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Identity and diversity of culturable endophytic fungi associated with *Capparis spinosa* L. in Iran

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Endophytic fungi play a crucial role in plant health, contributing to stress tolerance, disease resistance, and ecological adaptation. However, the diversity and richness of endophytic fungal communities associated with *Capparis spinosa* L. in the Alborz, Tehran, and Qom provinces—ranging from semi-arid and slightly temperate to arid and desert climatic conditions—have not yet been investigated. Using morphological and molecular methods, we identified a diverse fungal assemblage comprising 3 phyla, 7 classes, 14 orders, 28 families, and 36 genera. Among the genera shared across all three provinces, *Alternaria* (28.8%) was the most dominant among the isolates, whereas *Simplicillium* (1.6%) was the least abundant. Analysis of the isolates using diversity indices revealed that species distribution in all three provinces tended toward evenness, with a similar pattern observed across different tissues. Qom exhibited the highest diversity and richness of fungal species. Additionally, a detailed comparison of different plant tissues revealed that roots consistently harbored the greatest variety and the highest number of isolates compared to stems, leaves, and fruits. Diversity metrics suggest a potential link between climatic gradients and endophyte diversity. These findings enhance our understanding of fungal-plant interactions and provide insights into the microbial contributions to *C. spinosa* resilience in harsh environmental conditions.

Keywords Caper, Diversity indices, Mycoflora, Species richness, Symbiosis

Plants are complex ecosystems that host diverse microorganisms, such as bacteria, fungi, and viruses[1]. Among these are endophytic fungi that live symbiotically within plant tissues without causing harm. These fungi, present in numerous plant species, enhance growth and resistance to stress and produce bioactive compounds[2]. Moreover, their diverse distribution is influenced by both the host plant species and the environment in which they thrive[3]. Despite their evolutionary diversity, only approximately 150,000 of the estimated three million fungal species have been identified, highlighting untapped research opportunities[4].

The genus *Capparis*, established by Linnaeus in 1753, belongs to the *Capparidaceae* family and comprises 350 species, primarily in tropical and subtropical regions. *C. spinosa*, a hybrid of *C. orientalis* **Veill.** and *C. sicula* **Veill.**, thrives across diverse regions from the Canary Islands to Iran, North Africa, Europe, and beyond. With its robust root system, this plant adapts to varied environments, including arid, sandy, and nutrient-poor soils[5,6].

Endophytic fungi, harmlessly residing within plant tissues, are essential for ecosystem health and stability. Their diversity and population are shaped by host traits, interactions within plant parts, and environmental factors[7]. Climate change endangers global agriculture, causing abiotic stresses like drought and salinization that reduce productivity. An integrative approach, including the use of endophytes, particularly endophytic fungi, offers potential solutions to mitigate these stresses[8]. Endophytic fungi enhance root function, increasing water and nutrient absorption, particularly under water stress. They regulate plant growth and boost resilience to environmental challenges[9]. Furthermore, the diversity and functional roles of root-associated endophytic fungi contribute to promoting plant vitality under adverse conditions[10]. Endophytic fungi contain bioactive compounds with vital health benefits, such as antimicrobial, hepatoprotective, antidiabetic, anti-inflammatory, and anticancer effects, derived from components like flavonoids, phenolic acids, and alkaloids. Medicinal plants provide ideal conditions for endophytic fungi, enabling the production of unique bioactive substances[11,12].

Given *C. spinosa*'s resilience in challenging environments and its medicinal properties, it is crucial to investigate its largely unexplored endophytic fungi. Studying these fungi is vital for understanding the symbiotic relationships that bolster plant health and resilience. The objectives of this study included: (a) isolation of

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endophytic fungi from *C. spinosa* growing in Iran using culture-dependent methods, (b) identification of the isolated endophytic fungi from various plant organs using morphological and molecular methods, and (c) investigation of the impact of diverse tissues and geographical locations on the endophytic fungal communities.

Results

Isolation and taxonomic study of endophytic fungi

No fungal growth was detected on PDA (**Potato dextrose agar**) plates in the control group, confirming the effectiveness of the surface sterilization process. Therefore, all the fungi isolated in this study are confirmed to be endophytes, as pure single clones were successfully obtained from each tissue. In total, 711 endophytic fungal isolates were recovered from 1800 cultured tissue segments of *C. spinosa* collected from Alborz, Tehran, and Qom provinces. These isolates belonged to 3 phyla, 7 classes, 14 orders, 28 families, and 36 genera (Supplementary Fig. S1). After thoroughly examining the characteristics and growth patterns of the colonies, 76 distinct morphotypes were identified, reflecting the diversity among the total isolates (Supplementary Table S2, Fig. S2, S3). Relative abundance (RA%)of endophytic fungal isolates was determined by considering the number of isolates obtained from various tissues of *C. spinosa* across three different provinces: Qom, Alborz, and Tehran (Supplementary Fig. S4–S6). In Qom province, 243 isolates were obtained, resulting in a **colonization frequency (CF)** of 36.82% including 124 from root (75.15%), 80 from stem (48.48%), 35 from leaf (21.21%), and 4 from fruit (2.42%). In Alborz, 255 endophytic fungal isolates were obtained, with a CF of 42.50%. This includes 107 isolates from root (71.33%), 78 from stem (52.00%), 53 from leaf (35.33%), and 17 from fruit (11.33%). Similarly, from Tehran province, 213 strains were isolated, with a CF of 39.44%, consisting of 117 from root (86.67%), 58 from stem (42.96%), 34 from leaf (25.19%), and 4 from fruit (2.96%) (Supplementary Table S3). Alborz exhibited the highest CF at 42.50%, with the greatest number of endophytic fungal isolates ($n = 255$), followed by Tehran at 39.44% and Qom at 36.82%. Most isolates were obtained from the roots ($n = 348$), while the fruits yielded the fewest isolates ($n = 25$) (Supplementary Table S3).

The morphological and molecular characterization of 243 endophytic fungi isolated from Qom province revealed that they belonged to three phyla: *Ascomycota*, *Zoopagomycota*, and *Oomycota*. These fungi were categorized into 7 classes, 12 orders, 22 families, and 27 genera. Among them, 237 (97.5%) endophytic fungal strains were in the phylum *Ascomycota*, distributed across the classes of *Sordariomycetes*, *Eurotiomycetes*, *Dothideomycetes*, *Pezizomycetes*, and *Leotiomycetes*. Additionally, three strains (1.23%) were identified as *Basidiobolomycetes* from the phylum *Zoopagomycota*, and another three strains (1.23%) were classified as *Peronosporomycetes* from the phylum *Oomycota* (Fig. 1). Alborz province had 255 isolates, all classified within the *Ascomycota* phylum. These isolates are distributed across 3 classes, 6 orders, 12 families, and 13 genera (Fig. 2). The most strains were in the *Dothideomycetes* order (146 strains, 57.25%), followed by *Sordariomycetes* (60 strains, 23.52%) and *Eurotiomycetes* (49 strains, 19.21%). 213 endophytic fungi isolated from Tehran province belonged to *Ascomycota* phyla with 3 classes, 6 orders, 12 families, and 15 genera (Fig. 3).There were 93 (43.66%) strains in the *Dothideomycetes*, 74 strains (34.74%) in *Eurotiomycetes*, and *Sordariomycetes* with 46 strains (21.59%).

As shown in Supplementary Fig. S7, the genera *Alternaria*, *Aspergillus*, *Fusarium*, *Penicillium*, and *Simplicillium* are present in all three provinces. *Chaetosphaeronema*, *Cladosporium*, *Corynespora*, *Phoma*, and *Preussia* are common between Qom and Tehran provinces. *Chaetomium* and *Stilbocrea* are shared between Qom and Alborz, while *Devriesia* and *Neosetophoma* are found in both Tehran and Alborz provinces. Additionally, *Acremonium*, *Basidiobolus*, *Botryotrichum*, *Clarireedia*, *Coniothyrium*, *Lasiobolidium*, *Lophiostoma*, *Microascus*, *Neodidymelliopsis*, *Schizothecium*, *Paecilomyces*, *Pestalotiopsis*, *Phaeoacremonium*, *Phytophthora*, and *Stolonocarpus* were exclusive to Qom province. Several genera were specific to Alborz (*Acrocalymma*, *Diaporthe*, *Kalmusia*, and *Paramicrosphaeropsis*) and Tehran (*Bulbithecium*, *Myceliophthora*, and *Talaromyces*) provinces. The fungal endophytes of Qom province exhibited the greatest abundance and variety.

Alpha diversity assessment of endophytic fungi in *C. spinosa* across Qom, Alborz, and Tehran provinces

Fungal species isolated from each tissue corresponding to each province are presented in Figs. 4, 5 and 6.

The Shannon–Wiener index (H) and Simpson dominance index (D) revealed that the endophytic fungal population recovered from each province exhibits the highest diversity in root ($H = 3.34$, $D = 0.034$; $H = 2.886$, $D = 0.055$; $H = 3.084$, $D = 0.052$ respectively, Qom Alborz, Tehran provinces), while the lowest diversity is observed in fruit in Alborz ($H = 1.318$, $D = 0.243$), and leaf tissue in Qom ($H = 2.292$, $D = 0.076$) and Tehran ($H = 2.224$, $D = 0.89$). These results indicate that the diversity of culturable endophytic fungi species in *C. spinosa* is significantly influenced by the type of plant tissue (Table 1).

Understanding the relationship between abundance and diversity in fungal endophytic communities is crucial. Figure 7 demonstrates that the frequency distribution of fungal endophyte species isolated from Qom, Alborz, and Tehran provinces align with a Poisson distribution.

Fisher's alpha index is a highly valuable parametric measure that effectively determines species diversity within the framework of logarithmic series distribution models. The abundance of fungal endophyte species isolated from each province was calculated using Fisher's series for root, stem, leaf, and fruit tissues (the dashed line shown in Fig. 7). The correlation between observed and calculated data for fungal endophytes isolated from various tissues in all three provinces was significant.

Fisher's alpha index (Table 2) for all isolated fungal endophytes was 16.796 for Qom, 9.246 for Alborz, and 10.927 for Tehran province, indicating the highest diversity in Qom, followed by Tehran and Alborz provinces, respectively. Additionally, the highest diversity within each province was observed in root tissues, with values of 13.975 for Qom, 7.814 for Alborz, and 11.663 for Tehran. Conversely, the lowest diversity was recorded in fruit tissues, with values of 0.428 for both Qom and Tehran, and 1.648 for Alborz province.

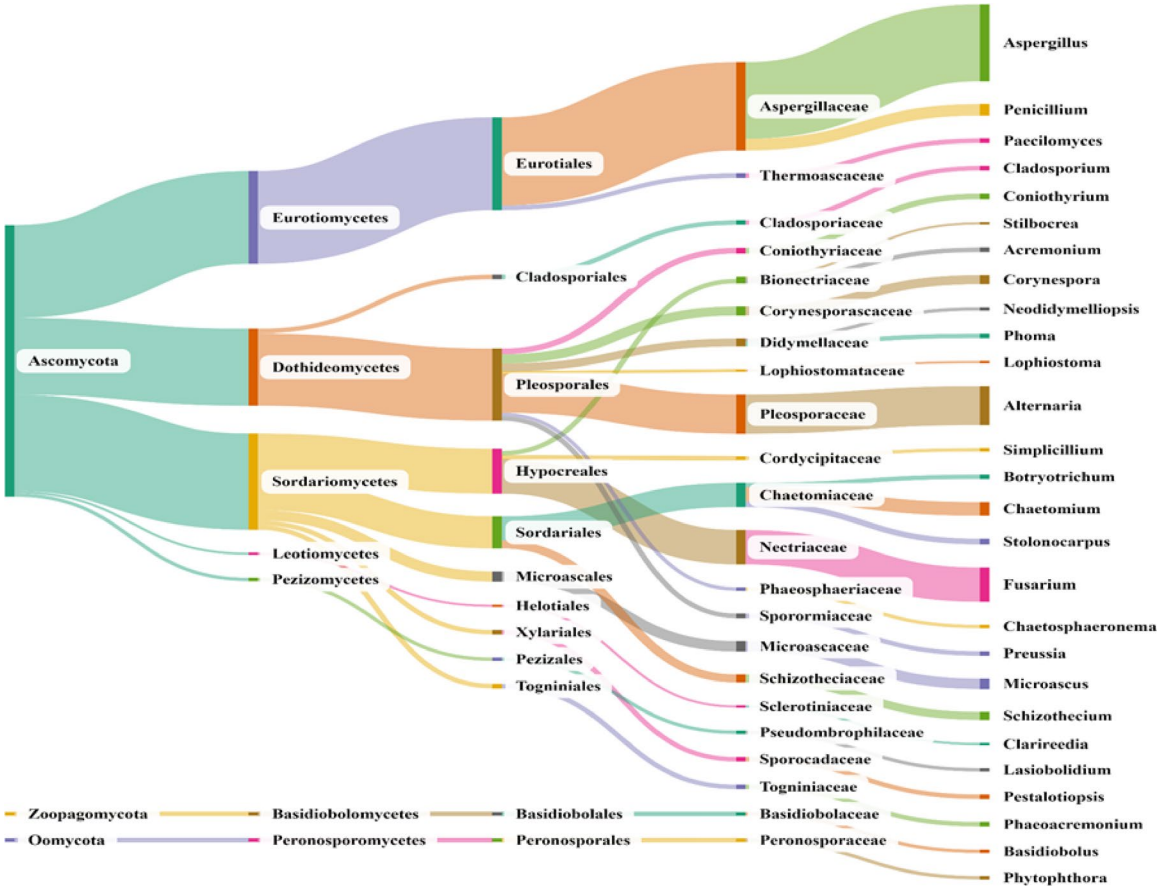

Fig. 1. Taxonomic relationships of species: The endophytic fungi isolated from *Capparis spinosa* in Qom province belonged to 3 phyla, 7 classes, 12 orders, 22 families, and 27 genera.

Beta diversity analysis of endophytic fungi in different *C. spinosa* tissues across three provinces

The values of the classic Sorensen and Jaccard indices, used to compare the endophytic fungal species composition across different tissues within each province and between provinces, revealed several notable similarities. Specifically, the endophytic fungal species in the root and stem tissues in Qom province were highly similar. In Alborz, the leaf tissues exhibited significant similarity with both the root and fruit tissues. In Tehran, the stem tissues were similar to both root and leaf tissues. Additionally, notable similarities were observed between the stem tissues from Qom and the leaf tissues from Tehran, as well as between the stem tissues from Qom and the root tissues from Alborz. Lastly, the root tissues of Alborz province showed significant similarity with the root tissues of Tehran province (Supplementary Table S4).

Richness of the species

Supplementary Table S5 illustrates the total count of species (S) as a direct measure of species diversity. The greatest number of species was recovered from Qom, followed by Tehran and Alborz provinces. When examining the number of individuals within each province, it was observed that root tissues had the highest number of isolated endophytic fungi, whereas fruit tissues had the lowest number of isolates.

It is important to note that the S is influenced by the sample size, which limits its utility as a comparative index. To evaluate species richness more effectively, Margalef and Menhinick indices, renowned for their lack of reliance on sample size, were employed. The indices exhibited higher values for endophytic fungi obtained from Qom in contrast to those from Tehran and Alborz provinces. Within each province, root tissues demonstrated the highest species richness compared to other tissues, such as stem, leaf, and fruit. When comparing isolates from the three provinces, Qom displayed the lowest Berger–Parker dominance index, followed by Alborz province. This index, along with its reciprocal, demonstrated the highest dominance and lowest diversity in endophytic fungi isolated from the roots in Tehran province, followed by Alborz and Qom provinces (Tables 1 and 2).

The Chao metric revealed that the endophytic fungal community collected from Qom demonstrated the greatest species abundance at 48.250, with Alborz exhibiting the least at 31.000 (Table 1).

Species evenness

The species distribution in the fungal communities isolated from all tissues in each province is depicted in Supplementary Fig. S8–S10.

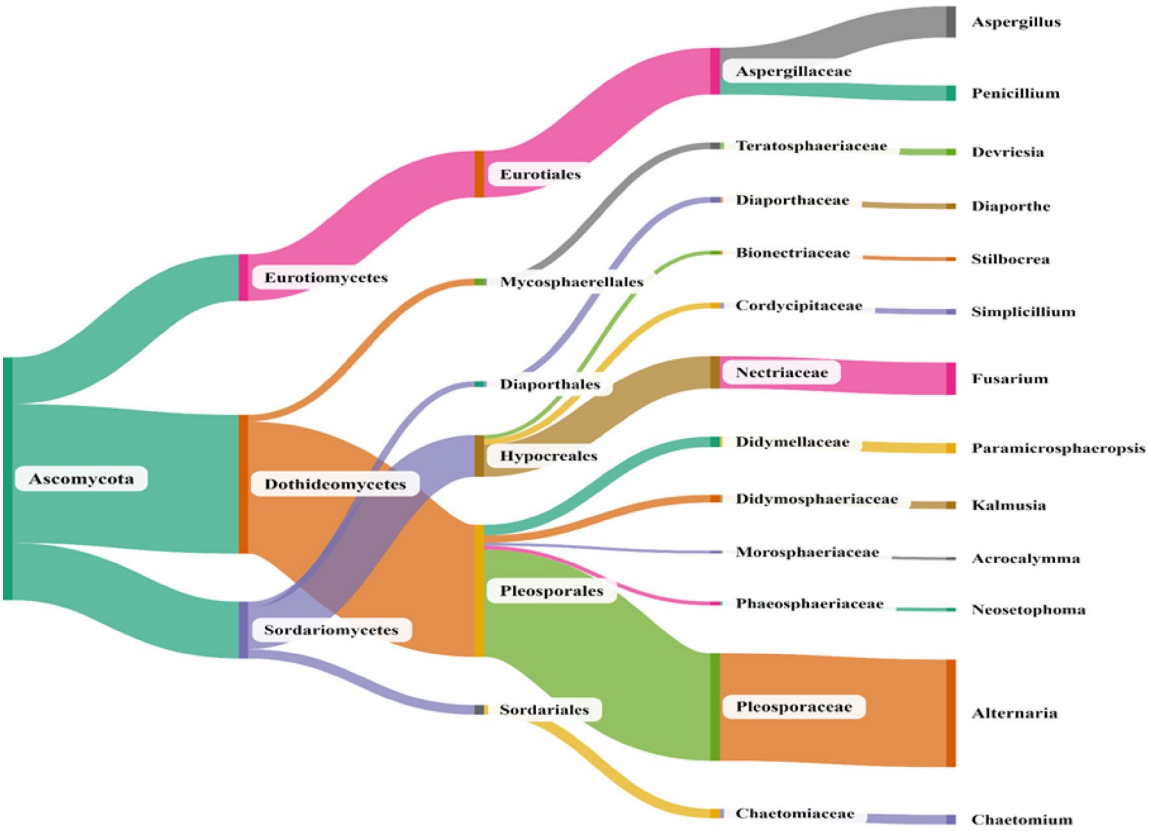

Fig. 2. Taxonomic relationships of species: The endophytic fungi isolated from *Capparis spinosa* in Alborz province belonged to 1 phyla, 3 classes, 6 orders, 12 families, and 13 genera.

The evenness (E) values for Tehran, Alborz, and Qom provinces were 0.686, 0.738, and 0.802, respectively. These values suggest that species distribution in these provinces leans towards evenness. A similar trend was observed across the root, stem, leaf, and fruit tissues in each province. Additionally, the Pielou Evenness index (J) values mirrored this pattern. In Qom province, the Pielou index values for root, stem, and leaf tissues were 0.956, 0.942, and 0.996, respectively, indicating that leaf tissues exhibit the highest evenness in the fungal community. In Alborz province, the J values for root, stem, leaf, and fruit tissues were 0.948, 0.951, 0.931, and 0.951, respectively, showing that stem and fruit tissues have the highest evenness, while leaf tissues have the lowest. For Tehran province, the J values were calculated as 0.926 for root, 0.928 for stem, and 0.966 for leaf tissues, revealing that leaf tissues demonstrate the highest evenness within the fungal community. Camargo's uniformity index was calculated for the fungal communities each province (Table 2). A uniform distribution of species was observed across all types of tissue in the provinces, particularly in the leaf tissues of all three provinces and the fruit tissues in Alborz province and the stem tissues in Tehran province.

The Simpson's Dominance values for all isolates obtained from each province are close to zero, indicating a stable fungal community structure. This trend was consistent across the endophyte fungal communities of plant tissues in each province, except for fruit tissues in Qom and Tehran. Consequently, it can be inferred that the fungal community structure is stable across all three provinces, except for the fruit tissue in Qom and Tehran provinces (Table 1). According to Table 1, the endophytic fungal community is diverse in the studied areas. This conclusion is based on the analysis of all isolates from the three provinces and each tissue type within those provinces, except for fruit tissue in Qom and Tehran provinces.

Principal Components Analysis (PCA) was conducted using diversity indices for the endophytic fungal species community isolated from Qom, Alborz, and Tehran provinces. The results indicate that the data align well with the model. The first principal component (PC1) explains 93.31% of the variation, showing high dominance coefficients for the Camargo, Simpson, and Berger-Parker indices. The second principal component (PC2) accounts for 6.69% of the total variation, with the Menhinick index richness index displaying a high positive coefficient for this component. According to Fig. 8 (A), Tehran province demonstrates the highest species dominance, while Qom province exhibits the highest species richness and, consequently, the greatest species diversity. Based on these findings, the primary and secondary principal components can be interpreted as factors estimating dominance and richness, respectively.

Based on the same diversity indices, a dendrogram was constructed (Fig. 8B), showing that Qom and Alborz provinces exhibit greater similarity to each other, as they clustered together at a lower dissimilarity level. In contrast, Tehran province shows less similarity to Qom and Alborz, joining the cluster at a higher dissimilarity

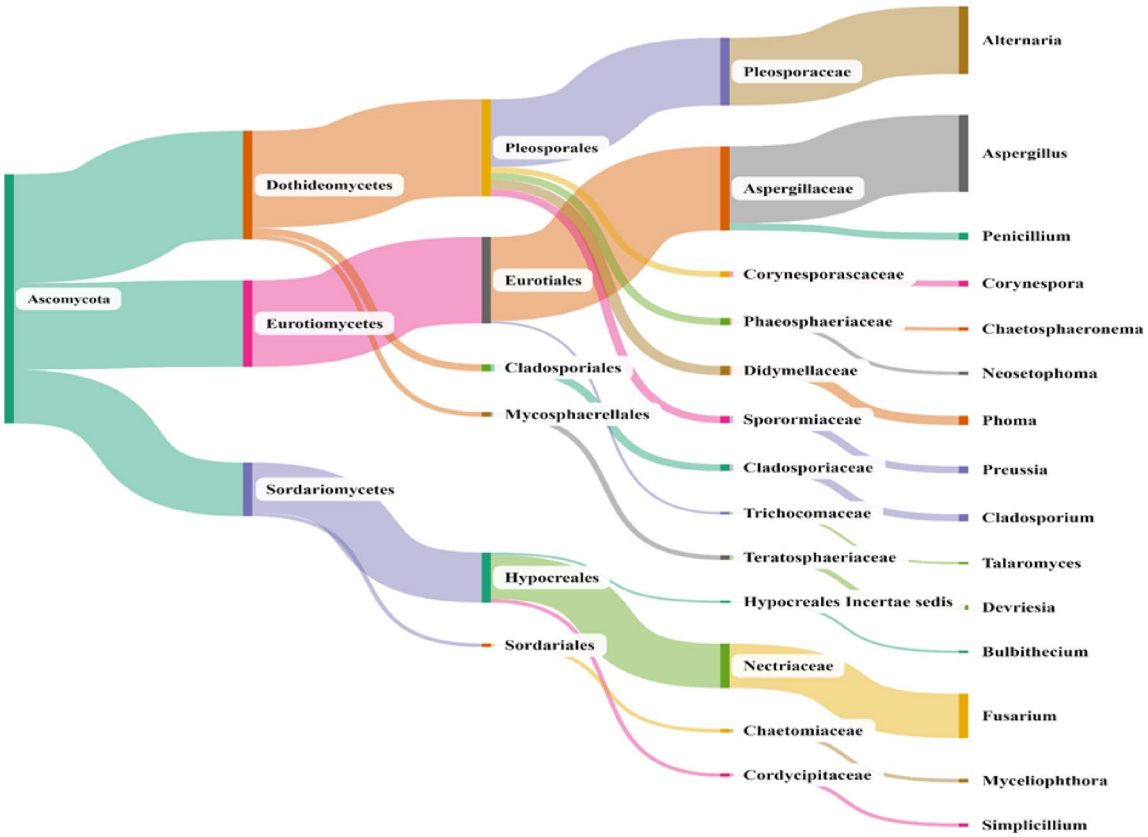

Fig. 3. Taxonomic relationships of species: The endophytic fungi isolated from *Capparis spinosa* in Tehran province belonged to 1 phyla, 3 classes, 6 orders, 12 families, and 15 genera.

level. This analysis helps in understanding the overall structure and diversity within the endophytic fungal communities, providing insights into the diversity and distribution of these fungi across different regions.

Community structuring and categorization

Cluster analysis, a tool for community classification, was used to examine the fungal communities in Qom, Alborz, and Tehran provinces, based on the Jaccard similarity index (Fig. 9). The dendrogram analysis indicates that Tehran and Alborz provinces are clustered together, while Qom has a distinct fungal community. This could indicate closer ecological relationships between Tehran and Alborz and a more unique environment in Qom province.

Specificity of community composition

Among the studied endophytic fungi, five common genera—*Alternaria*, *Aspergillus*, *Fusarium*, *Penicillium*, and *Simplicillium*—were found across Qom, Alborz, and Tehran provinces. Of these, the genus *Alternaria* was the most abundant in all three provinces. Additionally, the specific genera isolated from each province suggest that the climate of each region significantly influences the fungal community associated with *C. spinosa*. A heat map (Fig. 10) and bar chart (Supplementary Fig. S11) were created based on the relative abundance of endophytic fungal genera and isolates from Qom, Alborz, and Tehran provinces at the genus level. In general, these analyses revealed that Qom province exhibited the highest frequency of fungal genera, followed by Tehran and Alborz provinces, respectively. This indicates that the diversity and presence of endophytic fungi are more prominent in Qom compared to the other studied regions. Furthermore, when examining the relative abundance of specific genera, it was found that *Alternaria* dominated the fungal community. *Aspergillus* and *Fusarium* were also relatively abundant but to a lesser extent than *Alternaria*.

In addition, PCA was used to analyze the data and visualize the fungal genus diversity patterns. The PCA chart (Supplementary Fig. S12) presents the distribution of endophytic fungi abundance. The PC1 accounts for 61.24% of the total variance, while the PC2 accounts for 38.76%. The findings suggest that among the prevalent genera in the three provinces, *Alternaria* stands out as the most abundant, showing a notably significant presence in Alborz province. This dominance underscores the significant role of *Alternaria* in the fungal community of this region. Additionally, out of the 36 genera identified in this study, 15 were exclusively found in Qom province. This finding highlights the distinct regional differences in fungal diversity.

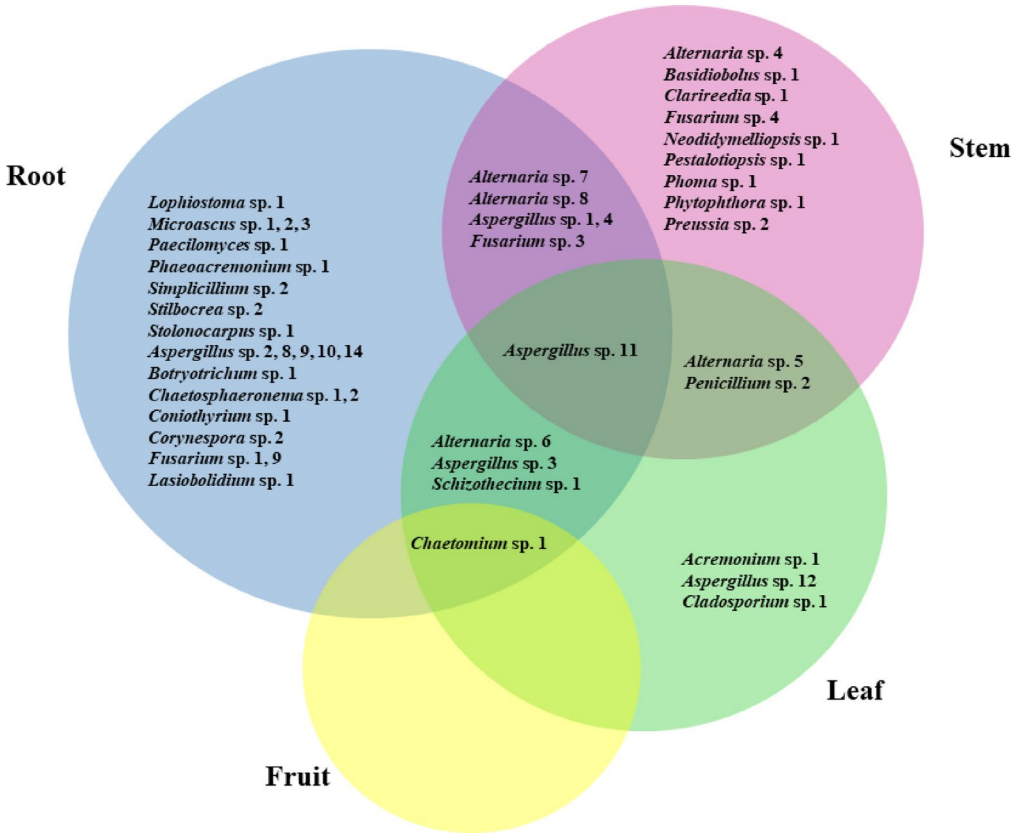

Fig. 4. Venn diagram of endophytic fungal species isolated from roots, stems, leaves and fruits of *Capparis spinosa* grown in Qom province.

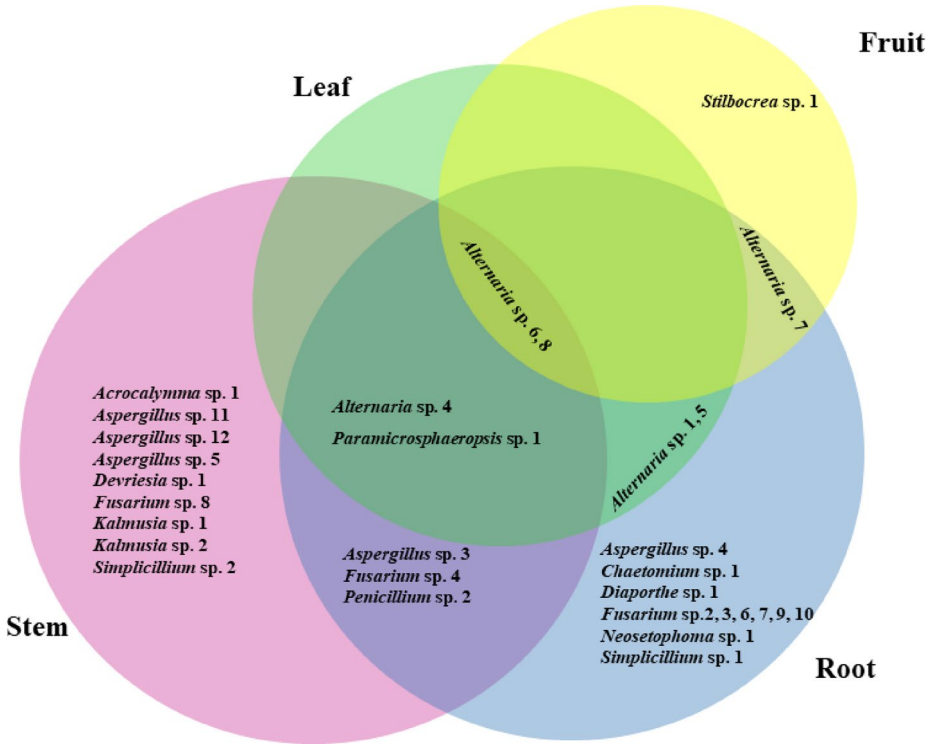

Fig. 5. Venn diagram of endophytic fungal species isolated from roots, stems, leaves and fruits of *Capparis spinosa* grown in Alborz province.

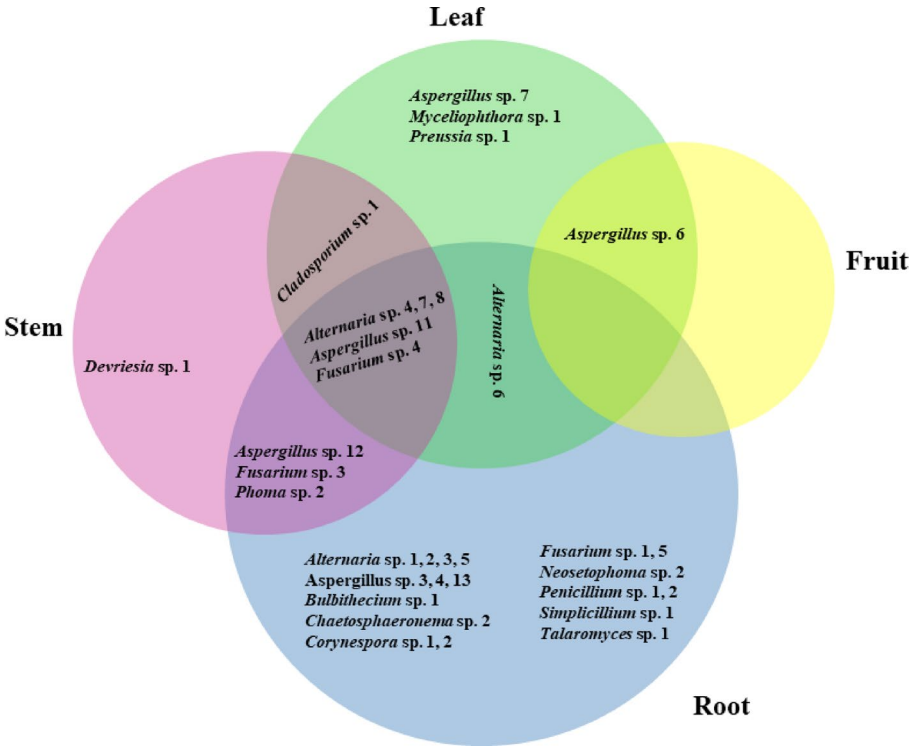

Fig. 6. Venn diagram of endophytic fungal species isolated from roots, stems, leaves and fruits of *Capparis spinosa* grown in Tehran province.

Tissues	Chao richness estimator	Margalef diversity index	Shannon-wiener diversity index	Simpson dominance index (D)	Simpson diversity index (1-D)	Reciprocal of simpson index (1/D)
Qom						
Total	48.250	8.192	3.608	0.030	0.970	33.680
Root	39.800	6.431	3.314	0.034	0.966	29.558
Stem	17.273	3.651	2.669	0.070	0.930	14.299
Leaf	55.000	2.531	2.292	0.076	0.924	13.222
Fruit	–	–	–	1	0	–
Alborz						
Total	31.000	5.414	3.130	0.053	0.947	18.774
Root	47.000	4.280	2.886	0.055	0.945	18.061
Stem	16.000	3.443	2.636	0.071	0.929	14.099
Leaf	9.000	1.259	1.669	0.192	0.808	5.220
Fruit	5.500	1.059	1.318	0.243	0.757	4.121
Tehran						
Total	35.333	5.969	3.120	0.058	0.942	17.196
Root	30.571	5.670	3.084	0.052	0.948	19.224
Stem	10.000	2.217	2.138	0.122	0.878	8.183
Leaf	11.200	2.552	2.224	0.089	0.911	11.220
Fruit	–	–	–	1	0	–

Table 1. Chao richness estimator, Margalef diversity index, Shannon-Wiener diversity index, Simpson dominance index (D), Simpson diversity index (1-D), and reciprocal of Simpson index (1/D) calculated for the endophytic fungal species community isolated from roots, stems, leaves and fruits of capparis spinosa collected from Qom, Alborz, and Tehran provinces.

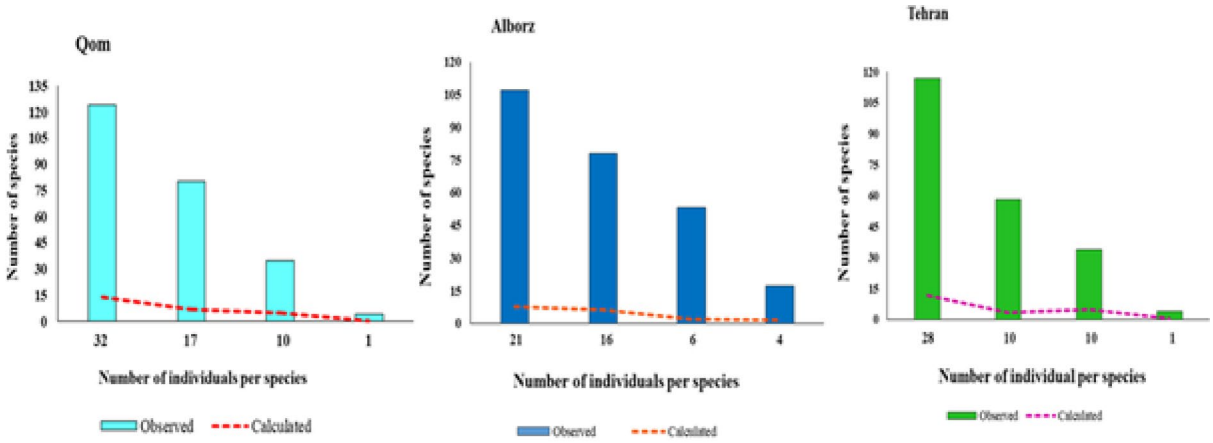

Fig. 7. Species abundance distribution for endophytic fungal isolates obtained from the roots, stems, leaves, and fruits of the *Capparis spinosa* from Qom, Alborz, and Tehran provinces.

Tissues	Berger–parker dominance index	Reciprocal of berger–parker index	Pielou evenness index	Menhinick index	Camargo index	Evenness (e^H′) /S	Fisher_alpha
Qom							
Total	0.091	11.045	0.881	2.951	0.339	0.802	16.749
Root	0.081	12.400	0.956	2.874	0.348	0.859	13.975
Stem	0.150	6.667	0.942	1.901	0.526	0.848	6.606
Leaf	0.114	8.750	0.996	1.690	0.592	0.990	4.676
Fruit	–	–	–	–	–	–	0.428
Alborz							
Total	0.137	7.286	0.813	1.941	0.515	0.738	9.246
Root	0.112	8.917	0.948	2.030	0.493	0.853	7.814
Stem	0.154	6.500	0.951	1.812	0.552	0.872	6.097
Leaf	0.283	3.533	0.931	0.824	1.213	0.884	1.739
Fruit	0.412	2.429	0.951	0.970	1.031	0.934	1.648
Tehran							
Total	0.164	6.086	0.802	2.261	0.442	0.686	10.927
Root	0.137	7.313	0.926	2.589	0.386	0.780	11.663
Stem	0.241	4.143	0.928	1.313	0.762	0.848	3.483
Leaf	0.176	5.667	0.966	1.715	0.583	0.925	4.774
Fruit	–	–	–	–	–	–	0.428

Table 2. Berger–Parker dominance index, reciprocal of Berger–Parker index, Pielou evenness index, menhinick index, Camargo index, and fisher alpha calculated for the endophytic fungal species community isolated from roots, stems, leaves, and fruits of capparis spinosa collected from Qom, Alborz, and Tehran provinces.

Discussion

The present study is unique because it reveals significant variations in endophytic fungal communities across different plant tissues and regions, providing valuable insights into the ecological and functional roles of these fungi. Distinctive patterns in the presence of endophytic fungal species were observed in *C. spinosa* plants collected from the three provinces (Fig. S1), highlighting the unique regional diversity of these fungi. As the climate gradient shifted toward higher temperatures and more arid regions—from Alborz to Tehran and then Qom—fungal diversity and richness increased. Similarly, previous studies have investigated the impact of geographical origin and increasing temperature levels on endophytic microbiomes[13,14].

The results showed that a wide range of fungal species coexist with *C. spinosa*. All 711 isolates were morphologically and molecularly classified into 76 morphotypes, with most belonging to the phylum Ascomycota. This aligns with the findings of Yeh and Kirschner[15], who identified three fungal phyla—Ascomycota, Basidiomycota, and Zygomycota—in their study, with Ascomycota being the dominant phylum among their isolates.

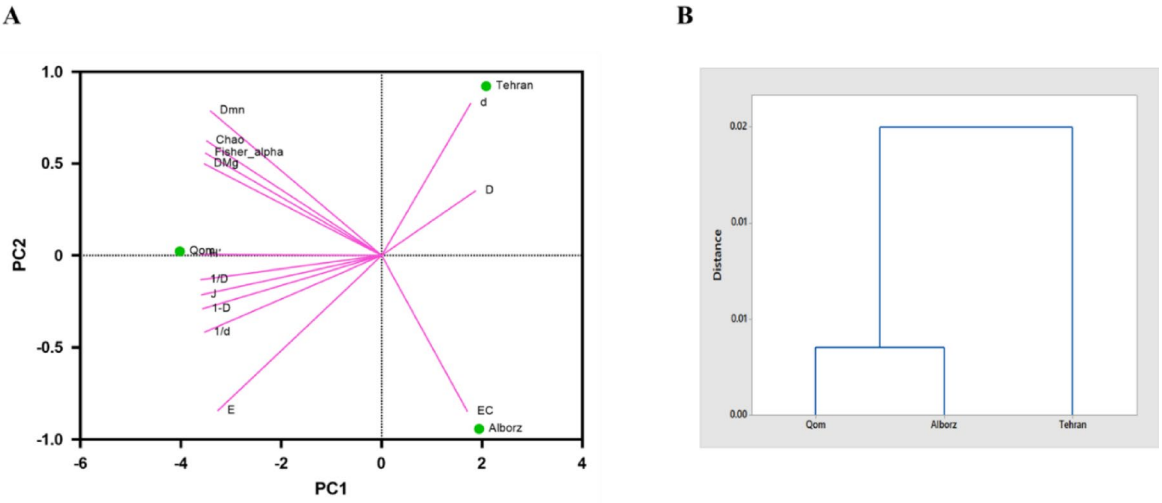

Fig. 8. Principal component analysis (**A**) and classification dendrogram (**B**) based on calculated diversity indices for fungal species from Qom, Alborz, and Tehran provinces.Chao, Chao richness estimator; DMg, Margalef diversity index; H′, Shannon–Wiener diversity index; D, Simpson dominance index; 1-D, Simpson diversity index; 1/D, Reciprocal of Simpson index; d, Berger–Parker dominance index; 1/d, Reciprocal of Berger–Parker index; J, Pielou evenness index; Dmn, Menhinick index; EC, Camargo index; E, Evenness_(e^H′)/S; Fisher alpha.

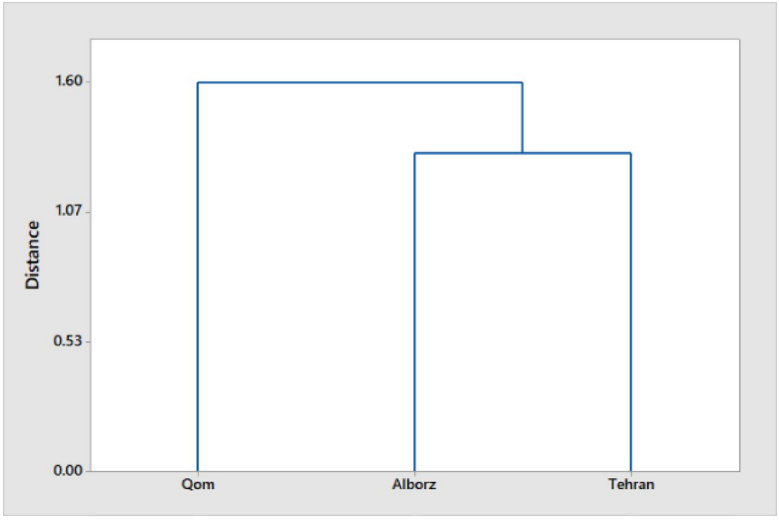

Fig. 9. Dendrogram of the classification of fungal species from Qom, Alborz, and Tehran provinces based on Jaccard similarity.

Different patterns in the presence of endophytic fungal species in *C. spinosa* were observed across the three provinces of Qom, Tehran, and Alborz, indicating a unique diversity and symbiotic association in plants from arid and desert regions. The diversity of host-specific endophytic microbiomes in desert plants has been previously reported[16]. In a previous study conducted in Iran, researchers collected four *C. spinosa* plant samples from ten regions—predominantly mountainous, cold, and dry environments—and successfully isolated 72 fungal strains belonging to ten genera[17]. While our study focused on warmer, semi-arid to arid environments, the unique distribution of endophytic fungal species in these climates may indicate a specific functional role of symbiotic fungi. Fungal endophytes are widespread symbionts in plants and can directly influence plant drought resistance. These effects include drought avoidance strategies, such as enhanced water uptake or reduced transpiration rates, as well as drought tolerance mechanisms through osmotic regulation[18,19].

The fungal frequency and diversity indices in the fruit, leaves, stem, and roots of *C. spinosa* showed that the abundance of endophytic fungi was significantly higher in the roots and in general, the diversity of endophytic microorganisms decreases after moving upwards or from the root to the fruits[20]. This can be attributed to the roots' direct contact with the soil, which acts as a microbial reservoir, and the soil's ability to buffer temperature

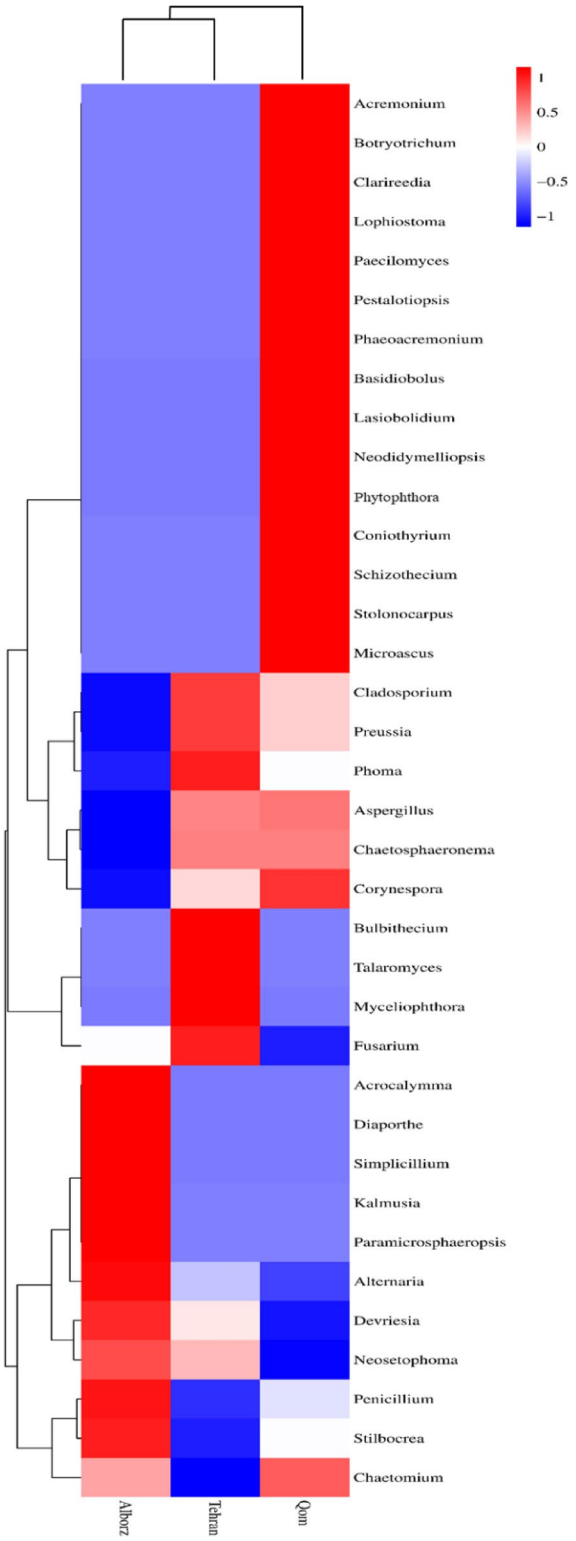

Fig. 10. Heat map shows the relative frequency of endophytic fungal genera isolated from *Capparis spinosa* in Qom, Alborz, and Tehran provinces.

and humidity fluctuations, thereby creating a stable environment for microbial colonization[21]. Plant roots play a crucial role in initiating plant-microbe interactions within the soil. Through root exudates, they facilitate the formation and stability of specialized microbial communities, tailored to support the host's needs. This highlights how the influence of root exudates extends the gene expression profiles of associated endophytes, shaping their functional dynamics[22]. The higher number of fungal species in the roots can also be explained

by the growth capability of the host plant in arid and semi-arid regions. In such environments, existing plants often possess sophisticated underground networks that create nutrient-rich zones. These extensive root systems, combined with the presence of various hosts and substrates, provide ample opportunities for fungi to survive. Consequently, desert environments with well-developed root structures may exhibit higher colony-forming units (CFU) and greater fungal species diversity in the roots compared to other plant parts[21,23].

Given the significance of *C. spinosa* in arid and semi-arid landscapes, this study aimed to provide a comprehensive assessment of its fungal diversity using a culture-dependent approach. Among these, various fungal genera were identified as endophytes in this study. Some of them, observed to be present without causing harm to the host in this research, may exhibit pathogenic behavior in other hosts. *Phytophthora*[24], for instance, is widely regarded as a plant pathogen, yet its presence as an endophyte in *C. spinosa* may be linked to host-dependent interactions and balanced antagonism. This implies that endophytes that engage in mutualistic interactions with their hosts under specific conditions may become pathogenic when the host is stressed, causing the balance of antagonism to shift in favor of the fungus[25].

This research facilitates the identification of novel fungi and their metabolites, while also aiding in research on biotic and abiotic stress resilience.

Conclusions

In total, 711 isolates of endophytic fungi were obtained from four different tissues sampled in three provinces. These isolates were classified in three phyla, seven classes, fourteen orders, 28 families, and 36 genera. Caper plant tissues in each province showed different fungal community composition, dominant genera, indigenous genera, and different levels of biodiversity. These patterns reflect the distribution of endophytes within different geographic regions and organ specialization or tissue preference (a factor contributing to diversity in the fungal community), which may vary depending on environmental conditions (e.g., seasonal changes or plant developmental stages). Investigating the diversity of endophytic fungi will not only contribute to the understanding of intricate plant-fungal interactions but also hold potential for stimulating plant growth, producing bioactive compounds, and protecting against biotic and abiotic stresses. Further studies using culture-independent metagenomics approaches may elucidate the entire range of these interactions and ultimately demonstrate the possibilities for endophytic fungi to expand their applications in agriculture and pharmaceuticals.

Materials and methods

Sampling and fungal isolation

Ten mature *C. spinosa* plants in the active growth stage were randomly sampled from Alborz, Tehran, and Qom provinces during summer and autumn 2022. Climate information for each region is provided in Table S1. The samples were legally collected from unprotected areas, and all procedures were conducted in strict accordance with relevant guidelines and regulations. The plant specimens were identified by Prof. Mostafa Assadi at the Research Institute of Forests and Rangelands in Tehran, Iran. The voucher specimens were deposited in the Central Herbarium of Iran (Alborz: TARI111953, Tehran: TARI111951, Qom: TARI111952), located at the National Botanical Garden of Iran. Samples, transported to the Plant Pathology Laboratory at Tarbiat Modares University within 48 h, were rinsed with water to remove dust and epiphytic fungi. After ensuring that the selected tissues were healthy and viable, plant surfaces (ripe fruits, leaves, stems, roots) were sterilized with ethanol before segmenting: leaves, fruits, and main roots into 5 mm^2 pieces; stems and finer roots into 3 × 5 mm pieces. Following a modified Kusari et al. [26] protocol, disinfection involved two rounds of ethanol treatment (70%), lasting 30 s to 2 min based on tissue type. Segments were rinsed thrice with sterilized distilled water, dried on filter paper, and cultured on PDA containing 250 mg/L Ampicillin. Sterility was verified by streaking the final rinse water onto PDA medium. Samples were incubated at 25 ± 2 °C for 3–20 days for fungal growth. Afterward, isolates were purified using the hyphal tip[27] or single spore[28] culture method.

Morphological identification of endophytic fungi

Fungal morphology was assessed both macroscopically and microscopically. Macroscopic observations included colony surface and reverse colors, tissue type, and growth patterns. Microscopically, fruiting bodies, conidiophores, spores, and their arrangements were examined using lactophenol-based staining under an Olympus BH2 microscope[29,30]. Photomicrographs were captured with an Olympus DP72 camera on a BX51 microscope with differential interference contrast. A series of genera were identified using identification keys and authoritative articles, focusing on microscopic assessment[31–46].

Molecular identification of endophytic fungi

After examining the microscopic slides of the isolates and identifying several genera based on reputable articles and identification keys, molecular identification was performed on the endophytic isolates that were mycelial or required final confirmation. In fact, morphological analysis was also supplemented with molecular techniques for isolates lacking conclusive identification. The ITS region was used for most mycelial isolates and some spore-producing ones that could be differentiated, while gene regions like *gpdh*, *β-tub*, *CaM*, and *EF-1α* were selected for samples requiring genus-specific differentiation. DNA extraction from mycelia cultured on PDA at 25 ± 2 °C for 7–10 days, as described by Safaie et al.[47]. PCR amplifications employed primers specific to target genes: ITS1/ ITS4 for ITS[48], gpdh1-F/gpdh2-R for *gapdh*[49], EF1/ EF2 for *EF-1α* [50], Btub2Fd and Btub4Rd[51] for β-tubulin and Cmd5/ Cmd6 for the *CaM*[52] genes.

The 25 µl PCR mixture included 9 µl sterile deionized water, 12 µl 2X PCR Master mix (Pishgam Co.), 1 pmol primers, and 2 µl of 30 ng/µl DNA. PCR was performed using a Thermal cycler (Epgradiant, Eppendorf) under the following conditions: initial denaturation at 95 °C for 3 min; 35 cycles of denaturation at 94 °C for

60 s; annealing at 58 °C (ITS), 52 °C (*gapdh*), 54 °C (*EF-1α*), 52 °C (*β-tub*), or 55 °C (*CaM*); extension at 72 °C for 60 s; and final extension at 72 °C for 10 min. PCR products were sequenced by Microsynth Company (www.microsynth.com).

After obtaining and editing the sequences, they were compared with existing sequences in the GenBank database using the BLAST search tool[53] to ensure data accuracy. After confirming the quality and read length of the fragment, the sequences were submitted to the GenBank database (NCBI), and accession numbers were obtained for them (Supplementary Table S2).

Diversity analysis of endophytic fungi

Diversity measures the variety of life forms within a site or ecosystem, combining species richness and evenness, which increase biodiversity[54,55]. The variety of endophytes within different *C. spinosa* plant tissues and regions was examined. The combination and redundancy of these endophytic fungi should be assessed by counting the quantity of fungal isolates (N) and using species as the statistical element. Macroscopic study allows for the differentiation of the morphotypes[21]. In this context, abundance (N) refers to the number of isolates, while richness (S) denotes the numerical values of species present within a particular sample population.

To evaluate the suitability of each tissue type of *C. spinosa* from various regions as a colonization substrate for the fungi, we enumerated the samples colonized by specific fungi. The colonization frequency (CF) was then calculated using Eq. (1) described by Hata and Futai[56].

$$CF\ (\%) = \frac{No.\ of\ colonized\ samples}{No.\ of\ plated\ samples} \times 100 \tag{1}$$

Isolation frequency (IF) measures the incidence of specific fungal species within a set of isolates. It's calculated by dividing the number of isolates for a particular species by the total number of isolates in all species, giving a percentage known as relative abundance (RA%). The formula is described by Huang et al.[57] and given in Eq. (2).

$$A\ (\%) = \frac{No.\ of\ isolates\ of\ a\ particular\ species}{Sum\ of\ isolates\ of\ all\ species} \times 100$$

The Chao1 estimator is a non-parametric method used to estimate species richness in a community. This method is based on the principle that rare species provide the most information about the number of undetected species. The Chao1 estimator emphasizes low-abundance species, utilizing only singletons and doubletons (species expressed by one and two individuals, respectively) to approximate the number of missing species. As a result, datasets with a bias toward species with low abundances derive the greatest advantage from this index. The Chao1 richness predictor is calculated using Eq. (3), which was described by Chao[58]. Where S_{obs}shows the observed number of species, $F_1$and F_2 denote the number of singletons and doubletons, respectively.

$$S_{chao1} = S_{obs} + \frac{F_1(F_1 - 1)}{2(F_2 + 1)} \tag{3}$$

Species richness of different fungal isolates was calculated using the Menhinick index (Dmn) utilizing the following formula (Eq. (4)) introduced by Whittaker[59]. In this formula, S represents the count of distinct fungal species per sample, and N shows the total number of fungal isolates within that specific sample.

$$D_{mn} = \frac{S}{\sqrt{N}} \tag{4}$$

The Camargo evenness index is a measure used to determine the evenness of species distribution within a community[60]. It is calculated using Eq. (5). Where D_{mn} is species richness.

$$EC = \frac{1}{D_{mn}} \tag{5}$$

The Shannon diversity index[61] is a fundamental metric used to assess the diversity within fungal endophyte communities in different tissues. It is expressed through Eq. (6).

$$H' = -\sum_{i=1}^{s} p_i ln p_i \tag{6}$$

Where p_i represents the relative abundance (RA) of each species within a sample. The index value H' can vary significantly. It considers both the number of species (richness) and the evenness of species abundances. It reaches 0 when only one species is present (indicating no diversity) and increases with higher diversity and a more even species distribution[57]. Thus, higher H' values signal greater uncertainty in predicting the species of an individual organism, reflecting a more evenly distributed community.

Pielou's evenness index, which is a common measure applied to assess how evenly individuals are distributed among species in a community[62], was calculated using Eq. (7).

$$J = \frac{H\prime}{ln\ (S)} \tag{7}$$

In this formula, represents Pielou's measure of species evenness, stands for the Shannon-Wiener index in the sample, and indicates the total number of species within the sample.

Equation (8), which was introduced by Muthukrishnan et al. [63], was utilized to compute the evenness index (E).

$$E = \frac{e^{H\prime}}{s} \tag{8}$$

Beta diversity is a concept introduced by Whittaker[64], and measures the difference in species composition between ecosystems within a specific region. Two of the most common non-parametric indices to calculate beta diversity are the Sorensen index (βSor)[65] and the Jaccard index (βJac)[66].

The Sorensen similarity index quantifies the ratio of the double count of common species between two communities to the overall species count within each community. This index was determined utilizing Eq. (9) [65]. Here, c represents the number of common fungal species found in both samples, while a and b indicate the species that are unique to each sample.

$$\beta_{Sor} = \frac{2c}{a+b+2c} \tag{9}$$

The Jaccard similarity index measures the proportion of species common between two communities relative to the total species count in both communities. This index is expressed using Eq. (10)[66].

$$\beta_{Jac} = \frac{c}{(a+b+c)} \tag{10}$$

Where c is the number of species common to both samples, a and b represent the number of species unique to each sample.

Both indices compare the presence and absence of species between two communities, emphasizing the shared species. However, they differ slightly in their calculations. The Sorensen index gives more weight to shared species, making it more sensitive to common species, while the Jaccard index treats all species equally.

Fisher's alpha is a parametric index used to estimate species diversity if the abundances of species follow a logarithmic distribution. This index was calculated using Eq. (11)[67].

$$S = \alpha\ \times\ ln\left(1 + \frac{n}{\alpha}\right) \tag{11}$$

Where defines the number of species in the sample, shows the total number of endophytic fungal isolates, and is Fisher's alpha, which represents the diversity index.

The Berger-Parker dominance index measures the relative abundance of the most dominant species in a community, highlighting its numerical importance.The formula for the Berger-Parker index is represented in Eq. (12)[68].

$$d = \frac{n_{max}}{N} \tag{12}$$

Where n_{max}represents the count of individuals in the most plentiful species, while N denotes the total count of individuals in the sample.

The reciprocal of the Berger-Parker index ($1/d$) is commonly used, and an increase in this value indicates greater diversity and reduced dominance by the most abundant species.

The Simpson dominance index was employed to evaluate species diversity, indicating the likelihood that two randomly chosen isolates from a sample will be of the same species (Eq. (13))[69].

$$D = \frac{\sum_1^s n_i\ (n_i - 1)}{N\ (N-1)} \tag{13}$$

Where $n_i$is the number of isolates of species, i and N are the total number of isolates across all species. Simpson's Dominance Index (D) ranges from 0 to 1. A value close to 0 indicates high species diversity and community stability, with no single species dominating. Conversely, a value near 1 suggests low diversity, dominance by one or a few species, and potential ecosystem instability due to ecological pressures[70].

Additionally, both the complement of the Simpson diversity index (1–D) and the reciprocal of the Simpson index (1–D) were computed. The Simpson diversity index (1-D) measures species diversity in a community. Higher values (close to 1) suggest high diversity, while lower values (close to 0) indicate low diversity[71,72].

Species richness was assessed with the Margalef index (Eq. (14))[73].

$$D_{Mg} = \frac{S-1}{ln\,(N)} \tag{14}$$

Where signifies the number of species and denotes the total count of isolates.

Eventually, data analysis and visualization were conducted using Minitab 18.1, Excel 2013, GraphPad Prism 10, and SR Plot (https://www.bioinformatics.com.cn/en. Accessed on Jan, 2025).

Data availability

Data is provided within the manuscript or supplementary information files.

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Author contributions

N.S. designed and supervised the project and validated the data. S.K. performed sampling, isolation, and identification of fungi. Sh.M. guided data processing. L.E. advised on fungal identification. N.S. and S.K. carried out diversity analyses. S.K. wrote the first draft of the manuscript. N.S. approved the final version. All authors contributed to the preparation and review of the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Permission

Capparis spinosa L. samples were collected legally from non-protected natural areas with permission from the relevant authorities.

Additional information

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