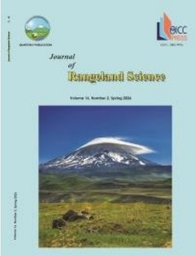

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Research Article

Fungal Community Associated With Two Declining Rangeland Plants (*Haloxylon Ammodendron* and *Astragalus Verus*) in Iran

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Abstract

Drylands are specific habitats for the growth of xerophyte plants. These lands are vulnerable to numerous threats, and all biotic and abiotic stresses can play essential roles. In 2022, declining symptoms were observed for *Haloxylon ammodendron* and *Astragalus verus* in the Alborz, Tehran, and Hamedan provinces. Therefore, this study aimed to characterize fungal communities associated with the decline of H. *ammodendron* and A. *verus* in drylands using ITS sequencing. Symptomatic plants were sampled and cultured on PDA media. After fungal purification, DNA extraction was performed, and the DNA in the ITS region was sequenced. The fungal genera identified in this study included *Alternaria, Arthrinium, Astragalicola, Biscogniauxia, Chaetomium, Cytospora, Fusarium, Mucor, Neoscytalidium, Paecilomyces, Pithomyces, Preussia, Sordaria,* and an undefined genus. Eleven identified genera of fungi were associated with H. *ammodendron* in dry areas of Tehran and Alborz provinces, while 10 genera were associated with A. *verus* plants in Hamadan province. The mycobiota profile showed a high relative frequency of fungal genera belonging to the *Paecilomyces* and *Biscogniauxia* genera in H. *ammodendron* plants and the *Astragalicola* and *Chaetomium* genera in A. *verus* plants. Additionally, seven genera of fungi were common to both hosts, while none of the seven other genera were shared by both hosts. Based on these results, Ascomycota comprised a large part of the mycobiota, and a minor part of the mycobiota belonged to Mucoromycota (only in A. *verus* plants). Our findings confirmed that the presence of the most important pathogenic genera of fungi in H. *ammodendron* and A. *verus* plants could be a threat to other dryland plants. This information can be valuable for conservation efforts and developing strategies to mitigate the threats posed by these pathogenic fungi to dryland ecosystems.

Keywords: Drylands, Decline, *Haloxylon ammodendron*, *Astragalus verus*, Mycobiota

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1. Introduction

Dryland ecosystems, encompassing arid and semi-arid regions, cover approximately 45% of Earth's terrestrial surface (Zhang et al., 2021). Defined by low precipitation, high evaporation, and extreme temperature fluctuations, these ecosystems are vital for global biodiversity and biogeochemical cycles (Middleton & Sternberg, 2013). However, their productivity is severely constrained by water scarcity, a challenge intensified by desertification and climate variability (Prăvălie et al., 2016). Within these fragile environments, interactions between plants, microbes, and soil underpin ecosystem stability and function, influencing nutrient cycling and resilience to environmental stress (Maestre et al., 2021).

The Haloxylon genus, belonging to Chenopodiaceae and consisting of 11 species worldwide, features vital desert shrubs like H. *ammodendron*, which play crucial ecological roles in arid desert ecosystems (Li et al., 2016). This genus is widely distributed across Central Asia, the Middle East, and Africa, where it has adapted to survive and flourish in extreme, hyper-arid environments with minimal water availability and harsh climatic conditions. (Ebrahimi et al., 2019). H. *ammodendron* helps stabilize soils, reduce erosion, and supports understory plant growth like Poa bulbosa by altering microhabitats, creating favorable conditions for diverse plant communities and ecological balance (Hu et al., 2021). Similarly, the genus Astragalus, comprising nearly 3,000 species, is one of the most extensive and diverse plant genera, predominantly found in temperate drylands and arid regions (Podlech & Zarre, 2013). Among these, species like A. *verus* exhibit remarkable resilience, tolerating environmental stresses such as high salinity, drought, and extreme temperatures (Madouh, 2022). However, these hardy species are increasingly threatened by climate change impacts, habitat destruction, overgrazing, invasive species, and pathogenic invasions (Yang et al., 2010).

Declines in woody plants, marked by reduced growth, chlorosis, and dieback, arise from complex abiotic-biotic interactions (Bettenfeld et al., 2020). Symptoms often manifest over decades, driven by pathogens such as fungi (e.g., *Fusarium, Rhizoctonia*), bacteria, and nematodes, whose virulence is amplified by drought and extreme weather (Jung et al., 2018; Colangelo et al., 2018). For example, prolonged drought weakens perennial grasses, inducing leaf wilting and root rot (Bondaruk et al., 2022). Pathogenic fungi also reshape ecosystem dynamics: shifts in plant competitiveness due to infections can reduce biodiversity, favor the establishment of invasive species, and destabilize communities (Bondaruk et al., 2022). Advancing disease management requires elucidating host-pathogen interactions within the "pathobiome" framework, where microbial consortia—not single pathogens—drive decline (Stewart et al., 2021). While traditional culturing methods detect <1% of microbiota, high-throughput sequencing offers comprehensive insights into fungal diversity, ecological networks, and co-occurrence patterns (Chen et al., 2020).

Understanding fungal pathogens in declining dryland plants like H. *ammodendron* and A. *verus* is critical to address ecosystem threats from climate change, biodiversity loss, and soil degradation, informing conservation strategies for vulnerable arid environments. Therefore, this study aimed to investigate the fungal communities associated with declining H. *ammodendron* and A. *verus* across multiple dryland regions using ITS sequencing. The other aims of the study were 1) to characterize species-specific mycobiota, 2) identify shared fungal taxa linked to analogous decline symptoms, and 3) evaluate ecological implications for dryland resilience. By integrating pathobiome theory and co-occurrence network analysis, our findings will inform strategies to mitigate plant decline in these vulnerable ecosystems..

2. Materials and methods

2.1. Symptoms and sampling

During the years 2021 and 2022, surveys were conducted across multiple natural dried areas of the Alborz, Tehran, and Hamedan provinces. H. *ammodendron* was evaluated in Tehran and Alborz Provinces, while A. *verus* was studied in Hamedan Province (Figure 1). Both hosts exhibited similar symptoms, including decrease, dieback, yellowing, and drying of aerial parts (Figures 2 and 3). Vascular discoloration was visible in cross-sections of the stems and large branches of both species. A total of 10 symptomatic branches or shoots were randomly sampled from each host species, totaling 20 samples, to ensure a representative collection of the fungal populations associated with the diseased tissues. These samples were placed in separate paper bags and moved to the Plant Pathology Department at Tarbiat Modares University, Tehran, and the Research Institute of Forests and Rangelands, Tehran, and were stored at 4°C until further analysis. The selection of 10 samples per host species was based on aiming for sufficient diversity to capture the variability of fungal communities while maintaining practical feasibility, ensuring the samples accurately reflected the health status of the plants and the range of fungi involved in the decline symptoms.

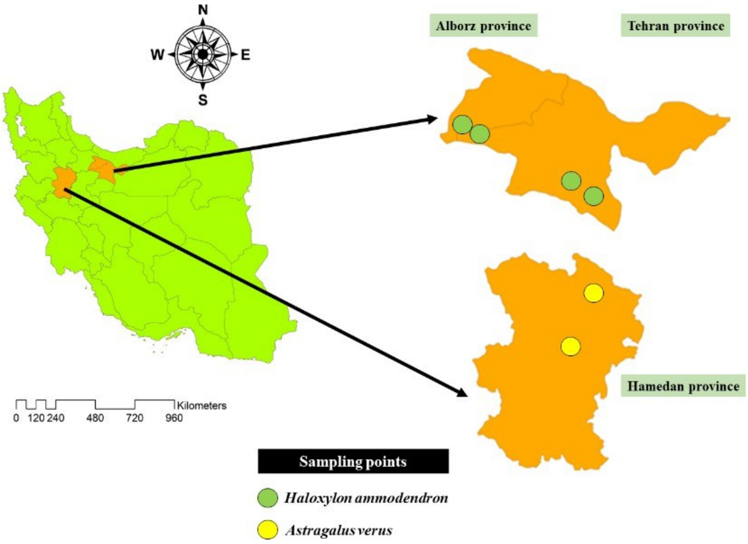

Figure 1. Sampling points in Alborz, Tehran, and Hamedan provinces. The green and yellow colors represent sampling points related to *H. ammodendron* and *A. verus* hosts, respectively. Geographically, the provinces of Alborz and Tehran are located in the north of Iran, while the Hamedan province is located in the west of the country

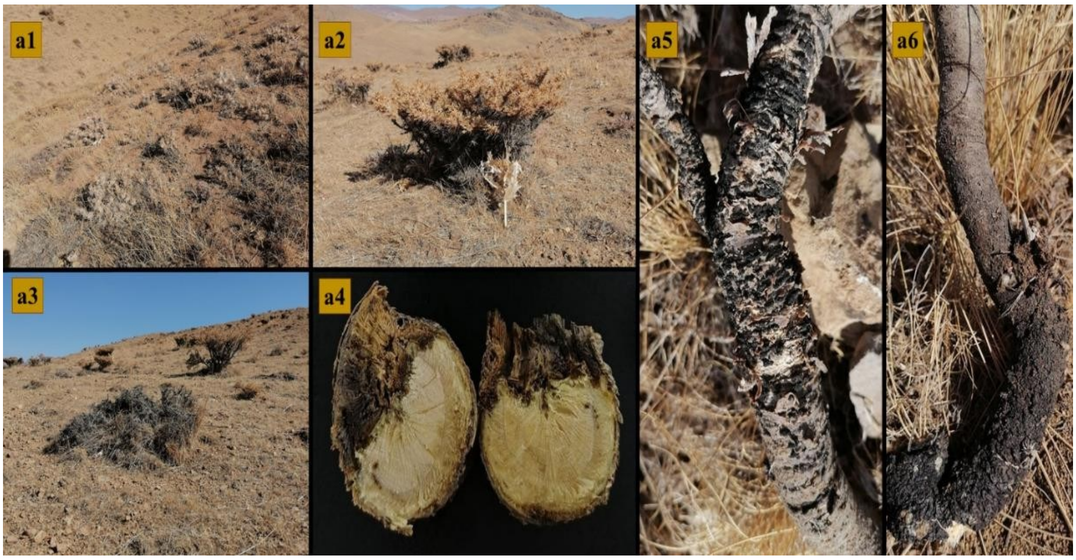

Figure 2. Symptoms of decline in *A. verus* in Hamedan province (a1, a2, and a3). Cross-section of branches displaying wood necrosis (a4). Dried branches (a5 and a6). Decline and wood necrosis symptoms were observed in all sampled points in young and mature *A. verus* plants in Hamedan province

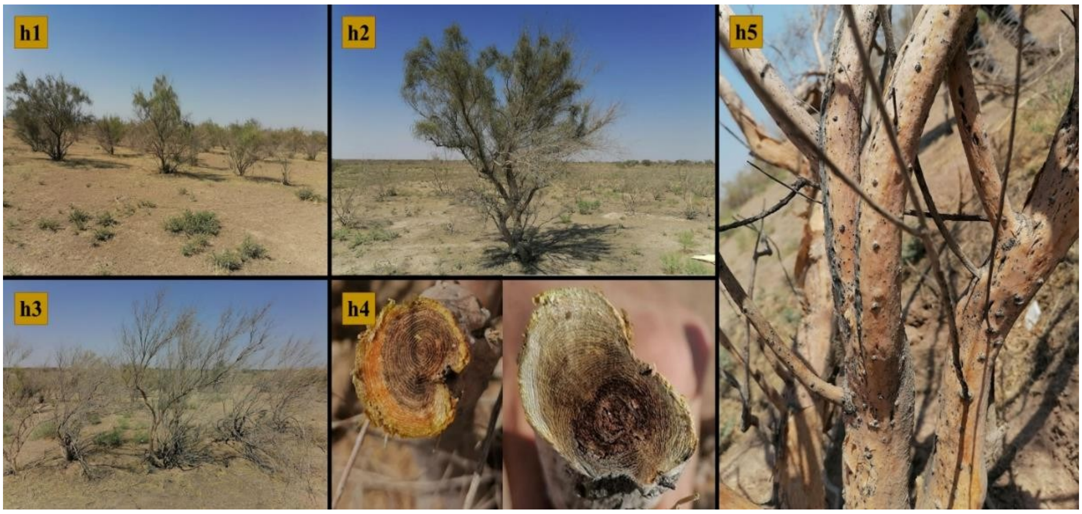

Figure 3. Symptoms of decline in *H. ammodendron* in Tehran and Alborz provinces (h1, h2, and h3). Cross-section of branches displaying wood necrosis (h4). Dried branches (h5 and h6). Decline and wood necrosis symptoms were observed in all sampled points in young and mature *H. ammodendron* plants in Alborz and Tehran provinces

2.2. Plant material

The samples were legally collected from unprotected dryland regions exhibiting decline and dieback symptoms in infected hosts across six locations in the Alborz, Tehran, and Hamedan provinces during 2021–2022, with the support of the Research Institute of Forests and Rangelands. A total of 20 samples were collected. Sampling involved randomly selecting 10 symptomatic branches or shoots from each host species, allowing for a representative assessment of the fungal communities associated with decline symptoms. The survey locations in urban and natural areas in Alborz, Tehran, and Hamedan provinces were chosen based on accessibility and known occurrences of symptoms, aiming to include a broad environmental and geographic variation.

2.3. Fungal isolation and purification

For fungal isolation, small segments (1 to 2.5 cm) of symptomatic tissues (branches and shoots) were surface-disinfected by soaking in 70% ethanol for 1 min, rinsed twice in sterile water for 2 min, and air-dried on sterile absorbent paper under a laminar flow hood for 40 min. The tissues were then cultured on potato dextrose agar (PDA) supplemented with 100 mg/liter ampicillin to suppress bacterial growth. Petri dishes were incubated at 27°C for 10 days to promote fungal colony development (Alizadeh et al. 2022). Following incubation, fungal colonies were subcultured onto fresh PDA. To obtain pure cultures, Water Agar (WA) was employed for purification: single hyphal tips from colonies grown on WA were transferred to PDA to establish axenic cultures. Purified fungal isolates were stored at 4°C in the Department of Plant Pathology at Tarbiat Modares University for further analysis. Isolates were photographed on PDA after 12 days of growth in a light box (Iranian-made) to document morphological features.

2.4. Morphotype selection

Fungi were grouped based on several key morphological and cultural characteristics, including colony color, texture, growth rate, and reproductive structures. The initial assessment involved examining colony morphology on PDA after a 10-day incubation period, where variations in colony shape, size, and pigmentation were documented. Additionally, the presence and type of reproductive structures (e.g., spores, conidia, and fruiting bodies) were recorded to further categorize each isolate. Selected morphotypes were based on distinct features that could suggest different species or taxonomic groups,ensuring that only representative morphotypes exhibiting notable differences were included for further analysis.

2.5. DNA extraction

DNA extraction was performed based on Alizadeh et al. (2022). After 10 days of fungal culture on PDA media, the fungi were crushed from the surface of the media by a sterile scalpel and transferred to a sterile mortar. Liquid nitrogen was added, and the samples were crushed for 20 seconds. These crushed fungi were immediately transferred to a 1.5 mL microcentrifuge tube. Then, 600 µl of DNA extraction buffer (TAE: Tris base-24.2 g; acetic acid-5.71 ml; EDTA-10 ml) was added to these tubes, which were immediately incubated in a warm bath at 65 °C for 15 min. Then, these tubes were incubated on ice for 15 min. The tubes were vortexed for 10 seconds. These tubes were centrifuged for 10 min at 11000 RCF at 4 °C to remove large particles. The supernatant was transferred to clean tubes. Then, 500 µl of cold isopropanol alcohol was added to the supernatant, which was slowly removed for 10 seconds. The tubes were centrifuged for 15 min at 13500 RCF at 20 °C. After removing the supernatant, the DNA pellet was allowed to dry at 25 °C. Approximately 50 µl of sterile DNase-free water was added to these pellets, which were subsequently stored at 4 °C until they were dissolved in sterile DNase-free water (Alizadeh et al. 2022).

2.6. PCR and sequencing

In general, 25 µL of PCR product consisting of 12.5 µL of Mastermix, 9 of deionized water, 1 µL of forward (ITS1: 5′-TCCGTAGGTGAACCTGCGG-3′) primer, 1 µL of reverse (ITS4: 5′-TCCTCCGCTTATTGATATGC-3′) primer (White et al., 1990), and 1.5 µL of DNA dissolved in deionized water was utilized. Each PCR run was started with the following settings: initial denaturation at 95 °C for 3 min; followed by 30 cycles of 94 °C for 40 s (denaturing), 58 °C for 45 s (annealing), and 72 °C for 1 min (elongation); and a final extension at 72 °C for 7 min (Alizadeh et al., 2022). The PCR products were visualized under UV light after gel electrophoresis in a 1% agarose gel containing GelRed at 90 volts for 25 min. The amplified PCR products were 600 base pairs (bp) (Figure 4). All PCR products were purified and sequenced by BGI Company in China (https://www.bgi.com/global).

2.7. Mycobiota evaluations and graphs

In this part, we selected one specific morphotype for similar fungi, and based on these morphotypes, the number of any fungi was determined to determine the relative abundance. Prism 6 (GraphPad Software, USA) was utilized for graphical visualization.

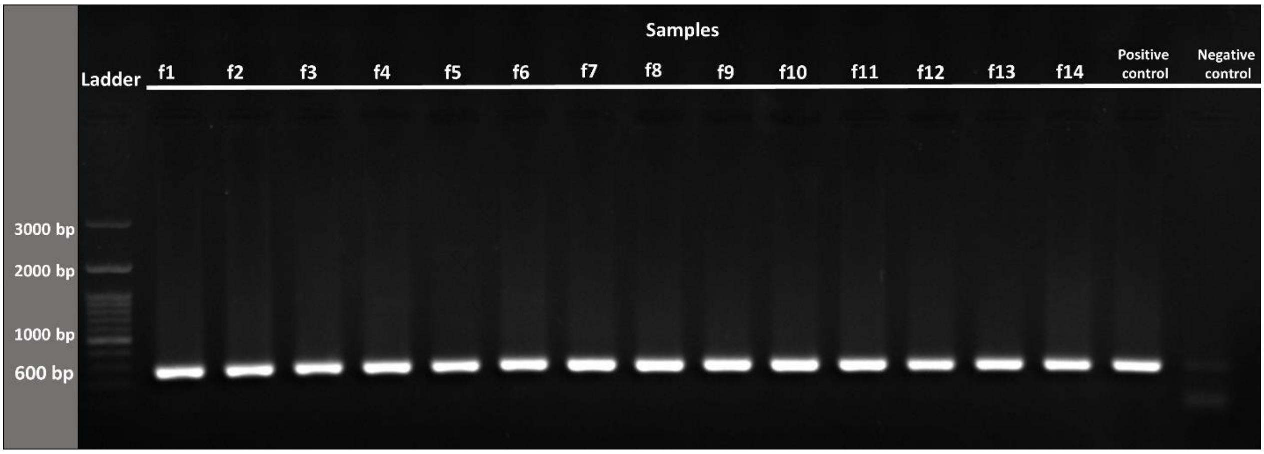

Figure 4. PCR products with 600 base pairs (bp). f1: *Alternaria*, f2: *Arthrinium*, f3: *Astragalicola*, f4: *Biscogniauxia*, f5: *Chaetomium*, f6: *Cytospora*, f7: *Fusarium*, f8: *Mucor*, f9: *Neoscytalidium*, f10: *Paecilomyces*, f11: *Pithomyces,* f12: *Preussia*. f13: *Sordaria*, f14: and an undefined genus associated with positive and negative controls

2.8. Phylogenetic tree with the present genera

The newly obtained internal transcribed spacer (ITS) sequences were selected for phylogenetic analyses. The dataset was updated by investigations in the database for acquiring accurate sequences. The outgroup taxa for the present dataset were taken based on the *Mucor* ITS region. All sequences were aligned by the Q-INS-i algorithm of MAFFT version 7 (http://MAFFT.cbrc.jp/alignment/server/) (Katoh & Standley, 2013), and the online version of Gblocks 0.91b (Castresana, 2000) was utilized to delete ambiguous parts of the alignment, with selection of all three options (including allowing smaller final blocks, allowing gap positions within the final blocks, and allowing less strict flanking positions) for less stringent selection (http://phylogeny.lirmm.fr/phylo_cgi/one_task.cgi?task_type=gblocks). The most suitable substitution model for the dataset was chosen using the Akaike information criterion (AIC) with PAUP∗/MrModeltest v2.2 (Nylander, 2004). A symmetrical model including a gamma distribution for rates (GTR+G) was selected for ITS analysis. Bayesian inference (BI) was performed using MrBayes v3.1.2 (Ronquist & Huelsenbeck, 2003) by selecting a random beginning tree and running the chains for 5 million for ITSs. After the burn-in samples were cast off, the residual samples were reserved for other analyses. The Markov chain Monte Carlo (MCMC) method within a Bayesian framework was utilized to assess the posterior probabilities of the phylogenetic trees (Larget & Simon, 1999) by the 50% majority rule. The acquired phylogenetic tree was generated via Dendroscope V.3.2.8 (Huson & Scornavacca, 2012) and then saved in Newick format. The Newick format was uploaded to Interactive Tree Of Life (iTOL) v4 (Letunic & Bork, 2021), and the final tree was drawn with all required components.

3. Result

3.1. Fungal morphotypes

The results of the morphological assessment revealed a diverse array of fungal isolates, categorized into 14 distinct morphotypes characterized by differing colony appearances for both hosts at 6 sampling locations. These morphotypes included ALT1 (*Alternaria*), ART1 (*Arthrinium*), AST1 (*Astragalicola*), BIS1 (*Biscogniauxia*), CHA1 (*Chaetomium*), CYT1 (*Cytospora*), FUS1 (*Fusarium*), MUC1 (*Mucor*), NEO1 (*Neoscytalidium*), PAE1 (*Paecilomyces*), PIT1 (*Pithomyces*), PRE1 (*Preussia*), SOR1 (*Sordaria*), and UNG1 (undefined genus) (Table 1). 12 and 8 morphotypes belonged to H. *ammodendron* and A. *verus*, respectively. The images of all morphotypes are shown in Figure 5.

3.2. Molecular identification

The ITS1-5.8S-ITS2 region of rDNA is the most broadly applied region for the identification of fungi. Newly obtained sequences were subjected to BLAST searches against the NCBI GenBank nucleotide database (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastnandPAGE_TYPE=BlastSearchandLINK_LOC=blasthome) to specify the closest sequences for the fungi isolated from both hosts. However, in this study, 14 morphotypes were characterized: *Alternaria*, *Arthrinium*, *Astragalicola*, *Biscogniauxia, Chaetomium, Cytospora, Fusarium, Mucor, Neoscytalidium, Paecilomyces, Pithomyces, Preussia, Sordaria*, and undefined genera. The sequences of the BLAST searches are shown in Table 1.

First, 162 and 97 fungal isolates were calculated to belong to H. *ammodendron* and A. *verus*, respectively. The names of genera and frequencies of fungi are listed in Table 1. Furthermore, the relative frequencies (RFs) were determined for the mycobiota in both H. *ammodendron* and A. *verus* hosts. In H. *ammodendron*, the RFs of fungal genera included *Arthrinium* (1.85%), *Astragalicola* (3.08%), *Biscogniauxia* (23.45%), *Chaetomium* (5.55%), *Cytospora* (1.85%), *Fusarium* (7.40%), *Neoscytalidium* (7.40%), *Paecilomyces* (49.38%), *Pithomyces* (3.08%), *Preussia* (3.08%), and *Sordaria* (1.23%). *Paecilomyces* and *Biscogniauxia* had greater numbers of RFs in H. *ammodendron* (Figure 6).

For A. *verus*, the RFs of fungal genera included *Alternaria* (4.12%), *Arthrinium* (3.09%), *Astragalicola* (35.05%), *Biscogniauxia* (5.15%), *Chaetomium* (27.83%), *Cytospora* (1.03%), *Fusarium* (7.21%), *Sordaria* (6.18%), and undefined genera (6.18%). On the other hand, most of the mycobiota of the A. verus host were from the *Astragalicola* and *Chaetomium* genera (Figure 7). Our results confirmed the presence of the Ascomycota and Mucoromycota phyla in A. *verus* and the presence of only the Ascomycota phylum in H. *ammodendron*. Although the present study revealed an enormous number of genera, undoubtedly many fungi were unidentified without microbiome analysis, revealing that the fungal biodiversity of the phyllosphere for both hosts is still unknown.

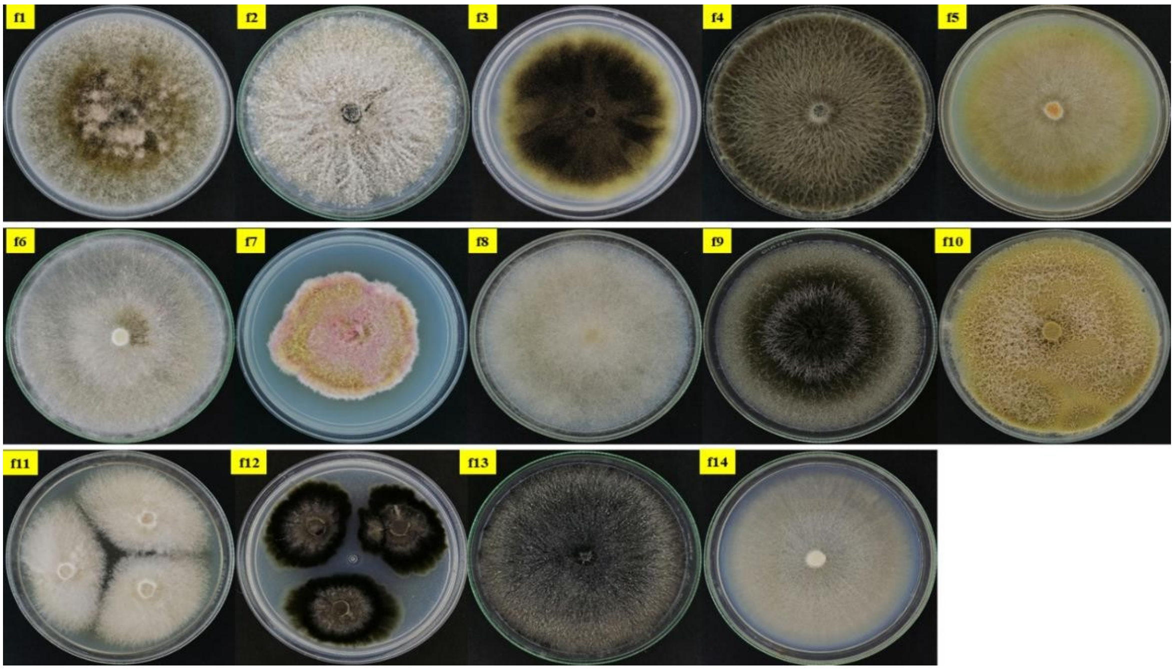

Figure 5. Fungal isolates on PDA medium. f1: *Alternaria*, f2: *Arthrinium*, f3: *Astragalicola*, f4: *Biscogniauxia*, f5: *Chaetomium*, f6: *Cytospora*, f7: *Fusarium*, f8: *Mucor*, f9: *Neoscytalidium*, f10: *Paecilomyces*, f11: *Pithomyces,* f12: *Preussia*. f13: *Sordaria*, and f14: undefined genus. These isolated fungi were photographed on PDA media after 12 days

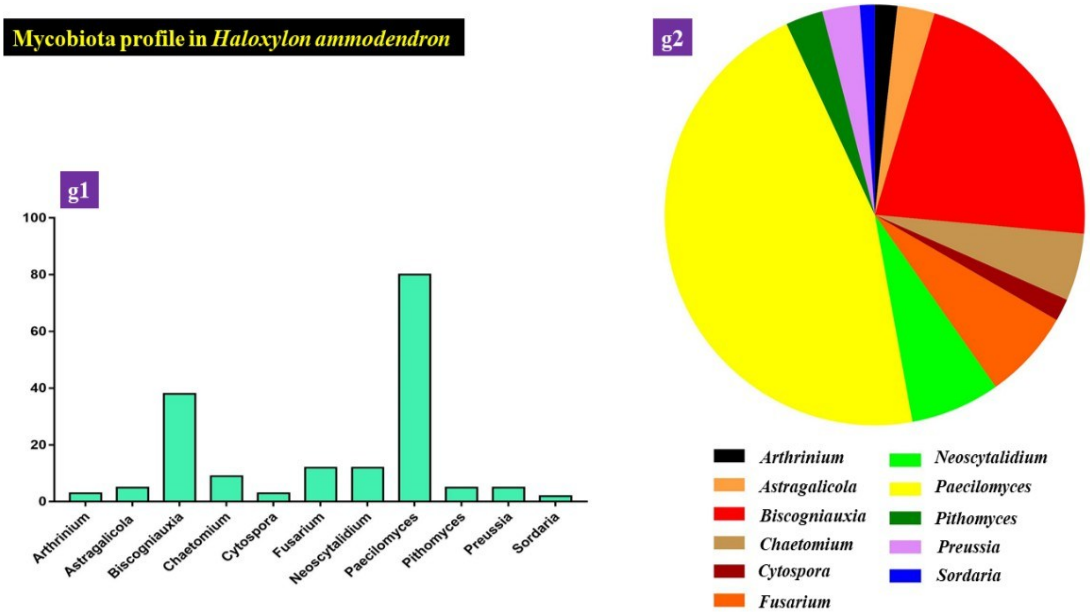

Figure 6. Number and frequencies of fungal isolates in *H. ammodendron*. g1: shows the number of isolated fungi. g2: represent the relative frequencies (RFs) of the identified genera in *H. ammodendron* plant. *Biscogniauxia* and *Paecilomyces* have been found to have a high frequency among isolated fungi

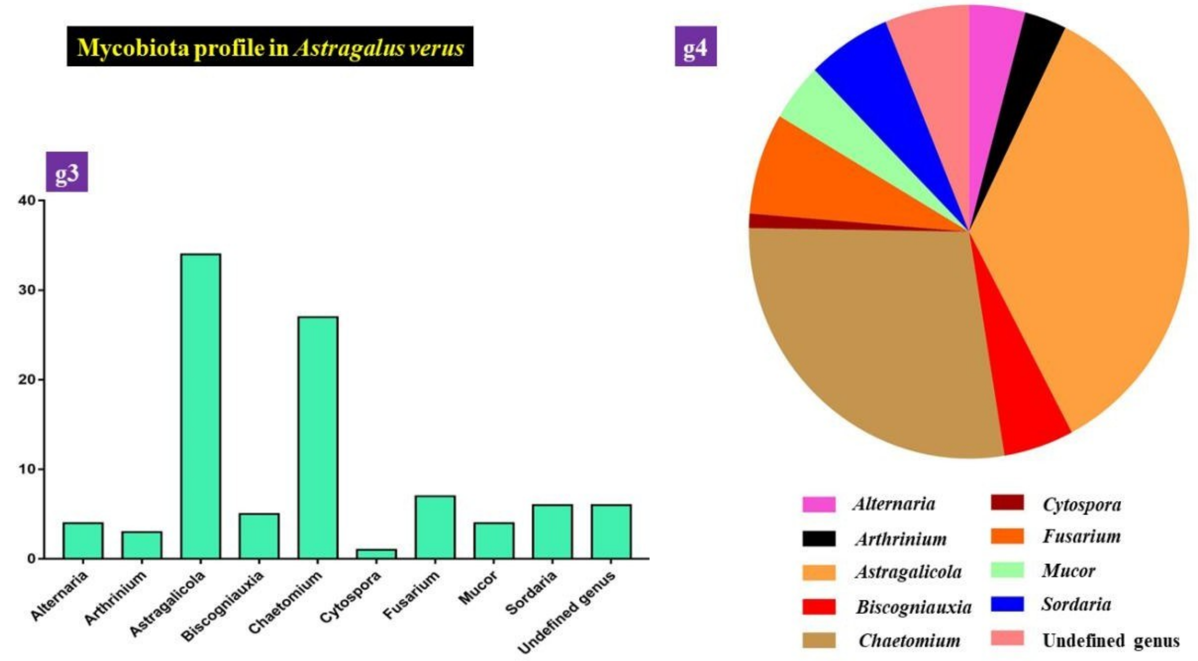

Figure 7. Number and frequencies of fungal isolates in *A. verus*. g3: shows the number of isolated fungi. g4: represent the relative frequencies (RFs) of the identified genera in *A. verus* plant. *Astragalicola* and *Chaetomium* have been found to have a high frequency among isolated fungi

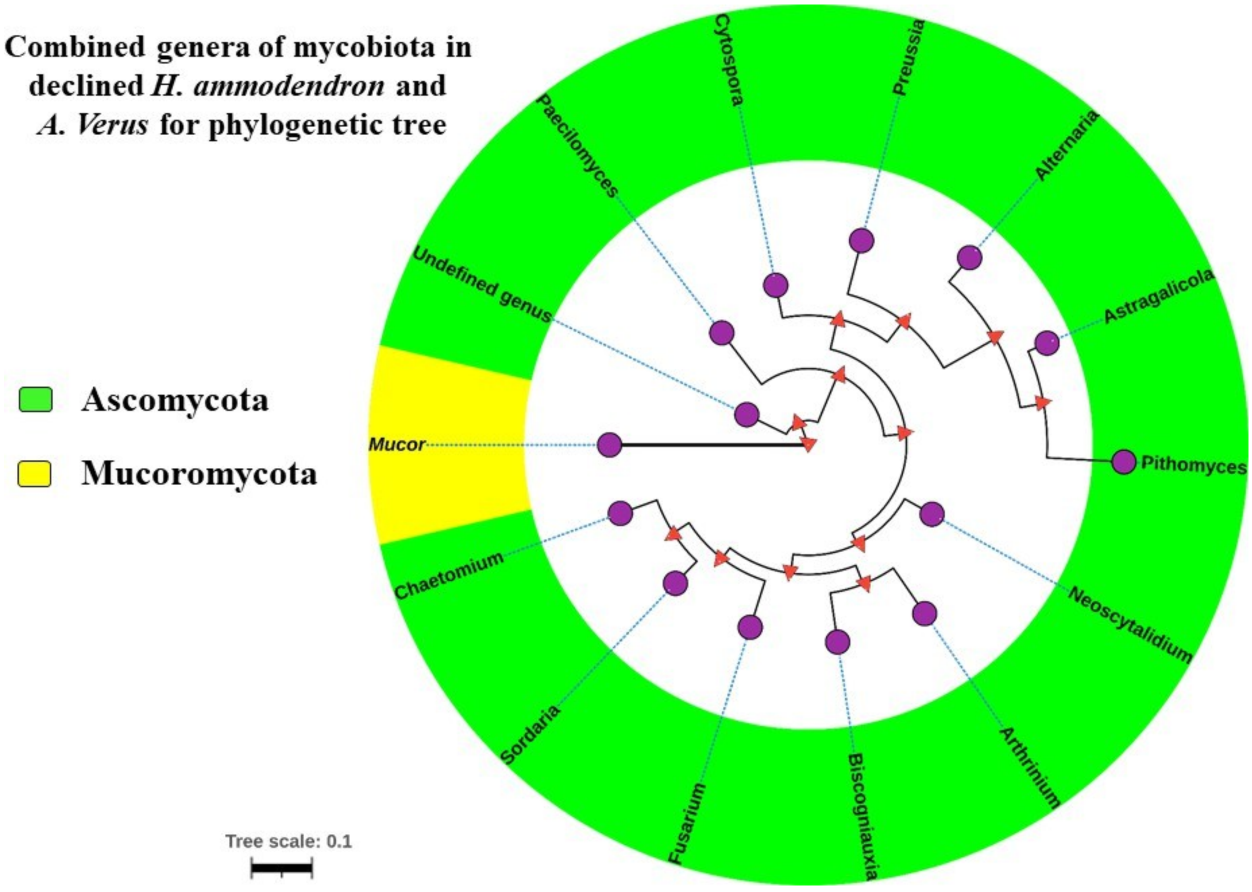

Figure 8. Phylogenetic tree showing the genera identified in this study. In comparison to Mucromycota, Ascomycota composed most of the fungal community. Ascomycota includes 12 identified genera, namely *Alternaria*, *Arthrinium*, *Astragalicola*, *Biscogniauxia*, *Chaetomium*, *Cytospora*, *Fusarium*, *Neoscytalidium*, *Paecilomyces*, *Pithomyces, Preussia,* and *Sordaria.* Furthermore, *Mucor* is the only genus within Mucormycota

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Table 1. Information about fungal isolates in both hosts as well as their classification

Row	Fungus genus	Morphotype	*H.ammodendron* (Isolate number)	*As.verus* (Isolate umber)	Classification
1	*Alternaria* sp.	ALT1	-	4	Ascomycota; saccharomyceta; Pezizomycotina; leotiomyceta; dothideomyceta; Dothideomycetes; Pleosporomycetidae; Pleosporales; Pleosporineae; Pleosporaceae
2	*Arthrinium* sp.	ART1	3	3	Ascomycota; saccharomyceta; Pezizomycotina; leotiomyceta; sordariomyceta; Sordariomycetes; Xylariomycetidae; Xylariales; Apiosporaceae
3	*Astragalicola* sp.	AST1	5	34	Ascomycota; saccharomyceta; Pezizomycotina; leotiomyceta; dothideomyceta; Dothideomycetes; Pleosporomycetidae; Pleosporales; Pleosporineae; Cucurbitariaceae
4	*Biscogniauxia* sp.	BIS1	38	5	Ascomycota; saccharomyceta; Pezizomycotina; leotiomyceta; sordariomyceta; Sordariomycetes; Xylariomycetidae; Xylariales; Xylariaceae
5	*Chaetomium* sp.	CHA1	9	27	Ascomycota; saccharomyceta; Pezizomycotina; leotiomyceta; sordariomyceta; Sordariomycetes; Sordariomycetidae; Sordariales; Chaetomiaceae
6	*Cytospora* sp.	CYT1	3	1	Ascomycota; saccharomyceta; Pezizomycotina; leotiomyceta; sordariomyceta; Sordariomycetes; Sordariomycetidae; Diaporthales;Valsaceae
7	*Fusarium* sp.	FUS1	12	7	Ascomycota; saccharomyceta; Pezizomycotina; leotiomyceta; sordariomyceta; Sordariomycetes; Hypocreomycetidae; Hypocreales; Nectriaceae
8	*Mucor* sp.	MUC1	-	4	Fungi incertae sedis; Mucoromycota; Mucoromycotina; Mucoromycetes; Mucorales; Mucorineae; Mucoraceae
9	*Neoscytalidium* sp.	NEO1	12	-	Ascomycota; saccharomyceta; Pezizomycotina; leotiomyceta; dothideomyceta; Dothideomycetes; Dothideomycetes incertae sedis; Botryosphaeriales; Botryosphaeriaceae
10	*Paecilomyces* sp.	PAE1	80	-	Ascomycota; saccharomyceta; Pezizomycotina; leotiomyceta; Eurotiomycetes; Eurotiomycetidae; Eurotiales; Thermoascaceae
11	*Pithomyces* sp.	PIT1	5	-	Ascomycota; saccharomyceta; Pezizomycotina; leotiomyceta; dothideomyceta; Dothideomycetes; Pleosporomycetidae; Pleosporales; Astrosphaeriellaceae
12	*Preussia* sp.	PRE1	5	-	Ascomycota; saccharomyceta; Pezizomycotina; leotiomyceta; dothideomyceta; Dothideomycetes; Pleosporomycetidae; Pleosporales; Sporormiaceae; Preussia/Sporomiella species complex
13	*Sordaria* sp.	SOR1	2	6	Ascomycota; saccharomyceta; Pezizomycotina; leotiomyceta; sordariomyceta; Sordariomycetes; Sordariomycetidae; Sordariales; Sordariaceae
14	Undefined genus	UNG1	-	6	Ascomycota; saccharomyceta; Pezizomycotina
Total			162	97	

3.3. Phylogenetic trees

The results of phylogenetic tree construction in MrBayes and iTOL showed that all genera created separate taxa (Figure 8). Undefined genera in the Ascomycota phylum indicated that they belonged to the phylum Pezizomycetes based on BLAST data. Additionally, the *Mucor* genus was selected as the outgroup based on its classification, and it was confirmed that it is outside the Ascomycota phylum and belongs to the Mucoromycota phylum. Phylogenetically, Ascomycota dominated both hosts, but A. *verus* uniquely harbored Mucoromycota. These differences highlight host-specific fungal associations and ecological niche partitioning in dryland ecosystems. The present phylogenetic tree shows the combined genera of the present sequenced fungi in both H. *ammodendron* and A. *verus* plants.

3.4. Effect of geographical location and host plant on mycobiota composition

The effects of geographical locations and host plant species revealed distinct milestones in shaping the mycobiota associated with these hosts, as observed in samples from ALT1, MUC1, NEO1, PAE1, PIT1, PRE1, and UND1. These differences are primarily related to the presence and dominance of specific fungal genera, including *Alternaria*, *Mucor*, *Neoscytalidium,*

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Paecilomyces, Pithomyces, Preussia, and an undefined genus, indicating that both environmental and biological factors influence fungal community composition. Variations in climate, soil conditions, and other environmental factors across different geographic sites likely contribute to the diversity and abundance of these fungal taxa. Additionally, host plant species may provide unique niches or defenses that select for specific fungal communities. Consequently, these findings demonstrate that both geographical location and host plant identity are key determinants of mycobiota composition, influencing ecological interactions, disease susceptibility, and overall ecosystem health in forest and dryland environments.

4. Discussion

The study of fungi in dryland ecosystems faces numerous challenges that impede comprehensive understanding. Sparse vegetation and uneven plant distribution complicate the collection of representative samples, potentially biasing data (Berdugo et al., 2022). Harsh environmental conditions—such as high temperatures, low moisture, and nutrient-poor soils—further influence fungal diversity and community structure, making it difficult to decipher fungi's responses to environmental stresses. Additionally, limited research focus, difficulties in species identification, and a lack of long-term data hinder progress. Our investigations on the mycobiota of H. *ammodendron* and A. *verus* are the first in Iran to compare these two hosts and to address the occurrence of decline and dieback across different regions. Based on ITS sequence results, we isolated and identified several pathogenic fungal genera from both hosts. In total, 14 genera were identified: 12 for H. *ammodendron* and 10 for A. *verus*. These included notable genera such as *Alternaria, Arthrinium, Astragalicola, Biscogniauxia, Chaetomium, Cytospora, Fusarium, Mucor, Neoscytalidium, Paecilomyces, Pithomyces, Preussia, Sordaria,* and some undefined genera. Our findings highlight the importance of pathogenicity testing and precise fungal identification to diagnose issues effectively. For example, we identified fungal genera such as *Arthrinium*, *Astragalicola*, *Biscogniauxia, Chaetomium, Cytospora, Fusarium*,and *Sordaria* in both A. *verus* and H. *ammodendron*, with *Fusarium, Rhizoctonia*, and *Alternaria* confirmed as significant pathogens affecting these species (Marin-Felix et al., 2019; Armitage et al., 2020; Raimondo et al., 2016). The study revealed that these fungal genera—many identified as plant pathogens—pose potential risks to plant health, aligning with reports from other studies (Marin-Felix et al., 2019; Armitage et al., 2020; Kwon et al., 2021; Raimondo et al., 2016; Norphanphoun et al., 2017; Zhu et al., 2020; Mirghasempour et al., 2022; Alizadeh et al., 2022; Heidarian et al., 2018). Given the ecological, economic, and societal importance of drylands (Adhikari et al., 2019), these findings highlight the need for targeted conservation strategies and integrated agricultural practices.

This study highlights the ecological roles and pathogenic potential of the dominant fungal genera identified, including *Paecilomyces, Biscogniauxia*, *Astragalicola*, and *Chaetomium*. The genus *Paecilomyces* is particularly notable for its dual role as both a saprobic and pathogenic organism. It predominantly functions as a saprobe in soil systems, contributing to organic matter decomposition and nutrient cycling. However, certain species have demonstrated pathogenic behavior toward plants, particularly under environmental stress, potentially impacting xerophytic species such as *Haloxylon ammodendron* in semiarid ecosystems. Similarly, *Biscogniauxia*, with its association with particularly dry environments, can be detrimental to plant health, causing diseases such as cankers in woody plants. The presence of *Biscogniauxia* species can indicate compromised plant vitality and environmental stress, leading to significant impacts on local dryland ecosystems. On the other hand, *Astragalicola* has shown symbiotic or endophytic relationships in certain contexts (Mattoo & Nonzom, 2023), yet it is also associated with pathogenicity, particularly affecting A. *verus* in the regions studied. Finally, *Chaetomium* species are involved in ecological succession and can act as plant endophytes under conditions that challenge plant resilience, such as drought or poor soil quality (Pan et al., 2024). The high frequency of these genera in the sampled plants highlights the complexities of biotic interactions in dryland habitats, where both beneficial and harmful fungal dynamics can significantly influence plant survival and ecosystem stability.

The phylogenetic analysis of the isolated genera revealed that the majority of the mycobiota belonged to the Ascomycota phylum, with a smaller proportion from Mucoromycota. As part of the complex and dynamic fungal community associated with declining dryland plants, these fungi play crucial roles in nutrient cycling, plant health, and ecosystem resilience (Liu et al., 2022; Yang et al., 2024). The community is primarily dominated by two major phyla: Ascomycota and Basidiomycota. Ascomycota are instrumental in degrading complex organic matter, which facilitates nutrient release into the soil-a vital process in nutrient-limited dryland environments are known for their ability to thrive in harsh conditions, forming mutualistic relationships with plants that enhance resistance to pathogens and promote growth, especially under stressors like drought (Liu et al., 2022; Yang et al., 2024). Conversely, Basidiomycota, although less abundant, contribute significantly by forming mycorrhizal associations that improve nutrient uptake and soil structure, further supporting ecosystem health. The presence of both phyla indicates a healthy and resilient ecosystem. Environmental factors such as soil moisture, temperature, and organic matter content influence the dynamics of the mycobiota, with drought conditions often shifting community composition towards drought-resistant species (Liu et al., 2022).

Various studies across different plant species reinforce these findings. For instance, in pear trees, Ascomycota were predominant alongside Zygomycota and

Basidiomycota (Ren et al., 2019), while in ash trees, Ascomycota accounted for 60.5% of the mycobiota (Bakys et al., 2022). Research on Cnidoscolus aconitifolius showed that Ascomycota constituted the entire fungal community, and in Tectona grandis, 43 morphotypes (99.7%) belonged to Ascomycota compared to only two morphotypes (0.3%) of Basidiomycota (Singh et al., 2017). Additionally, studies on poplar tissues affected by vascular wilt revealed a dominance of Ascomycota and Basidiomycota, with smaller contributions from Glomeromycota and Zygomycota (Kwaśna et al., 2021). Overall, these findings underscore the prevalence and critical ecological roles of Ascomycota across diverse plant ecosystems, highlighting their importance in maintaining ecosystem stability, particularly in the face of changing climate conditions.

Various fungi have been identified as causal agents or associates of diseases in Astragalus species across multiple studies. These include genera such as Embarrisia sp., *Fusarium* oxysporum, *Fusarium* chlamydosporum, *Fusarium* avenaceum, *Fusarium* solani, *Fusarium* semitectum, *Fusarium* verticilloides, Conostachys rosea, Cladosporium herbarum, *Alternaria* alternata, and other *Alternaria* species (Li et al., 2007). Specific pathogenic fungi, like Embellisia astragali, have been found on Astragalus adsurgens (Li & Nan, 2009), while *Fusarium* spp., including F. oxysporum and F. solani, have been linked to root rot in A. membranaceus (Chen et al., 2011) and A. mongholicus (Li et al., 2021). Additionally, F. solani has been associated with root rot in A. membranaceus var. mongholicus (Ren et al., 2016), and multiple fungi have been detected in the roots of Astragalus plants (Niu et al., 2016; Tang et al., 2017). Colletotrichum spinaciae was identified as the causative agent of anthracnose in A. membranaceus (Jin et al., 2021). A study conducted in Isfahan Province, Iran, revealed the presence of pathogenic fungi such as Verticillium dahliae, Rhizoctonia solani, F. solani, and F. oxysporum in Astragalus plants showing decline and dieback symptoms (Nasr Esfahani et al., 2018). Despite extensive research into these fungal pathogens, few studies have specifically addressed the pathogenicity of fungi on A. *verus*, primarily focusing instead on underground hosts with root rot symptoms. Consequently, future research should prioritize investigating the aerial parts of A. *verus* and other Astragalus species-manifesting decline and dieback symptoms to better understand disease dynamics.

Numerous studies have identified specific biotic and abiotic factors contributing to the decline of Haloxylon species. Sabbagh Sharafabadi et al. (2002) confirmed that fungal pathogens such as *Fusarium* solani, F. *oxysporum*, F. *culmorum*, Pythium aphanidermatum, *Alternaria* alternata, and Rhizoctonia fragariae are pathogenic to Haloxylon spp. Additionally, Tavakoli Neko et al. (2019) demonstrated how abiotic factors like salinity and nutrient deficiency exacerbate plant drying and decline in these ecosystems. The main pests affecting H. *ammodendron* include Aceria haloxylonis (Li et al., 2016), Julodis variolaris (Song, 2008), Loxostege stieticatis (Chen et al., 2007), Desertobia heloxylonia (Li et al., 2007), Lacydes spectabilis (Yang et al., 2010), Chromonotus sp. (Zang, 1986), and Anomala exoleta (Chen et al., 2004). Most research on H. ammodendron has focused on its genetics (Batkhuu et al., 2019; Hu et al., 2021), responses to abiotic stresses (Hu et al., 2021), interactions with biotic agents (He et al., 2021a), organic materials (Rigi Pardad et al., 2021), culture studies (He et al., 2021b), and nutrition (Zhao et al., 2021). Despite this extensive research, the pathogenicity of various biotic agents or phytopathogens specifically affecting H. ammodendron remains understudied. Therefore, further research is essential to identify the causes of decline and dieback symptoms in regions where Haloxylon species are prevalent, which will improve our understanding of disease dynamics and inform management strategies.

In H. ammodendron, all isolated fungi belong to the Ascomycota phylum, whereas A. *verus* hosts both Ascomycota and Mucoromycota, reflecting differences in fungal community composition. These insights are vital for addressing latent pathogens in drylands and devising management strategies. Traditional culturing methods are increasingly being supplemented—or replaced—by next-generation sequencing (NGS), which can detect numerous taxa within a single sample more efficiently (Ruiz Gómez et al., 2019). Microbiome analysis, by identifying beneficial microorganisms that promote plant growth and disease resistance (biocontrol), can help improve crop yields and reduce reliance on chemical inputs (Bonatelli et al., 2021). However, these ecosystems face limitations like the difficulty of collecting representative samples due to sparse vegetation and harsh environmental conditions, which affect fungal diversity and community responses to stresses. To overcome these challenges, innovative methods and interdisciplinary collaboration are essential. Improved understanding of ecosystem responses to environmental fluctuations, such as temperature and precipitation changes, is critical for managing plant health and preventing decline (Yang et al., 2024).

The study of fungi in dryland ecosystems faces several significant challenges that hinder comprehensive research. Sparse vegetation and uneven plant distribution complicate the collection of representative samples, potentially biasing results (Berdugo et al., 2022). The harsh environmental conditions-high temperatures, low moisture, and poor soils—also influence fungal diversity and community structure, making it difficult to understand how fungi respond to environmental stresses. Additional limitations include limited research focus, difficulties in species identification, and a lack of long-term data, which are crucial for monitoring ecosystem dynamics. Current gaps in knowledge involve understanding the types and distribution of plant arrangements across arid regions and the roles of biotic and abiotic factors influencing these variations. Moreover, research on water and nutrient availability in desert ecosystems remains limited. Our findings on fungal pathogens associated with declining plants such as *Haloxylon ammodendron* and *Astragalus verus* underscore the importance of pathogenicity testing and accurate fungal identification for effective diagnosis and management. These insights can inform policymaking and guide the development of targeted environmental and agricultural programs—such as risk assessment frameworks, conservation strategies, and integrated pest management (IPM). Implementing sustainable practices, including the use of beneficial microbes for biocontrol, can enhance plant resistance and soil health, ultimately promoting ecosystem resilience, biodiversity, and sustainable land use in drylands. Overcoming these challenges with innovative research methods and interdisciplinary collaboration is essential for better understanding and managing these fragile ecosystems.

5. Conclusion

This study offers vital insights into the fungal communities associated with the declining H. *ammodendron* and A. *verus* in Iran's drylands, highlighting the presence of multiple pathogenic and potentially latent fungal genera. Sanger sequencing of the ITS region primarily revealed a dominance of Ascomycota, including genera such as *Paecilomyces, Biscogniauxia*, *Astragalicola*, and *Chaetomium*, although their roles in host decline remain poorly understood. To advance this research, we recommend several key steps: conducting pathogenicity testing through controlled inoculation experiments and applying Koch's postulates—especially for high-abundance taxa like *Paecilomyces* and *Astragalicola*—to clarify their direct contributions to plant mortality; employing NGS-based profiling methods such as metagenomics and ITS metabarcoding to capture the full spectrum of mycobiota, including unculturable and rare taxa, and to understand microbial interactions and community shifts under environmental stress; establishing long-term monitoring to link fungal dynamics with climatic and soil parameters, enabling ecological modeling to predict climate change impacts; and exploring biocontrol strategies with beneficial microbes or microbiome engineering to bolster host resilience, thereby integrating ecological understanding with practical conservation approaches to mitigate pathogen threats and support the sustainability of these vulnerable ecosystems under global change.

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Authors Contribution

All the authors have participated sufficiently in the intellectual content, conception and design of this work or the analysis and interpretation of the data (when applicable), as well as the writing of the manuscript.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Conflict of interests

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.

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