust. *Physiol Mol Plant Pathol* 138:102705. <https://doi.org/10.1016/j.pmpp.2025.102705>
- Salmi D, Riou C, Issawi M, Titouche Y, Ambrosini V, Smail-Saadoun N, Abbaci H, Houali K (2021) Antibacterial and antioxidant activities of endophytic fungi and nettle (*Urtica dioica* L.) leaves as their host. *Cell Mol Biol* 67(3):204–211. <https://doi.org/10.14715/cmb/2021.67.3.33>
- Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, Consortium FB, List FBCA, Bolchacova E (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proc. Natl. Acad. Sci.* 109(16):6241–6246. <https://doi.org/10.1073/pnas.1117018109>
- Teimoori-Boghani Y, Ganjeali A, Cernava T, Müller H, Asili J, Berg G (2020) Endophytic fungi of native *Salvia abrotanoides* plants reveal high taxonomic diversity and unique profiles of secondary metabolites. *Front Microbiol* 10:3013. <https://doi.org/10.3389/fmicb.2019.03013>

- Turdiboev OA, Shormanova AA, Sheludyakova MB, Akbarov F, Drew BT, Celep F (2022) Synopsis of the central Asian *salvia* species with identification key. *Phytotaxa* 543(1):1–20. <https://doi.org/10.11646/phytotaxa.543.1.1>
- Wang GK, Yang JS, Huang YF, Liu JS, Tsai CW, Bau DT, Chang WS (2021) Culture separation, identification and unique anti-pathogenic fungi capacity of endophytic fungi from Gucheng *Salvia miltiorrhiza*. *In Vivo* 35(1):325–332. <https://doi.org/10.21873/invivo.12263>
- Wang Y, Cai S, Tao Z, Peng J, Li D, Li L, Cao X, Jiang J (2025) Isolation of endophytic fungi and effects on secondary metabolites in hairy roots of *Salvia miltiorrhiza*. *J Microbiol Biotechnol* 9(35):e2411051. <https://doi.org/10.4014/jmb.2411.11051>
- Zhou LS, Tang K, Guo SX (2018) The plant growth-promoting fungus (PGPF) *Alternaria* sp. A13 markedly enhances *Salvia miltiorrhiza* root growth and active ingredient accumulation under greenhouse and field conditions. *Int J Mol Sci* 19(1):270. <https://doi.org/10.3390/ijms19010270>
- Zimowska B, Bielecka M, Abramczyk B, Nicoletti R (2020) Bioactive products from endophytic fungi of sages (*Salvia* spp). *Agriculture (Basel)* 10(11):543. <https://doi.org/10.3390/agriculture10110543>

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